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Population analysis of the European Green Toad (*Bufotes viridis*) in Donaufeld, Vienna

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Abstract

Amphibians are the worlds most threatened animal group. Loss of habitat is one of the main reasons for the decline. In Austria, the European Green Toad (*Bufotes viridis*) well-known in urban areas, is one of these endangered species. The study area Donaufeld is big and open with fields, but a big urban expansion with residential buildings is planned there. The transformation of their habitat Donaufeld in Vienna poses a potential threat to the Green Toad population there.

The present study is a capture-recapture study on the current Green Toad population in the Donaufeld. Data were collected during the breeding season (March until July) of 2023 and include sex, body mass, snout-vent-length of captured toads. The toads were captured, measured, photographed and released again after data collection. For population analysis the Jolly-Seber-Schwarz-Arnason (JSSA) model for open populations was used.

96 individual toads (66 males, 30 females) were captured during this study. The males were in average lighter and smaller than females but the SMI suggests no difference in body condition of the sexes. Overall *B. viridis* in the Donaufeld show a comparably high body condition in comparison to toads of other Viennese populations. The calculated population size of 2023 in the Donaufeld with 172 individuals (CI: 128 – 232) is smaller than calculations of 2021 and 2022, suggesting a decline. The apparent survival rate Phi was lowest from 2022 to 2023.

The high SMI and recapture rate also indicate a well-established population with mature, healthy toads. The Donaufeld apparently provides an optimal habitat for the European Green Toad, resulting in good body condition when compared to other Viennese populations. Nevertheless, the population size is likely decreasing, probably partly due to the ongoing construction work. As the habitat Donaufeld is changing, the question remains if this population of Green Toads will be able to adapt.

In conclusion, this study underscores the importance of continued monitoring and conservation efforts on the Donaufeld Green Toad population to ensure its long-term survival.

Key words: urban habitat, Bufonidae, population monitoring, demographic analysis

Zusammenfassung

Amphibien sind die am stärksten gefährdete Tiergruppe der Welt. Der Verlust des Lebensraums ist einer der Hauptgründe für ihren Rückgang. In Österreich ist die Wechselkröte (Bufotes viridis), bekannt in städtischen Gebieten, eine dieser bedrohten Arten. Das Untersuchungsgebiet Donaufeld ist groß und offen mit Feldern, aber eine große Erweiterung mit Wohngebäuden ist dort geplant. Die Transformation ihres Lebensraums Donaufeld in Wien stellt eine potenzielle Bedrohung für die Wechselkrötenpopulation dort dar. Die vorliegende Studie ist eine Fang-Wiederfang-Studie an der aktuellen Wechselkrötenpopulation im Donaufeld. Daten wurden während der Laichsaison (März bis Juli) 2023 gesammelt und umfassen Geschlecht, Körpermasse, Schnauzen-Anus-Länge der gefangenen Kröten. Die Kröten wurden gefangen, vermessen, fotografiert und nach der Datensammlung wieder freigelassen. Für die Populationsanalyse wurde das Jolly-Seber-Schwarz-Arnason (JSSA) Modell für offene Populationen verwendet. In dieser Studie wurden 96 individuelle Kröten (66 Männchen, 30 Weibchen) gefangen. Die Körpermasse und Schnauzen-Anus-Länge der Männchen waren leichter und kleiner als die der Weibchen, aber der SMI deutet auf keinen Unterschied in der Körperkondition der Geschlechter hin. Insgesamt zeigen *B. viridis* im Donaufeld eine hohe Körperkondition im Vergleich zu anderen Wiener Populationen. Die berechnete Populationsgröße von 2023 im Donaufeld ist mit 172 Individuen (CI: 128 - 232) kleiner als Berechnungen von 2021 und 2022, was auf einen Rückgang hinweist. Die Überlebensrate Phi war von 2022 bis 2023 am niedrigsten. Der hohe SMI und die Fang-Wiederfang-Rate deuten ebenfalls auf eine etablierte Population mit ausgewachsenen, gesunden Fröschen hin. Das Donaufeld bietet anscheinend einen optimalen Lebensraum für den Europäischen Grünfrosch, was zu einer guten Körperkondition im Vergleich zu anderen Wiener Populationen führt. Dennoch nimmt die Populationsgröße wahrscheinlich ab, möglicherweise teilweise aufgrund der laufenden Bauarbeiten. Da sich der Lebensraum Donaufeld verändert, bleibt die Frage, ob diese Population von Grünfröschen sich anpassen wird. Zusammenfassend betont diese Studie die Bedeutung der kontinuierlichen Überwachung und des Naturschutzes der Grünfroschpopulation im Donaufeld, um ihr langfristiges Überleben sicherzustellen.

Schlagwörter: urbanes Habitat, Bufonidae, Populationsmonitoring, Populationsanalyse

Introduction

Amphibians are the world's most threatened animal group. Out of all amphibian species 41% are listed as critically endangered, endangered or vulnerable (Luedtke et al., 2023). Most amphibians require both terrestrial and aquatic habitats during their different life stages (Stöck et al., 2008; Glandt, 2015, 2018). This complex life cycle makes them vulnerable to habitat loss, as they are dependent on two very different habitats (Glandt, 2018). Habitat loss due to infrastructural development, human disturbance or agriculture are the main reasons for the decline in amphibians (Luedtke et al., 2023).

An amphibian species often found in urban areas in central Europe is the European Green Toad, *Bufotes viridis* (Laurenti, 1768), which was first described from Vienna. The Green Toad is found throughout most of Central and East Europe, but is also common across Anatolia and the Caspian Depression to Siberia (Dufresnes et al., 2019). Its habitats primarily consist of open steppes with little vegetation and loose soils (Stöck et al., 2008; Glandt, 2015). As their natural habitat is degrading, *B. viridis* has become well-known in urban areas such as parks.

Bufotes viridis is regarded as a pioneer species that inhabits new water bodies and habitats relatively quickly (Cabela et al., 2001; Glandt, 2015; Mazgajska & Mazgajski, 2020). It is a thermophilic species that prefers to breed in shallow, warm and often temporary waters such as puddles (Glandt, 2015; Mazgajska & Mazgajski, 2020). Because of data on population decline *B. viridis* is listed as vulnerable in Austria (Gollmann, 2007). The species is considered to be critically endangered in Vienna (Tiedemann & Häupl, 1994). Vienna is an expanding city with intensive development which subsequently leads to habitat loss for Green Toads and other species due to sealing of green spaces (Tiedemann & Häupl, 1994). *Bufotes viridis* is not only endangered in Austria but is also listed in the Annex IV of the European Council Habitats and Species Directive (Umweltbundesamt, 2019) which includes species for which specific protective measures must be taken (Council Directive 92/43/EEC, 2013). In several Austrian states, conservation programs have been implemented to ensure the survival of this endangered species, such as the "Wechselkrötenaktionsplan" in Vienna (Gollmann, 2007; Rienesl, 2017).

In Vienna, one can see the Green Toads ability to adapt to new environments and urban habitats quite well. There are several well-known populations within the city area. One example is the "Nordbahnhofgelände" in Vienna (Csarmann, 2012; Wappl & Heyer, 2016;

Wolensky, 2023). The area is close to the city centre and offers open ground spots and fallow land (Csarmann, 2012). The toads use the water bodies in the Rudolf-Bednar-Park as breeding sites (Csarmann, 2012; Wappl & Heyer, 2016). Populations of *B. viridis* are also known in gardening and agricultural areas like the Simmeringer Haide (Staufer, 2018; Horvath, 2023) and in the open fields of the Donaufeld (Sistani et al., 2021). A recent paper comparing data from the last few decades by Landler et al. (2023) also showed that the presence of Green Toads was found to be especially high on construction sites.

Even though *B. viridis* is regarded as a pioneer species and well known in urban areas, it stays an endangered species and in the last decades the population size of the Green Toad is declining in many European cities (Kühnel & Krone, 2003; Herczeg et al., 2012; Konowalik et al., 2020). Habitat fragmentation may impede migration of toads and temporary breeding sites often dry out too quickly for the tadpoles to develop fully in urban areas (Herczeg et al., 2012; Konowalik et al., 2020). Roadkill might also be one of the reasons for the decline of *B. viridis* in urbanised areas (Herczeg et al., 2012; Staufer, 2018; Konowalik et al., 2020; Horvath, 2023). Furthermore urbanization often reduces the size of populations and hinders the gene flow between them (Johnson & Munshi-South, 2017). This process led to lower genetic diversity in some urban populations of *B. viridis* (Vargová et al., 2023).

The Donaufeld in Vienna is a transforming area, which makes monitoring of its breeding population quite interesting. Moreover, the breeding population of *B. viridis* in the Donaufeld was not reported until a few years ago. A monitoring of spawning areas of amphibians in Vienna from 2016 (Schweiger et al., 2016) did not list the Donaufeld as an area where *B. viridis* was found. In the Donaufeld, Green Toads were first recorded in 2017 by C. Riegler (HFDÖ), leading to the suggestion that the Donaufeld as a transforming area might have not been a suitable habitat before. Sistani et al. (2021) conducted the first study on this population of *B. viridis*. Since then, the yearly monitoring of the toads was done by students during an university course (Liebhart, 2021; Gurtner, 2022; Zanghellini, 2022). In 2022 *B. viridis* in the Donaufeld showed a higher SMI (Scaled Mass Index) compared to the populations in the Rudolf-Bednar-Park and Simmeringer Haide (Zanghellini, 2022). The higher SMI indicates a greater body condition (Landler et al., 2022) and could indicate long living adults (Zanghellini, 2022). The SMI might be because the open, sunny fields of the Donaufeld provide a characteristic urban habitat for European Green Toads (Schweiger et al., 2021)

In the Donaufeld, further big urban expansions with residential buildings for thousands of people are planned (IBA Wien, 2020a). The City Council approved the zoning and development plans for the project "An der Schanze" in 2017 (Stadt Wien, 2024). This development poses a potential threat to the Green Toad population located there. Plans include the construction of 1500 new flats, student housing, as well as the implementation of a new tram and bus line. Despite the high protection status of *B. viridis* in Vienna the construction work has already begun in some areas. The construction work includes a new-build house and plowed gardens, which are situated close to known breeding sites of the Green Toads.

The construction activities directly threaten the toads, as one of the breeding sites (a shallow, temporary puddle) is affected by the construction plan and is going to be removed in the building process (IBA Wien, 2020a). As amphibians migrate in a small radius of only few kilometres (Rienesl, 2021), destruction of the habitats can have significant impact on the population. If their habitat were destroyed and no suitable habitat provided, the population could be threatened with extinction (Mazgajska & Mazgajski, 2020). Compensatory measures, such as the creation of a compensation pond, are therefore being carried out in the Donaufeld. Still, it remains uncertain if these measures will work.

This master thesis is a continuation of the capture-recapture studies that were conducted in the Donaufeld in the last years since discovery of the population(Liebhart, 2021; Sistani et al., 2021; Gurtner, 2022; Zanghellini, 2022). Data acquisition of the current size and status of the population of the European Green Toad in the Donaufeld is crucial, due to ongoing construction, which makes it uncertain how the population is faring. Despite construction, there is a chance of shallow pools and potential new breeding sites emerging. Regular area-wide monitoring is essential to observe the population. Given that *B. viridis* is listed as vulnerable in Austria (Gollmann, 2007), monitoring this endangered population in Donaufeld is important for species conservation efforts.

Material and Methods

Study species

Sexually mature adults of the European Green Toad (*Bufotes viridis*) are usually between 50 and 80 mm long. Among the most striking features of Green Toads are coloration and pattern by which the individuals can also be distinguished from another. The primary colour is light cream to greenish with light green to dark green patterns on the back (Stöck et al., 2008; Glandt, 2018; Rienesl, 2021). Females are usually larger and less warty, while males have callused and dark thumbs during mating season (Rienesl, 2021). Males also emit distinct mating calls during the mating season (Stöck et al., 2008; Rienesl, 2021). During mating, the male clasps the female around the back and holds its hind legs between the female's thighs to inseminate the spawn. This mating position is called amplexus (Stöck et al., 2008). The breeding season of *B. viridis* starts usually at the end of March and ends at end of June. Each female spawns about 5000 to 15000 eggs in egg strings into the water of shallow ponds. After three to four days tadpoles with gills hatch out of the eggs. After around twelve weeks of development, they leave the water as air breathing metamorphs (Glandt, 2018; Rienesl, 2021).

Study area

The study area Donaufeld is located in the 21st district of Vienna (Austria), north of the Danube.

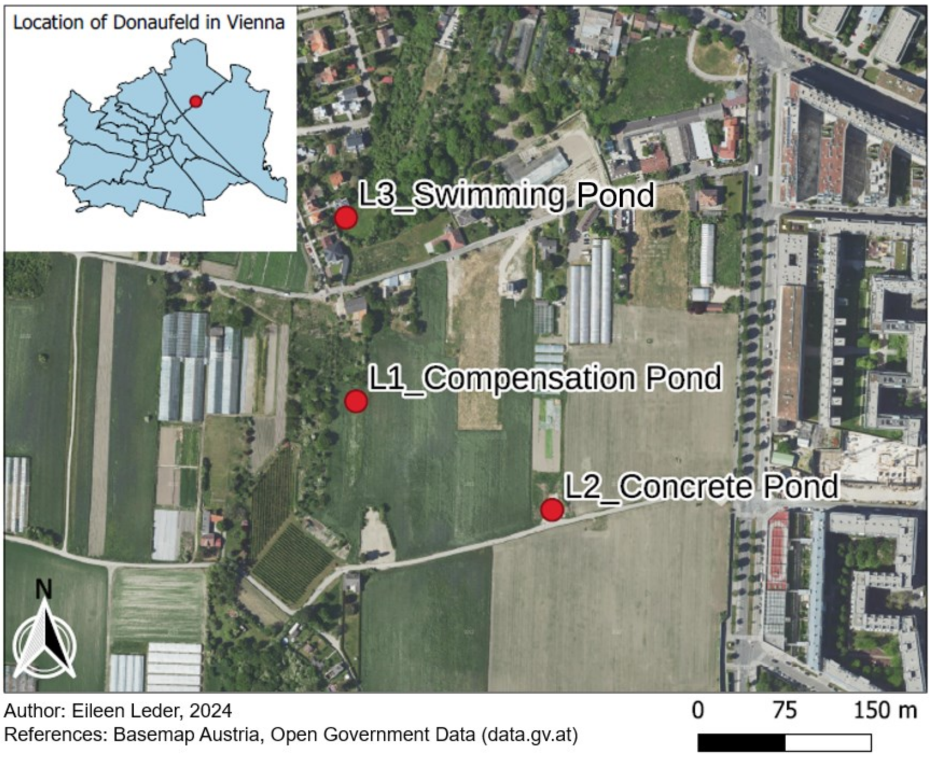
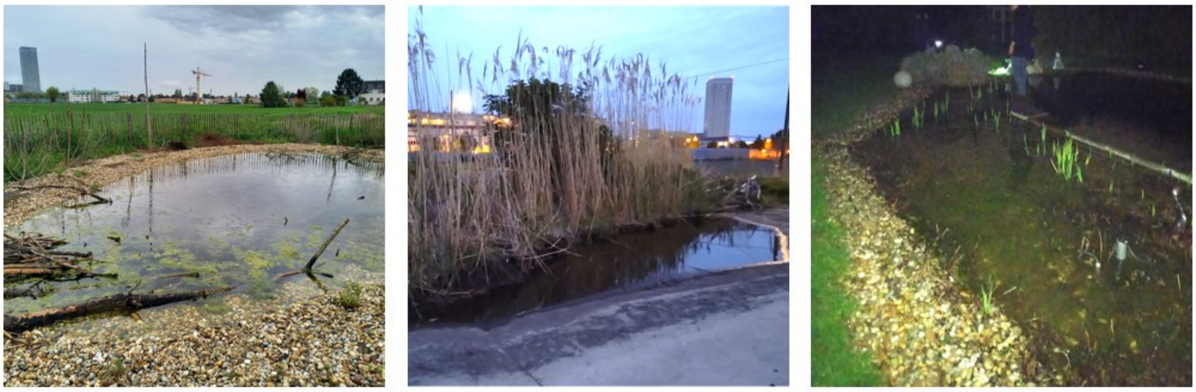


Figure 1: Map of the study area Donaufeld with the three main breeding sites and additionally an overview map of the location of Donaufeld in Vienna.

The area was flat, open and the land-cover consisted mostly of small farms growing vegetables, private allotment gardens, arable lands and fallows.

In the study area I monitored three breeding sites of *B. viridis* (Figure *1*). The Compensation Pond (L1) and Concrete Pond (L2) were located next to fields in the open area, whereas the third location, a private Swimming Pond (L3) was inside a garden (Figure *2*). In addition, all toads found outside of a pond, mostly on the streets “An der Schanze” or “Nordmanngasse” were recorded as well.



L1 Compensation Pond L2 Concrete Pond L3 Swimming Pond

Figure 2: Pictures of the three monitored breeding sites in the Donaufeld

Pond L3 was not monitored before 2023. When I surveyed the whole area to find possible new breeding sites, I discovered this Swimming Pond due to male toads calling. I further heard males calling from another pond inside another garden which was not accessible to me and therefore not monitored.

<u>Field work</u>

I collected the data during the main breeding season of *B. viridis*, between 25 March to 3 July 2023. I visited the area two to three times each week after sunset, when it was dark out; between 19:30 and midnight. Since toad activity only starts at around 10°C and more (Stöck et al., 2008), I primarily went out on warm evenings and omitted days with evening temperatures below 10°C. In total I went into the field 32 times. During the first four weeks of the breeding season, I surveyed the whole area by listening to toad calls and locating new breeding sites. Breeding sites L1 and L2 were monitored in previous studies (Sistani et al., 2021; Gurtner, 2022; Zanghellini, 2022).

I measured snout vent length (SVL), body mass (BM) and noted location and sex of all captured toads. The snout vent length was measured with callipers (in mm, to one decimal place). The body mass was measured with a digital micro scale (in g, to one decimal place, Brifit –

Professional Digital Mini Scale KA25/UF200H). The sex was determined by morphological features and behaviour of the toads. I recorded whether a toad was found in one of the three breeding sites or somewhere else (e.g. a street nearby). I also recorded whether the toad was found on land or in water.

Furthermore, I took photographs of the head and dorsal pattern of the toads, so they could be identified and compared to toads caught previously. The pictures were taken with a mobile phone (Resolution: 48MP) while the toads were inside a Photobox (Model: Puluz Foldable LED Ring Light Studio) that produced consistent lighting and backdrop for the pictures.

When I encountered toads in amplexus I only took pictures of them, I did not record any other data. I did this to put the pair back into the pond immediately after photography. The temperature was recorded at the start and the end of each field day. I used weather data from the nearby weather-station Donaufeld (ZAMG - Wien, 2023) to calculate the average temperature during each monitoring session. Furthermore, interesting observations, such as new spawn, tadpoles or metamorphs (young toads), other amphibians or predators were documented. I also found Common Toads, for which I recorded SVL, body mass, sex, location, and took a picture. However, I didn't calculate body condition or population analysis for the Common Toads.

Body condition

To evaluate the body condition of the toads, the data on their size and mass were used to calculate the Scaled Mass Index (SMI). I calculated the SMI of the toads using the approach by Landler et al. (2022), which allows comparisons between green toad populations. Landler et al. (2022) suggest using 3 as a scaling coefficient when calculation the SMI of *B. viridis*. The formula which was used to calculate the SMI is the following:

$SMI = BM \times (60/SVL)^3$

To evaluate the body conditions of the toads over the season, I used a Linear Model (LM). I used data of all toads captured at least twice in 2023. The function used was glmmTMB from the package glmmTMB. In one calculation, the SMI value was used as the response factor, while in another, the body mass was used. I calculated the difference between the first and last values of SMI/body mass. These were then correlated with the temporal distance (days) between the measurements. SMI/body mass was the response variable, and the time interval was the independent variable.

Photo analysis

All photos were edited with the free photo software Gimp (GNU Image Manipulation Program, GIMP 2.10.34) to make sure nothing but the toad was visible on the picture. This was especially important for pictures of toads in amplexus because the background and the second toad had to be removed to have a clear picture of each individual toad.

To match the dorsal pattern, the software IBEIS (Crall et al., 2013) was used (Burgstaller et al., 2021; Sistani et al., 2021). I matched the pictures of all toads caught in 2023 to the data of toads from the years 2020, 2021 and 2022 (provided by my supervisors). I manually went through the best possible matches (provided by IBEIS algorithm) and identified all correct matches as TRUE (Figure 3). Toads with no match were new captures and got a new ID.

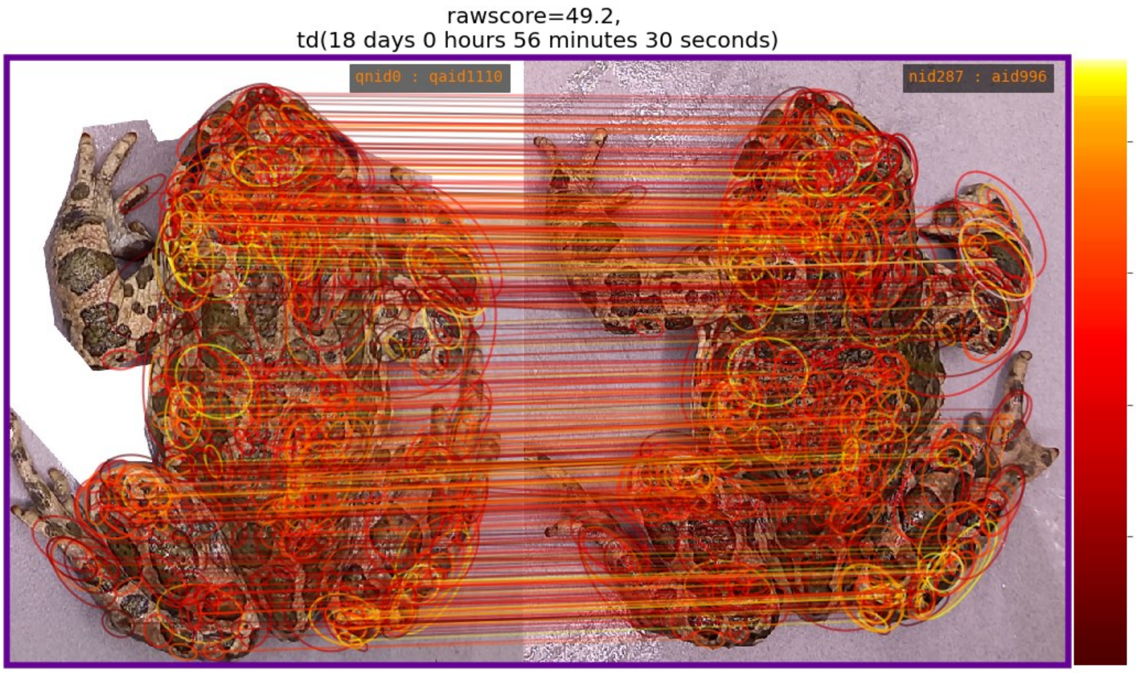


Figure 3: Example of a true match. The reference picture (left) is compared with all other pictures. The software shows the match with the highest score and therefore highest possibility of being a match. Red ellipses show similar patterns in the toads and are connected with lines. There are many lines and ellipses between the two pictures, indicating a true match.

Demographic analysis

The statistical analysis was done with the software R (version 4.2.3) (R Development Core Team, 2020). I used the Jolly-Seber-Schwarz-Arnason (JSSA) model which is usable on open populations where animals can leave or join the population for my demographic analysis (Schwarz & Arnason, 1996). The same model was also employed in a previous master's thesis on *Bufotes viridis* done in the Donaufeld (Sistani et al., 2021). For the demographic analysis of data from the years 2020 to 2023 I used the JSSA model in the package openCR (Efford, 2023).

The JSSA-test estimates the recapture probability p, the apparent survival rate ϕ and the population size SuperN. The recapture probability p shows the probability of an individual toad being recaptured at a separate occasion. The apparent survival ϕ shows the probability of an individual surviving and remaining in the population until the next season. This parameter can only be calculated for data on at least two seasons. The parameter SuperN is an estimation of the total number of individuals present in the population during the survey time.

Results

On 21 out of 32 sampling days, toads were captured. In total, 258 toads were captured during the sampling period of 2023. I later identified them as 96 individuals (30 females, 66 males). I found that 56% of toads were new captures that had not been caught in previous seasons.

Most individuals were only captured once during this study (Figure 4). Forty-two toads were captured at least two times during the study. Six toads were captured more than 10 times. One toad was caught 15 times during this study. All 30 females were only captured once this season.

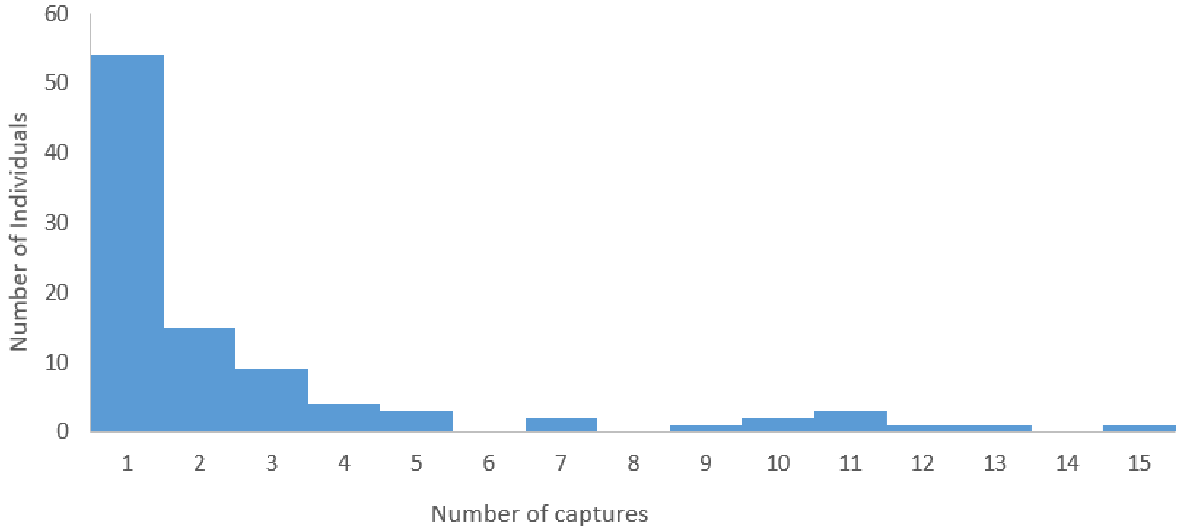


Figure 4: Number of toads captured how often in 2023.

The mean temperature during the time of my field work was low at the beginning of April with only 6.7 °C (Figure *5*). Most toads were captured between mid of April to mid of May. Most captures were recorded on 17 May with 41 individuals caught. On this date the temperature was low (9.2 °C) compared to the other days in May. The field day with the second highest captures was 30 April (12.4 °C) with 38 individuals caught.

The last day of fieldwork was July, but the last adult toads were encountered almost one month earlier on 6 June.

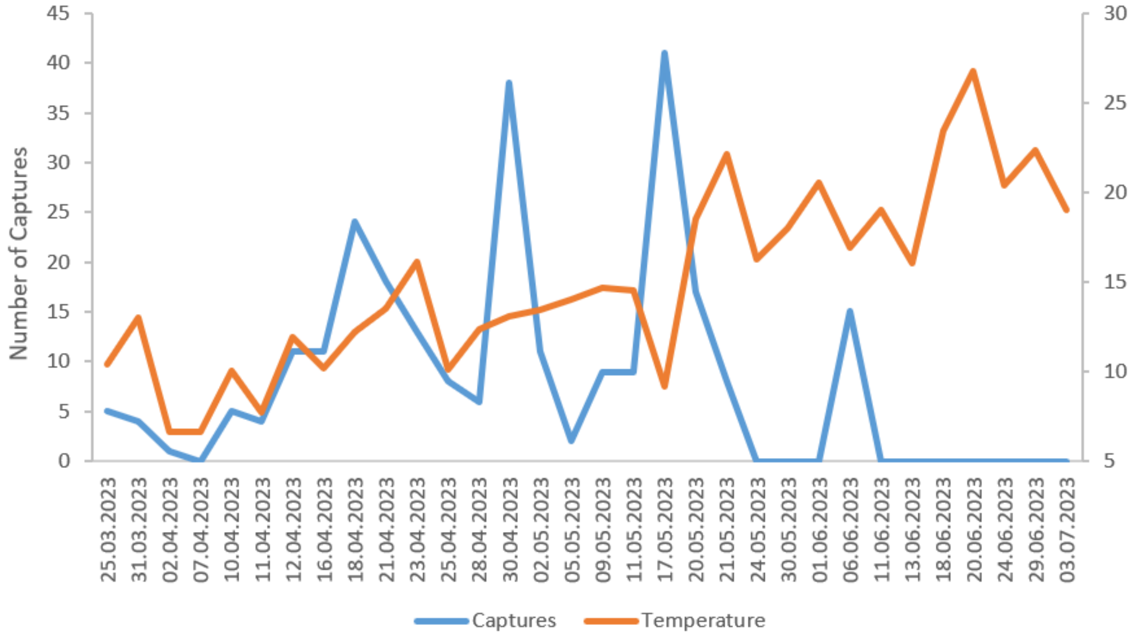


Figure 5: Temperature and total number of toads captured on each occasion in 2023.

I found the first spawn in L1 on 31 March. The first tadpoles were seen there on 23 April. Due to algae in the water, I could not see many tadpoles during May and June. I recorded the first metamorphs on 11 June. Each following field day until the end of the season I could observe 20 to 50 metamorphs in L1 or close by. The toads spawned in L2, whenever it was full of water (after rain). On 18 April eight pairs in amplexus were recorded there. But as L2 dried out quickly after the rain no tadpoles developed there but I did transport spawn from L2 to L1 before the Compensation Pond dries out. I found spawn in L3 on 2 May but no metamorphs.

On 12 field days I encountered ducks swimming in L1 and on three days in L3. There were dragonfly nymphs in L1 and L3. I also found a dead female toad in L3 on the 17 May. Two toads (probably male and female) were found as roadkill on 23 April.

The majority (93.2%) of captured toads were found in the water. Only 18 individuals (6.8%) were found either on land close to the ponds or on the streets “An der Schanze” or “Nordmanngasse” (). Five of the toads were found on the road. In the Alois-Negrelli-Gasse, I discovered two dead toads (roadkill). All other toads were found on land, very close to the breeding sites. Most individuals were found in the Concrete Pond L2 (59%), 23% of toads were found in L1 and 19% in L3.

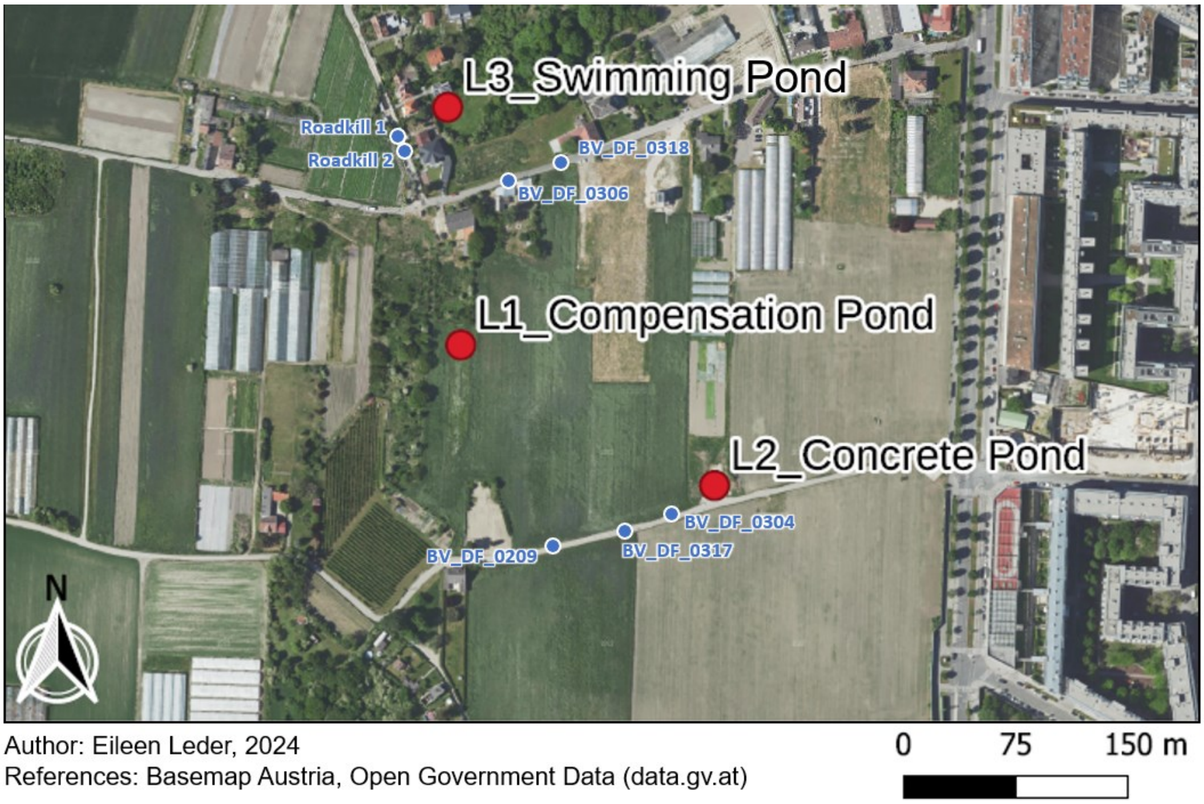


Figure 6: Location and ID of the toads captured on land, and not close by to a breeding site.

In addition to Green Toads, I also recorded Common Toads (*Bufo bufo*) in all three breeding ponds. There were a total of 15 *B. bufo* individuals recorded, 13 males and two females. The majority of Common Toads (eleven individuals) were found in L1, while three were captured in L3 and only one in L2. On two occasions, I observed amplexus between a male *B. viridis* and a female *B. bufo*: once on 19 April in L1, and again on 17 May in L3.

<u>Body condition</u>

Males and females differed in size (Figure *7*, Table *1*). Males were, with an average SVL of 71.99 mm, smaller than females, with a SVL of 80.03 mm. Males also weighed less (42.97 g) compared to females (66.42 g).

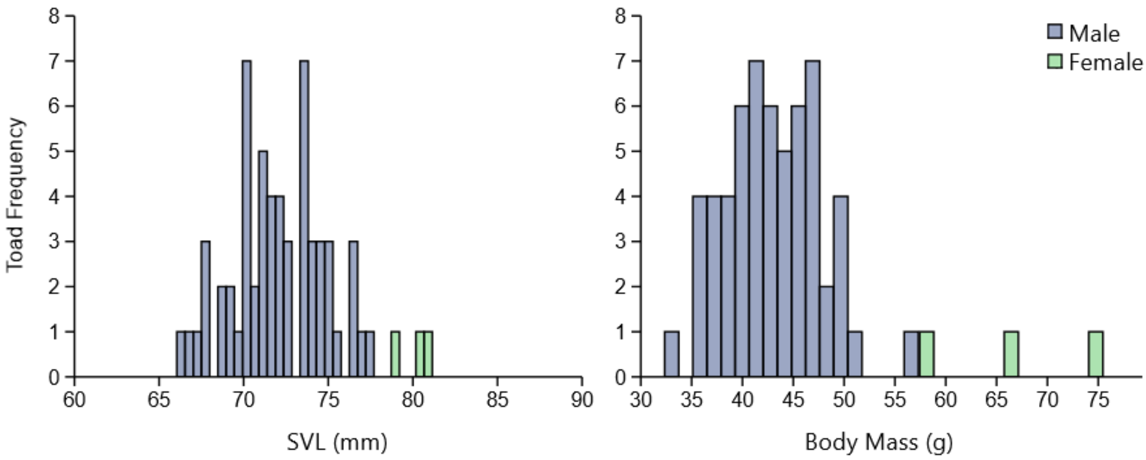


Figure 7: Distribution of snout-vent length and body mass of Green Toads.

The body condition data of only three females were used in the calculation. All other 27 females were caught in amplexus and no data on SVL and BM were collected. For all males that were recaptured multiple times the mean of all measurements was used for this calculation. The confidence Intervals of the SMI values for males and females overlap (Table *1*). Even though females were larger and heavier than males (Figure *7*) the SMI does not suggest differences in body condition (Figure 8).

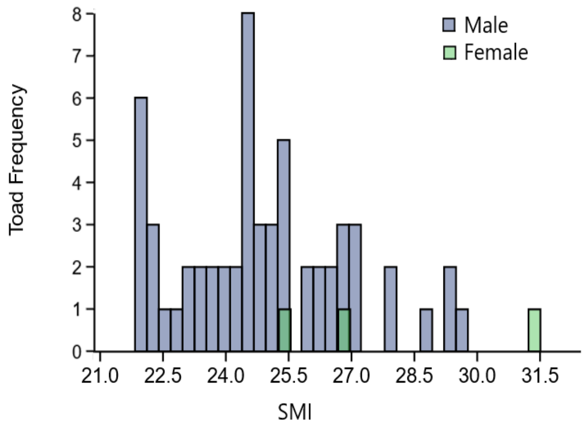


Figure 8: Distribution of Scaled-Mass-Index of Green Toads.

Table 1: Mean and confidence Interval (95%) of SVL, Body Mass and SMI for male and female toads.

Sex	**Sample Size N**	**SVL**		**Body Mass**		**SMI**	
		Mean	cI	Mean	cI	Mean	cI
Male	N = 59	71.99	0.68	42.97	1.18	24.94	0.51
Female	N = 3	80.03	1.10	66.42	8.19	27.94	2.90

The linear regression shows a significant negative effect of time on body mass (p=0.0207). The results imply that the toads lost weight over the course of the season. However, when the SMI value is used as the response, the result is not significant.

Demographic Analysis

For the demographic analysis, data on the Green Toads in the Donaufeld from 2020 to 2023 were used. In 2020 the lowest number of toads in total was caught (Figure 9). Each year fewer females than males were caught. In 2023 the highest number of female individuals (30) was captured. In 2022 the highest number of toads was captured.

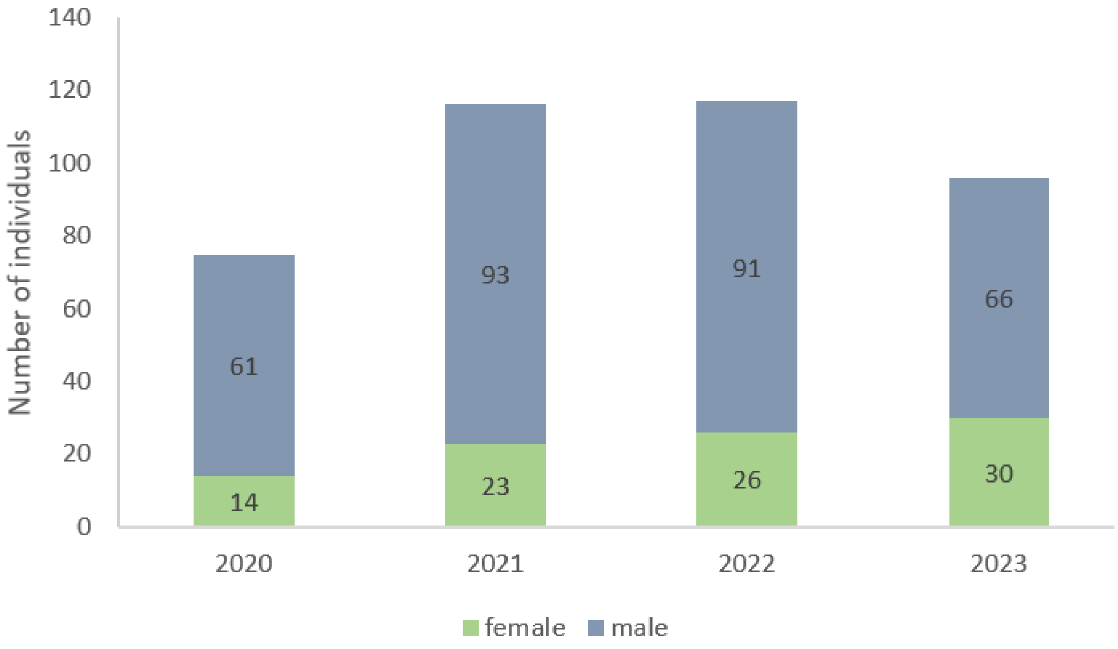


Figure 9: Total number of captured individuals divided into males and females from the years 2020 to 2023.

The potential models were assessed using Akaike's Information Criterion (AIC). Only the best models, characterized by a dAICc value of less than 2, were used and averaged (Table 2).

In model ranked 1, the recapture probability (p) depends on the sex. Apparent survival rate (Phi) and population size (N) depend on the non-linear timely dependent (t). In model 2, p depends on the sex, Phi depends on t and the linear timely dependent Session. N is dependent on t.

Table 2: Model based AIC evaluation of the population models with their parameters and predictors (P = recapture probability; Phi = apparent survival rate; N = Super N; Session = linear timely dependent, t = non-linear timely dependent)

Model Rank	Parameters and Predictors	AICc	dAICc
1	p~sex + t phi~t N~t	5927.573	0
2	p~sex + t phi~Session N~t	5929.04	1.467

The recapture probability p was consistently higher for males (Figure 10). It was always more than 10% for the males (highest in 2021 with 22%). In 2023 the recapture probability p for male toads was 15.7%. In 2023 only one female toad was captured twice. The recapture probability for females was 1.4% in 2023. For females it was highest in 2021 with 2.1%.

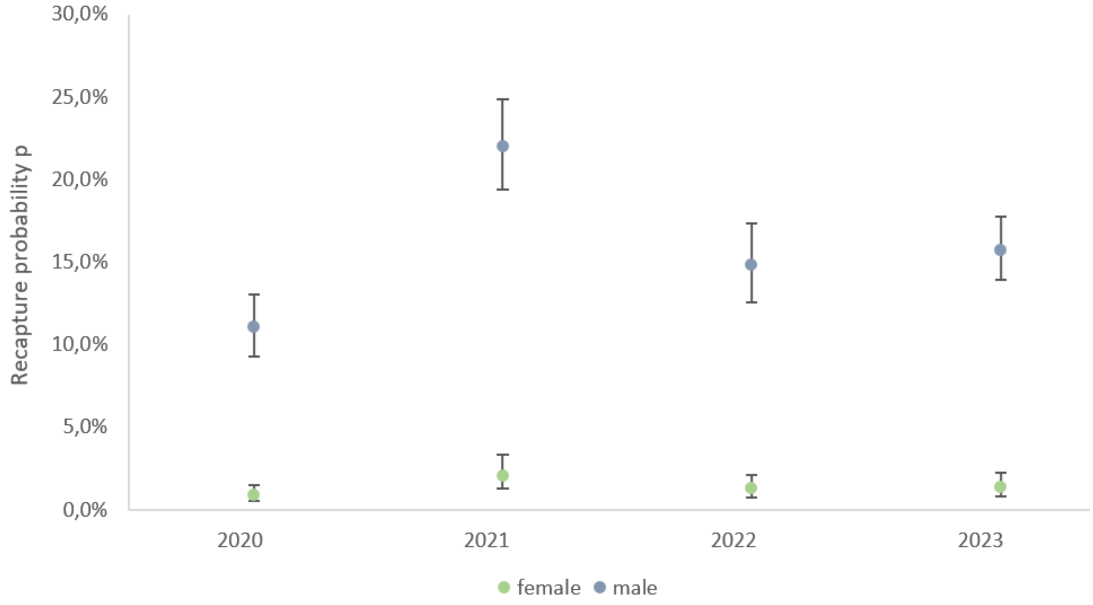


Figure 10: Recapture probability p of the toad population in the years 2020 to 2023 with Confidence Interval as error bars (divided in males and females).

The years 2021 to 2022 had the highest apparent survival estimate rate Phi (58.7%). The lowest apparent survival rate was from 2022 to 2023 (37.9%) (Figure 11).

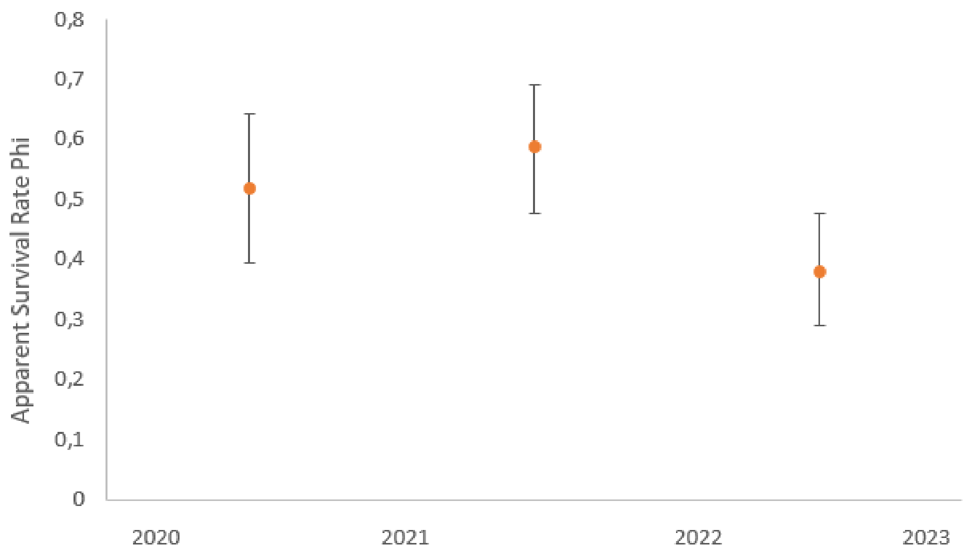


Figure 11: Apparent survival rate (Phi) of the toad population in the years 2020 to 2022 with Confidence Interval as error bars.

In 2020 the estimated size of the green toad population in Donaufeld was 151 individuals (CI: 111 – 215). In 2022 the estimated size was 271 (CI: 204 – 361) individuals, the highest of all four compared years. In 2023 the estimated size was 172 (CI: 128 – 232) individuals, almost 100 individuals less than the estimated number of the previous season (Figure 12).

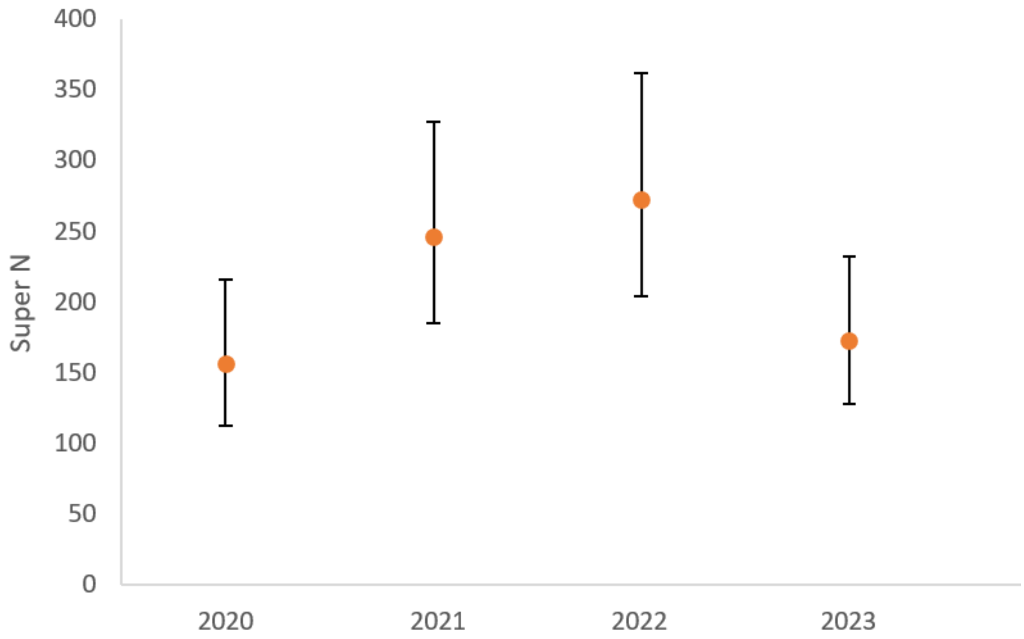


Figure 12: Total number of individuals (Super N) present in the toad population in the years 2020 to 2023 with a 95% confidence Interval as error bars.

Discussion

In total more male than female toads were caught during the observation period in 2023. The recapture probability p was also higher for males than females (Figure 10). The sex ratio is typical for studies of *B. viridis* conducted at the breeding site (Staufer et al., 2023). While male toads visit the breeding sites frequently during the season to attract females with their call, female toads only spend few days at the breeding site (Kovács & Sas, 2010; Rienesl, 2021). Females migrate there shortly before spawning, stay at the breeding site for a few days and leave soon after spawning (Rienesl, 2021). Male toads stay at the breeding site for three weeks up to the whole breeding season to maximize reproduction (Sinsch et al., 2007; Kovács & Sas, 2010; Rienesl, 2021). My findings indicate that the majority of toads were captured only once, a pattern commonly observed in literature and typical for capture-recapture studies of Green Toads (Sistani et al., 2021; Amon, 2022; Wolensky, 2023).

Bufotes viridis mainly becomes active at night temperatures of around 10°C (Stöck et al., 2008; Rienesl, 2021). At lower temperatures the toads stay in their land habitat (Rienesl, 2021). Therefore, the low temperatures at the beginning of the 2023 breeding season could explain the low numbers of toads encountered at the end of March and start of April. Comparing the data for the highest capture rate of the last years should demonstrate this correlation between temperature and toad activities. In 2020 the day with the highest capture rate was on 4 May (Sistani et al., 2021), in 2021 it was earlier at the end of April (Liebhart, 2021). In 2022 the most toads were captured on 7 April (Gurtner, 2022), very early in the season. All days with the highest catch numbers were quite warm days where the air temperature was around 17°C in Vienna at 7 p.m. On 4 May 2020, it was 17.4°C, on 30 April 2021, it was 17.8°C, and on 7 April 2022, exactly 17°C (ZAMG - Jahrbuch, 2024). In 2023, the day with the highest catch numbers was a cold, rainy evening (9,2°C). The nevertheless high catch number can be explained by the rain in the preceding days. L2 was filled, allowing for a large number of toads to be caught there. Summing up it is uncommon for the Donaufeld to have the date with the highest capture rate comparable late in the season (Figure *5*) which can be related to the low night temperatures in April.

According to literature the breeding season of *B. viridis* lasts normally until end of June (Glandt, 2015; Rienesl, 2021). However, in 2023, there were only few toads captured in June (Figure *5*). As other studies of the Donaufeld show a drop of capture numbers in June as well

(Liebhart, 2021; Sistani et al., 2021; Gurtner, 2022) and e.g in 2021 no toads were captured in June (Liebhart, 2021) this could be a peculiarity of the toad population in Donaufeld. I think that the toads no longer spawn due to the high temperatures. Green Toads often spawn in temporary water bodies, which frequently dry up in the summer (Mazgajska & Mazgajski, 2020). Therefore, spawning early in the season is the best way to make sure that the tadpoles develop. Perhaps the toads in Donaufeld have adapted to the high temperatures in June and rapid drying of the Concrete Pond by spawning early.

The analysis of snout-vent length (SVL) and body mass measurements of captured toads in 2023 reveal a size and weight distribution similar to that reported in the literature for both sexes (Figure *7*; Table *1*). For males the mean SVL was 71,99 mm, which lies within the range given in literature which is between 50 and 80 mm (Stöck et al., 2008). The size range in the literature is quite large and can vary for each population. In previous years *B. viridis* in Donaufeld has also been on the upper end of this size range (Zanghellini, 2022). For females the observed SVL of 80,03 mm in mean also corresponds to literature data (Stöck et al., 2008). In general, the males of the European Green Toads are on average smaller and lighter than the females which could be confirmed for the toad population of Donaufeld (Stöck et al., 2008; Glandt, 2015; Rienesl, 2021).

The SMI suggests no difference between the body condition of males and females (Table *1*, Figure 8) due to the large CI of the SMI of the females. Landler et al. examined the SMI value and the best method for its calculation for *B. viridis* in Europe (Landler et al., 2022). The body mass (and therefore the SMI) differs depending on if females were captured before or after spawning (Landler et al., 2022; Zhelev et al., 2020). Female green toads loose up to 30 % of their body mass after the spawn deposit (Castellano et al., 2004). Since only data of three females could be used for my calculation, the meaningfulness of these results is in question.

The data presented indicate that the SMI of toads in the Donaufeld was higher compared to other green toad populations in Vienna (Horvath, 2023; Wolensky, 2023). For example, research in 2022 (Zanghellini, 2022) showed that the SMI of Donaufeld toads exceeds that of populations in areas like Simmeringer Haide (Horvath, 2023) or Bednar-Park (Wappl & Heyer, 2016; Wolensky, 2023). The big and heavy toads in the Donaufeld indicate a long-lived, old population as age and body size are positively correlated (Castellano et al., 1999). European Green Toads can become sexually mature at the age of two years (Stöck et al., 2008; Sinsch et al., 2007) These animals have a lifespan of up to 11 years (with females usually living two years longer than males) and reproduce until eight years (m) or ten years (f) old (Sinsch et al., 2007). Competition, food availability, climatic conditions and environmental quality are all factors that play a role in the size and body mass of anurans (Zhelev et al., 2020).

The male toads of the Simmeringer Haide with their average mass of 21.42 g (cI: 0.31) (Aiglsperger, in prep.) were notably lighter than the males in Donaufeld (body mass: 42.97 g; cI: 1.18). They were also smaller with 61.83 mm (cI: 0.29) SVL compared to the 71.99 mm (cI: 0.68) from Donaufeld. This year, approximately 46% of the captured toads in the Donaufeld were recaptures from previous years. In the Simmeringer Haide, only about 13% of toads were recaptures (Aiglsperger, in prep.). The lower number of recaptures suggests a population with lower average age (Sinsch et al., 2007). The captured toads in Simmeringer Haide were therefore probably young and in one of their first breeding seasons, explaining the low recapture rate and the small individuals in the Simmeringer Haide. The toads in Donaufeld are a well-established toad population with bigger and older individuals.

Sinsch et al. showed that the age and size of toads differ among populations living in habitats of varying quality (Sinsch et al., 2007). The Donaufeld likely offers a good urban habitat for the Green Toads. Open space habitats within cities (such as the Donaufeld) can provide suitable habitats for amphibian species (Hutto & Barrett, 2022). However, since these areas are often associated with real estate projects (as in the Donaufeld), they are usually temporary. Additionally, roads running through the habitat pose a direct threat to amphibians (Hutto & Barrett, 2022). In 2023 I found two dead toads on the streets in the Donaufeld (). There are probably only few roadkills in Doaufeld because toads are generally rarely found on the road. Furthermore, the roads are equipped with speed limits of 30 kilometres per hour, one-way streets or driving bans, and are marked with amphibian warning signs (per sobs.). In contrast, in Simmeringer Haide, mortality due to cars is very high for Green Toads (Staufer, 2018).

The linear regression shows a significant negative effect of time over mass (p=0.0207) when using the body mass as a response over time. Body mass of male toads decline over the season. Initially, the toads were significantly heavier than during their last measurement. There are fluctuations in the decline, sometimes even upwards. This inconsistency is to be expected, partly due to variations in the filling of the digestive tract, and partly due to measurement errors (Landler et al., 2022). Other studies in Vienna also show that toads lose weight throughout the breeding season (Landler et al., 2022; Wolensky, 2023), likely because they focus on reproduction and spend less time foraging for food (Wolensky, 2023). The males spend most of the night calling for females and have less time foraging for food further away from the breeding site (Cherry, 1993). Cherry showed that higher call rates in males correlated with greater weight loss in *Bufo rangeri* (Cherry, 1993).

When calculating the SMI as the response factor there is no significance between SMI and time (p=0.510). Since the SMI is calculated with SVL and body mass (and body mass is known to be decreasing), the SVL must be the issue here. Measuring the length of the toads is considerably more prone to measurement errors, than measuring the body mass because the toads move a lot. Measurement errors of up to 2 mm are common when measuring the SVL of living toads (Raney & Lachner, 1947). I suspect that the linear regression calculation with SMI as the response yielded no results because of measurement errors of the SVL.

The estimated population size of European Green Toads in the Donaufeld for 2023 was smaller than in previous years. Because of the confidence interval, there isn't a distinct decrease in the population evident. For example, a population size of 200 individuals would be possible with the calculations in all years. There were more individuals recorded than in 2020, although data of 2020 are not entirely comparable. The population estimate was smallest in 2020, followed by 2023 (Figure 12).

Over the four years, variations in the extent and time of fieldwork, weather conditions, consistency and accessibility of water bodies, as well as potential differences in the endurance and skill of the surveyors, may have contributed to unequal levels of population assessment. In 2020 the data acquisition started in late April due to lockdowns (Sistani et al., 2021) and might explain the smaller population size estimates of the population size in 2020 the following seasons. In 2023 I included a private swimming pond (L3) which was not monitored in previous studies. All toads breeding in L3 have not been in the calculated population size from previous years, so these sizes are probably underestimated. Furthermore, the Concrete Pond (L2) was less accessible in 2023 due to the ongoing construction work (pers. obs.).

The apparent survival rate was lower from 2022 to 2023 compared to previous years (Figure 11) as well. Fewer toads could be captured in the Concrete Pond (L2) probably due to limited accessibility of the pond – not because the toads were not there. In fact, data of captures show that the L2 pond was frequently used by toads also in 2023. Since in previous years, most toads were captured in L2 (Gurtner, 2022; Liebhart, 2021; Sistani et al., 2021), it makes sense that now, with its limited accessibility, a lower recapture rate is the result.

Interestingly, I captured more females in 2023 than in all previous years (Figure 9). Since L2 was only filled up for a few days after the rain, all females living in the surrounding area had to spawn during few nights, probably leading to a higher number of captured females in these nights than usual.

Another reason for less captures of toads in 2023 could be the habitat transformations in Donaufeld. The construction work for the residential buildings in the Donaufeld has already begun. In the summer of 2022 a housing complex in the road Nordmanngasse was completed (IBA Wien, 2020b). This house and the adjacent gardens and fields are close to all three breeding sites and the area was probably used by the toads to migrate. The construction of this building and the ploughing of the neighbouring gardens might have hindered the toads in their natural migration to known breeding sites. The plants on the fields between L1 and L2 grew up to one meter high in June (pers. obs.), probably making it hard for the toads to migrate.

Construction sites are open habitats with little vegetation or sealed ground similar to early succession or wasteland habitat types. Construction work can be beneficial for toads as there might be possible new breeding sites for the toads during the construction work (Landler et al., 2023). While my data may not be entirely comparable to previous years, it still shows a declining trend of population size. This decline is probably also due to the building activities in the Donaufeld. As the construction plans include ruining the breeding pond L2 and sealing the fields and hiding spots of the toads (IBA Wien, 2020a) the construction will not beneficial for the toads and poses a threat for the population.

In L1, successful reproduction occurred in 2023. Several young toads were observed during metamorphosis and leaving the water in June. In 2021, some dead metamorphs were found, apparently dried out on the hot stones next to the water body (Liebhart, 2021). Such observations were not made this year.

Interestingly, in both the Swimming Pond (L3) and Concrete Pond (L2), no metamorphs were found in 2023. In L2 this was because it dried up quickly after the rain and spawn did not survive. I observed spawn and tadpoles developing in L3. Nevertheless, there were no metamorphs registered during field study. The Swimming Pond is surrounded by grass and it could be that I simply did not see any metamorphs there. Also, they could have metamorphosed after the study period ended. But if no tadpoles turned into toads, it might have been because of the pumps and filters in the artificial pond. Predation could also have been a reason why the tadpoles did not metamorphose as it is a big mortality factor of tadpoles or young amphibians (Laurila & Aho, 1997). Ducks could be observed twelve times at L1 and L3. In L1 and L3 there also were dragonfly nymphs and waterbugs in the water. Dragonfly nymphs are well known predators for tadpoles (Jara & Perotti, 2010). These predators, along with the pumps, could have been the reason why there was no successful metamorphosis in L3.

The occurrence of mismatched mating between *B. viridis* and *B. bufo*, as observed by me, is not unprecedented. In 2020, a similar event was documented (Sistani et al., 2021). Since then, no hybrids have been observed in the Donaufeld, suggesting no successful reproduction between these two species in the study area. A study conducted in 2017 revealed that there were no viable hybrids between *B. Bufo* and *Bufotes balearicus*; all tadpoles were deformed and did not reach metamorphosis (Canestrelli et al., 2017). For *B. viridis* and *B. bufo* hybridisation is possible, both in natural settings and in laboratory conditions, but the mortality rate of tadpoles is exceptionally high (Duda, 2008). Typically, due to differences in breeding seasons, hybridization rates between *B. bufo* and *B. viridis* are low, as *B. bufo* breeds early in the year, whereas Green Toads breed later in the season (Canestrelli et al., 2017). However, climate change and warmer springs may play a role in promoting hybridization events by facilitating an overlap in breeding activities (Canestrelli et al., 2017).

Conclusion

In conclusion, this study suggests a possible declining trend in the population size of European Green Toads in the Donaufeld. The data indicate a slight decrease in population size observed in 2023, although the population appears to be generally healthy. The Concrete Pond remains the most used breeding site, with most spawning toads found there. However, if L2 becomes unusable as a breeding pond in the future, it could potentially threaten the population, as alternative ponds (L1 and L3) are not solely used by toads but also predators.

If the observed trend of population decline continues, additional construction activity and further loss of open habitats could pose significant threats to the local Green Toad population in Donaufeld. Great care should be taken to ensure the availability of suitable breeding habitats, and close monitoring of this population is needed in the following years. Collaboration between researchers, conservation organizations and local stakeholders is going to be crucial to protect this endangered species in the urban habitat Donaufeld.

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