Superfrogs in the city: 150 year impact of urbanization and agriculture on the European Common Frog

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Abstract
Despite growing pressure on biodiversity deriving from increasing anthropogenic disturbances, some species successfully persist in altered ecosystems. However, these species' characteristics and thresholds, as well as the environmental frame behind that process are usually unknown. We collected data on body size, fluctuating asymmetry (FA), as well as nitrogen stable isotopes (δ15N) from museum specimens of the European Common Frog, Rana temporaria, all originating from the Berlin–Brandenburg area, Germany, in order to test: (a) if specimens have changed over the last 150 years (1868–2018); and (b) if changes could be attributed to increasing urbanization and agricultural intensity. We detected that after the Second World War, frogs were larger than in pre-war Berlin. In rural Brandenburg, we observed no such size change. FA analysis revealed a similar tendency with lower levels in Berlin after the war and higher levels in Brandenburg. Enrichment of δ15N decreased over time in both regions but was generally higher and less variable in sites with agricultural land use. Frogs thus seem to encounter favorable habitat conditions after pollution in postwar Berlin improved, but no such tendencies were observable in the predominantly agricultural landscape of Brandenburg. Urbanization, characterized by the proportion of built-up area, was not the main associated factor for the observed trait changes. However, we detected a relationship with the amount of urban greenspace. Our study exemplifies that increasing urbanization must not necessarily worsen conditions for species living in urban habitats. The Berlin example demonstrates that public parks and other urban greenspaces have the potential to serve as suitable refuges for some species. These findings underline the urgency of establishing, maintaining, and connecting such habitats, and generally consider their importance for future urban planning.

1 | INTRODUCTION

"Habitat loss and degradation", "climate change" as well as "excessive nutrient load and other forms of pollution" represent three of the major threats to biodiversity worldwide, with urbanization and agriculture being important drivers for all three (Secretariat of the Convention on Biological Diversity, 2010). Due to city growth, more and more natural surfaces are sealed, increasing numbers...
of roads and railways are fragmenting the landscape, and cities become heat islands characterized by higher temperatures compared to their surrounding (Liu et al., 2016; Ward et al., 2016). The world’s human urban population has grown rapidly and is associated with an expansion of urban areas but also with an increase of global food demand, and accordingly, with an agricultural expansion.

Agriculture is a major cause for deforestation, increase in greenhouse gas emissions, and the consumption of a substantial amount of freshwater (Ramankutty et al., 2018). The invention of the Haber Bosch procedure in the early 20th century permitted the synthesis of nitrogen fertilizer from atmospheric nitrogen. The resulting increased use of fertilizer, in combination with pesticides, led to a boost in agricultural productivity but with the result in decrease of freshwater quality with detrimental effects to freshwater ecosystems and their terrestrial surroundings (Ramankutty et al., 2018).

However, some species, such as wild boars, red foxes, house sparrows, American toads, or whistling tree frogs, persist in habitats altered by urbanization or intense agriculture (Bateman & Fleming, 2012; Hamer & McDonnell, 2010; Isaksson, 2018; Koumaris & Fahrig, 2016; Stillfried et al., 2017). They are traditionally referred to as urban-adapters (McKinney, 2002). Species in both types of these modified ecosystems have usually been considered generalists with a broad environmental tolerance. Although scientific attention has rather focused on identifying reasons for the exclusion of certain species from human-modified landscapes (Ducatez et al., 2018; Hassall, 2014; Howard et al., 2020), the characteristics behind a species’ ability to persist, as well as the limits in environmental changes that still allow persistence, have rarely been investigated (Hamer & McDonnell, 2008; Jung & Threlfall, 2016; Marques et al., 2019).

However, it is essential to understand the organisms’ responses to anthropogenic impacts and environmental features facilitating their persistence in order to develop sustainable management strategies, which might promote and maintain biodiversity and associated ecosystem services in human-modified landscapes (Alberti, et al., 2017; Donihue & Lambert, 2015; McDonnell & Hahs, 2015). General responses of organisms to rapid environmental changes, natural as well as those deriving from human actions, are either moving into more suitable areas, or stay and adjust physiology, behavior, life-history, and/or morphology (Alberti, 2015; Alberti et al., 2017; Lowry et al., 2013; Sparkman et al., 2018). These adjustments are facilitated via phenotypic plasticity and contemporary evolution, that is, evolution occurring over less than a few hundred years (Palkovacs et al., 2012), with humans acting as major driving forces (Hendry et al., 2008; Hendry et al., 2017). Phenotypic trait changes, in turn, can alter ecosystem function and, in case of heritability, create the potential for eco-evolutionary feedback, with consequences for human well-being (Alberti, 2015; Alberti, et al., 2017; Rivkin et al., 2019; Rudman et al., 2017).

The study of potential adaptive trait changes in persisting species requires long-term data, which are not easily accessible for physiological, behavioral, or life-history traits. However, changes in morphological and biochemical traits of preserved specimens may be used as a proxy for species’ responses to rapid environmental changes (Holmes et al., 2016; Kern & Langerhans, 2018; Meineke et al., 2018; Pergams & Lawler, 2009; Schmitt et al., 2018; Stumpf et al., 2016). Several morphological characters have been shown to be affected by urbanization and agricultural land-use, for example, body sizes of birds, amphibians, and arthropods as an indicator of habitat quality and life history (Jennette et al., 2019; Meillère et al., 2015; Merckx et al., 2018). Fluctuating asymmetry (FA), defined as small, random deviations from perfect bilateral symmetry, has been used as a measure of developmental instability caused by environmental stress in reptiles and fish (Lazić et al., 2013; Lutterschmidt et al., 2016). Additionally, stable isotopes can serve as an indicator of nitrogen enrichment in amphibians, fish, invertebrates, and plants (Donázar-Aramendía et al., 2019; Jefferson & Russell, 2008). Notably, so far only few studies investigating human-induced morphological changes in mammals and fish included the temporal component (Kern & Langerhans, 2018; Pease et al., 2018; Pergams & Lawler, 2009; Tomassini et al., 2014).

We herein aim at identifying specific trait changes reflecting the changing living conditions of a species that have persisted in rapidly changing environments. To this end, we tested the hypotheses that in the widespread European Common Frog, *Rana temporaria* Linnaeus, 1758, morphological traits, that is, body size and FA, and nitrogen stable isotopes (δ15N) as a reflection of the environment the frogs lived in, (a) have changed over a period spanning the last 150 years (1868–2018); and (b) that changes in these traits can be attributed to urbanization and agricultural intensity. More precisely, we expected decreasing body sizes, increasing levels of FA and increasing δ15N values in response to decreasing habitat quality, increasing environmental stress and enrichment of nitrogen through artificial fertilizers and air pollution, respectively. If we could detect such changes, we assumed that they were associated with the change of specific land use features.

We selected *R. temporaria* for three reasons. First, amphibians have highly specific ecosystem demands, low dispersal abilities either due to physical constraints, a high breeding site fidelity, or to anthropogenic barriers, and are therefore particularly affected by landscape modifications (Arntzen et al., 2017; Hamer & McDonnell, 2008; Stuart et al., 2008). They rely on terrestrial and aquatic environments due to their biphasic life cycle, making them very vulnerable to changes in both ecosystem types (Becker et al., 2010). Consequently, they have experienced a global decline since the second half of the 20th century (Beebee & Griffiths, 2005). Second, the European Common Frog has persisted in a large range of habitats (Sillero et al., 2014), including cities and agricultural landscapes, during the entire Anthropocene, despite fundamental environmental changes (Carrier & Beebee, 2003; Schlüpmann et al., 2004), making the species suitable for tracking the effects of environmental changes (Vander Wal et al., 2013). Third, historical series of museum-preserved specimens were
available, making long-term analyses possible. We chose specimens originating from the Berlin-Brandenburg region, Germany, which comprises both urban areas and areas with intense agricultural activities, and thus potentially huge environmental change during the last 150 years.

2 | MATERIALS AND METHODS

2.1 | Study area

The study area comprises Germany’s capital, the city of Berlin (52°31’N, 13°24’E), covering an area of 891 km² with a current population of 3.65 million inhabitants (Amt für Statistik Berlin-Brandenburg, 2019), and the surrounding rural Federal State of Brandenburg. Since the early phase of industrialization, Berlin has grown continuously. It reached its highest population in 1943. At the end of the Second World War in 1945, 50% of Berlin’s residential and one-third of its industrial area were destroyed and the population decreased to 2.8 million. After 1945, Berlin was rebuilt consistently (Senate Administration for Urban Development, 2002). Today the city contains 53% built-up area (i.e., residential or industrial areas and roads or railway tracks), 36% green infrastructure, such as forests, grassland or urban greenspaces (i.e., parks, gardens, cemeteries), and 6% water bodies. These calculations are based on the habitat type mapping of Berlin (Senate Department for Urban Development and Housing, 2014). Brandenburg, on the other hand, is mainly characterized by agricultural land (45%) and forest (37%; Ministry of Rural Development, Environment, & Agriculture of the Federal State of Brandenburg, 2016; Statistisches Bundesamt (Destatis), 2017). The Berlin sites used in this study are predominantly characterized by a certain amount of built-area and developed urban green spaces. Very few Berlin sites are located within forested areas or close to agricultural fields at the edge of the city. The Brandenburg sites are predominantly, but not exclusively, characterized by agriculture. Some are also located close to forested patches, within grasslands (not to be confused with developed urban greenspace) or urbanized areas (e.g., the city of Potsdam).

2.2 | Specimen selection

We used all ethanol-preserved adult voucher specimens of *R. temporaria*, unambiguously originating from the Berlin-Brandenburg area in the collection of the Museum für Naturkunde Berlin (ZMB, https://doi.org/10.7479/5tm4-9r29). Snout-vent length (SVL) of at least 5 cm was used as a criterion to define adults (Dittrich et al., 2018; Miaud et al., 1999). This provided us with an initial sample size of $n = 124$ specimens, divided into $n = 56$ specimens collected at 15 different locations in Brandenburg, and $n = 68$ specimens collected at 17 different locations in Berlin, covering a time span from 1868 to 2017.

2.3 | Morphological traits and stable isotopes

Body size was measured as SVL (Watters et al., 2016) with a digital caliper, always by the same observer. In addition to the measurements of preserved specimens, we also took SVL of 34 live adults from Berlin in 2018. Total sample size for SVL analysis over time was $n = 158$.

For the assessment of FA whole preserved frogs were removed from ethanol, wrapped in bubble wrap, transferred to a dry plastic tube and scanned with non-destructive micro-3D-computed tomography ($\mu$CT; Niemeier et al., 2019). Images were generated using a Phoenix|X-ray nanotom of the company GE Sensing & Inspection Technologies GmbH at 90 kV and 150 $\mu$A with fast scan settings for upper and lower body scans, acquiring 1,000 projections per scan. Effective voxel size ranged between 19 and 21 $\mu$m for each scan. Volumetric reconstructions were made in Datos|x-reconstruction software (GE Sensing & Inspection Technologies GmbH). We measured each specimen at the right and left side of: humeri from the highest point of the head to the end of the capitulum, radio-ulnae from the olecranon process to the styloid process of the ulna, femora from the medial condyle to the femur head, and tibio-fibulae from the intercondylar eminence to the tip of the medial malleolus of the tibia (Figure 1a–e). We used VG Studio Max 3.0 with the distance measurement tool for the measurements. Broken bones were excluded from analyses.

For determining nitrogen stable isotope values, thigh muscle tissue was extracted from preserved frogs ($n = 104$). Samples were dried at 60°C in a drying chamber for 72 hr. Stable isotope

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**FIGURE 1** Limb characters measured for fluctuating asymmetry assessment in *Rana temporaria* (male, ZMB 87968). (a) Micro-3D-computed tomography scan of entire body with, (b) right humerus (purple), (c) right radio-ulna (yellow), (d) right femur (green), and (e) right tibio-fibula (orange)
analysis of 1 mg dried tissue per frog was performed with a THERMO/Finnigan MAT V isotope ratio mass spectrometer (Thermo Finnigan), coupled to a THERMO Flash EA 1112 elemental analyzer via a THERMO/Finnigan ConFlo IV-interface in the stable isotope laboratory of the Museum für Naturkunde, Berlin. Stable isotope ratios are expressed in the conventional delta notation (δ¹⁵N) relative to atmospheric nitrogen (Mariotti, 1983). Standard deviation for repeated measurements of laboratory standard material (peptone) was generally better than 0.15 per mille for nitrogen.

2.4 | Land use features

We assessed the proportion of built-up area (impervious surfaces e.g., buildings, roads, industrial areas), greenspace (public parks, cemeteries, private gardens, sports grounds), and agricultural fields (arable land i.e., intensively/extendively cultivated fields and fallow arable land excluding meadows and orchards) in a 1 km buffer zone around the vouchers’ collection sites using the open source geographic information system (QGIS) (QGIS Development Team, 2018). Calculations for the pre-World War II vouchers were based on the digitized maps of the Preußische Luftaufnahmen (1927–1940) provided by the Technical University of Berlin (Figure 2a). Calculations for the recent samples (1968–2018) were based on the habitat type mapping of Berlin (Senate Department for Urban Development and Housing, 2014) and Brandenburg (Landesamt für Umwelt Brandenburg, 2009; Figure 2b).

2.5 | Data analysis

Prior to FA analyses, measurement error (ME) was quantified by repeating measuring bones on both sides (right and left) in a subset of n = 20–22 individuals per limb character (see Niemeier et al., 2019). Significant ME outliers were identified using the Grubb’s test (Grubbs & Beck, 1972) leading to the exclusion of two tibio-fibula measurements. We then applied a mixed-model ANOVA (R package “lme4”; Bates et al., 2015) with Side as a fixed factor, Individual as a random factor, and the Side by Individual interaction as a mixed effect. Significance in the fixed factor Side would indicate directional symmetry, which has to be excluded. To verify that FA exceeded ME, variance components (σ²) were extracted from the random effects and signal (FA)-to-noise (ME) ratios calculated (Graham et al., 2010; Knierim et al., 2007). The variance component for the interaction (σ²ME) is an estimate for FA. The residual random variance (σ²ME) is an estimate for ME (Table 1).

To check if ME was of similar magnitude for each character, variations in the degree of ME were tested with a mixed-model ANOVA with Character as a fixed factor and Individual as a random factor (Figure S1). Absence of antisymmetry was validated for the whole dataset by examining the frequency distributions of the signed FA values that is, right side minus left side (R − L), visually for symmetry and kurtosis (Figure S2) and by using the Anscombe–Glynn kurtosis test (R package “moments”; Komsta & Novomestky, 2015; Table S1). Character-size dependency was tested by Spearman’s rank correlation between absolute values of FA (|R − L|) and character-size (averaged (R + L)/2; Table S1). Directional asymmetry was further excluded for the whole data-set by comparing deviations of the mean FA (R − L) from zero for each character with a one-sample t test (Table S1). Characters suitable for FA analysis were finally selected according to the following criteria: (a) a significant level of FA; (b) a signal-to-noise ratio > 1 to avoid that FA was masked by ME; (c) no significant variation in the degree of ME; (d) absence of directional asymmetry and antisymmetry; and (e) no character-size dependency in signed FA values. Only the humerus and radio-ulna met all the criteria (Table 1). In a recent study, selection pressure on the symmetry of functionally important characters, such as the hind limbs of anurans, has been proposed as an explanation for that observation (Didde & Rivera, 2019). Therefore, we excluded the femur and tibio-fibula from FA analyses.
TABLE 1 Summary of Rana temporaria limb characters’ suitability examination for fluctuating asymmetry analysis. A two-way mixed model ANOVA (side = fixed factor, individual = random factor, side × individual = mixed interaction) on subset of untransformed repeated measurements was applied.

| Character     | Source of variation | df  | Expected MS | Variance component $\sigma^2$ | Signal/noise ratio | F/LRT | p value |
|---------------|---------------------|-----|-------------|------------------------------|-------------------|-------|---------|
| Femur         | Side (S)            | 1   | 0.0000      | —                            | 0.99              | 0.005 | .94     |
|               | Individual (I)      | 21  | 50.6155     | 12.6486                      | 134.336           | <.001 |         |
|               | S × I               | 21  | 0.0211      | 0.0070                       | 9.144             | <.01  |         |
|               | ME                  | 44  | 0.0071      | 0.0071                       |                   |       |         |
| Tibio-fibula  | Side (S)            | 1   | 0.0003      | —                            | 0.57              | 0.039 | .85     |
|               | Individual (I)      | 17  | 65.9387     | 16.2304                      | 115.010           | <.001 |         |
|               | S × I               | 17  | 1.0171      | 0.0050                       | 3.616             | .06   |         |
|               | ME                  | 36  | 1.0071      | 0.0087                       |                   |       |         |
| Humerus       | Side (S)            | 1   | 0.0024      | —                            | 3.92              | 0.645 | .43     |
|               | Individual (I)      | 19  | 34.1485     | 8.0281                       | 104.593           | <.001 |         |
|               | S × I               | 19  | 2.0361      | 0.0145                       | 33.035            | <.001 |         |
|               | ME                  | 40  | 2.0071      | 0.0037                       |                   |       |         |
| Radio-ulna    | Side (S)            | 1   | 0.0000      | —                            | 1.26              | 0.015 | .9      |
|               | Individual (I)      | 19  | 12.9405     | 2.4812                       | 101.363           | <.001 |         |
|               | S × I               | 19  | 3.0157      | 0.0043                       | 11.107            | <.001 |         |
|               | ME                  | 40  | 3.0071      | 0.0034                       |                   |       |         |

Individual FA values of the humerus and radio-ulna were combined to a composite FA index across both characters (CFA) to increase the reliability of FA as a detector of stress. Therefore, each FA (|R – L|) value was divided by the average absolute FA value of the respective character and then summed up (Leung et al., 2000).

To analyze morphological changes and isotopic composition over time, the response variable SVL was related to the years from 1868 to 2018 (including 2018 measures from live specimens), CFA and $\delta^{15}$N to the years from 1868–2017 using generalized additive models (GAM) (R package “mgcv”; Wood, 2019). To fit models 1/SVL transformation was used and for CFA Gamma family with inverse link function was specified. Year was included as fixed smooth term and Location of the sample was added as a random effect smooth term. Model fit was checked visually using diagnostic plots.

Trait changes were further analyzed by comparing pre- (1868–1938) and post- (1968–2017) World War II periods and their interaction with the regions Berlin and Brandenburg. Therefore, we used linear mixed-effects models for 1/SVL (in this analysis only museum vouchers, no live specimens) and $\delta^{15}$N were related to the calculated percentages of built-up area and greenspace within the 1 km buffer zone around each site as predictive variables. Therefore, generalized additive models were used again with the same family and random effect smooth term specification.

To investigate the effect of agriculture, all sites with a proportion of arable land, that is, intensively/extensively cultivated fields and fallow arable land excluding meadows and orchards, within the surrounding 1 km buffer zone greater than 2% were classified as agricultural sites ($n = 33$), regardless of the region (Berlin or Brandenburg). These sites covered a range from 2% to 39% of arable land. $\delta^{15}$N values of these sites were compared to the sites without agricultural land use, that is, 0%–1.3% ($n = 71$) using a linear mixed-effects model. Location of the sample was included as a random effect. We decided to use the 2% threshold because it has been shown that agricultural land cover along with the contamination of soils and waterbodies with chemicals from fertilizers can affect amphibian populations over large spatial scales up to at least 1 km due to their migration between aquatic and terrestrial habitats and the circulation of contaminants in surface water systems (Babini et al., 2018; Boissinot et al., 2019; Koumaris & Fahrig, 2016; Marsh et al., 2017). We thus speculated a priori that a proportion of 2% arable land, which was usually located at the edge of the 1 km buffer zone adjacent to agricultural fields continuing outside the buffer zone, might be sufficient to detectably affect our specimens. Additionally, it was tested if there was a difference in the variability of the $\delta^{15}$N values between these two land use types by comparing the coefficient of variation (R package “cvequality”; Marwick & Krishnamoorthy, 2019).
All statistical analyses were performed using R (R Core Team, 2018). The significance level was set to \( p = .05 \). Visualizations of model outputs were done using ggplot2 (R package “ggplot2”; Wickham, 2016).

### 3 | RESULTS

Body sizes decreased between 1868 and the beginning of the Second World War in 1939 but increased afterward (GAM adjusted \( R^2 = .55, p < .01 \); Figure 3a). Comparison of the two time periods including the interaction with the factor Region revealed that body size differed between the two time periods \( t = -3.98, p < .001 \), and that there was a significant interaction with the regions Berlin and Brandenburg \( t = 2.693, p < .05 \); Table 2). Specifically, postwar individuals became larger in Berlin \( t = 3.932, p < .01 \), but not in the rural Brandenburg \( t = 0.337, p = .98 \) as revealed by post hoc pairwise comparisons (Figure 3b).

There was no general trend detectable for the development of FA over time (GAM adjusted \( R^2 = 0.03; p = .25 \); Figure 3c). Comparison of pre- and postwar time periods including the interaction with the factor Region revealed a tendency toward lower CFA levels in Berlin after war, and higher postwar levels in Brandenburg \( Time period: t = 2.221, p < .05 \); Time period \( \times \) Region:

![Figure 3](image)

**TABLE 2** Comparison of *Rana temporaria* traits from pre- (1868–1938) and post- (1968–2017) World War II periods and their interaction with the region (Berlin and Brandenburg). Linear (1/SVL and \( \delta^{15}N \)) and generalized (CFA) mixed-effect models were used. Location of the sample was included as the random effect.

| Dependent variable | Independent variable | df  | \( t \)   | \( p \) value |
|-------------------|----------------------|-----|---------|--------------|
| 1/SVL             | Time period          | 21.445 | -3.987 | <.001        |
|                   | Region               | 28.889 | -0.345 | .73          |
|                   | Time period \( \times \) Region | 35.956 | 2.693  | <.05         |
| CFA               | Time period          | -   | 2.221  | <.05         |
|                   | Region               | -   | 1.638  | .10          |
|                   | Time period \( \times \) Region | -   | -2.699 | <.01         |
| \( \delta^{15}N \)| Time period          | 21.846 | -1.601 | .12          |
|                   | Region               | 24.129 | 0.391  | .67          |
|                   | Time period \( \times \) Region | 38.446 | -1.999 | .05          |
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$\text{t} = -2.699, p < .01; \text{Table 2})$. However, post hoc pairwise comparisons in $p$ value adjustment led to insignificance of the results (Berlin: $z = -2.221, p = .09$; Brandenburg: $z = 1.575, p = .32$; Figure 3d). Enrichment of $\delta^{15}N$ decreased over time (GAM adjusted $R^2 = .69; p < .001$; Figure 3e). We identified a significant interaction between the factors $\text{Time period}$ and $\text{Region}$ ($t = -1.999, p < .05$; Table 2), but only in Brandenburg the $\delta^{15}N$ decrease remained significant after post hoc comparisons with $p$ value adjustment (Berlin: $t = 1.596, p = .35$; Brandenburg: $t = 5.623, p < .0001$; Figure 3f).

Largest individuals originated from areas with moderate degree of impervious surfaces ($>35\% < 70\%$; GAM adjusted $R^2 = .55, p < .05$; Figure 4a). We could not detect a relationship between CFA or $\delta^{15}N$ and degree of impervious surfaces (Figure 4c,e). However, we detected a significant relationship between body size (GAM adjusted $R^2 = .54, p < .01$), $\delta^{15}N$ (GAM adjusted $R^2 = .64, p < .01$) and the amount of greenspace (i.e., public parks, cemeteries, private gardens, sports grounds) around the frog sites (Figure 4b). The higher the proportion of greenspace the larger the frogs, and the lower were the $\delta^{15}N$ values (Figure 4b,f). FA tended to decrease with increasing amount of greenspace but the relationship was not significant (GAM adjusted $R^2 = .02, p = .23$; Figure 4d).

Individuals originating from agricultural sites ($>2\%$ arable land) had significantly higher $\delta^{15}N$ values than individuals from non-agricultural sites ($df = 21.599, t = 2.269, p < .05$; Figure 5). The overall variability of $\delta^{15}N$ values obtained from individuals from the agricultural sites was significantly lower than from non-agricultural sites ($D'AD = 14.136, p < .001$).

**FIGURE 4** Change of the *Rana temporaria* traits body size (snout-vent length [SVL], including measures of 2018 live specimens), fluctuating asymmetry (CFA) and nitrogen stable isotopes ($\delta^{15}N$) along built-up area (a, c, e) and greenspace (b, d, f) gradient. Illustrated are predicted smoothed regression lines with confidence intervals from generalized additive mixed-effects models. Percentage of built-up area/greenspace within 1,000 m buffer zone around the samples’ sites was used.

**FIGURE 5** Effect of agriculture on the nitrogen stable isotopes’ signature ($\delta^{15}N$) and variability (difference between coefficients of variation) in *Rana temporaria* from agricultural and non-agricultural sites; small numbers represent sample sizes of the groups.
In our study, we aimed at examining potential trait changes across a long-term temporal scale in a species, which has persisted in habitats altered by urbanization and intense agriculture. We herein demonstrated trait change in the European Common Frog, *R. temporaria*, from the Berlin–Brandenburg area collected over the last 150 years. Our initial prediction assumed that environmental conditions have worsened and, given the effect of climate change, temperatures have risen. Tadpoles consequently would develop faster, reach smaller sizes at metamorphosis, and, consequently, adult body sizes would shrink (Jennette et al., 2019). We further assumed that FA (CFA) and δ15N-ratio would both increase as a result of increasing environmental stress and nitrogen enrichment, respectively. In contrast, frogs became larger after World War II, showed unchanged levels of CFA, and δ15N-ratio decreased. However, by separating the samples by region, urban Berlin versus rural Brandenburg, it became obvious that this only applied to the city of Berlin. In Brandenburg, δ15N-ratio decreased equally, but frog sizes remained stable and levels of CFA tended to increase (albeit not significantly). These findings suggest that habitat quality improved compared with prewar conditions and environmental stress as well as nitrogen pollution decreased in Berlin, despite increasing housing construction and densification of urban infrastructures after war with a construction peak in 1997 (Jefferson & Russel, 2008; Keinath et al., 2020; Lazic et al., 2013; Merckx et al., 2018; Senate Administration for Urban Development, 2002; Senate Administration for Urban Development and the Environment, 2011).

The factor built-up area, representing the level of urbanization, was not the main environmental factor associated with the observed changes. Largest individuals originated from areas with moderate degree of impervious surfaces (>35% < 70%), implying that *R. temporaria* actually encountered more favorable environmental conditions in habitats with intermediate urbanization degrees compared to very urban (i.e., densely built-up areas with dense road network), but as well to barely urbanized areas (i.e., sites characterized by arable land, forest or grassland, which should not be confused with developed urban greenspace). Although seemingly counterintuitive, these findings are in accordance with other studies, which reported larger body sizes and lower levels of CFA in urban areas, compared to natural habitats for amphibians and reptiles (French et al., 2018; Hall & Warner, 2017; Iglesias-Carrasco et al., 2017; Winchell et al., 2019). Explanations given in these studies vary from reduced predation pressure, more stable water temperatures to lower population densities, and thus lower competition and lower risk of infectious diseases in cities (Saenz et al., 2014; Scheffers & Paszkowski, 2015).

It is well known that amphibians grow continuously and body size thus is dependent of the age of an individual. To estimate amphibian ages skeletochronology techniques can be used (Leclair & Castanet, 1987). This method turned out to be inapplicable in the case of preserved museum specimens because the bones splinter when trying to cut them and destruction of the irreplaceable vouchers should be minimized. Furthermore, even in cases of successful identification of Lines of Arrested Growth (LAGs), results indicated to provide unreliable ages for example, large adult individuals with only one or two LAGs (unpublished data). However, there is less evidence for variation in the ages of breeding adults among different populations (Jennette et al., 2019). In our study we only used adult that is, breeding individuals with an SVL of at least 5 cm. To this end, we expected that age classes over time and between regions would be comparable and their role for changes in overall body sizes would be negligible. Even if the observed increase of body sizes was supposed to be driven by older ages of the specimens, this would imply that the frogs on average survived longer, which in turn would be the result of a change of the conditions they lived in for example, habitat quality and predation pressure. Furthermore, female Common Frogs tend to be larger than males at the same age (Miaud et al., 1999). However, we did not detect significant size differences between the sexes within the pre- and postwar groups neither in Berlin nor in Brandenburg.

Notably, the study sites with intermediate urbanization degrees (i.e., intermediate amount of built-up area), where largest individuals occurred, are concurrently characterized by a relative high amount of developed greenspace (i.e., public park, cemeteries, private gardens, sports grounds). We found an effect of the proportion of greenspace at frog sites on all three traits. Body sizes increased, CFA tended to decrease and nitrogen enrichment decreased with increasing amount of greenspace. The effects of urban greenspaces were highlighted by several studies, for example, to enhance biodiversity in cities (Beninde et al., 2015; Hamer & Parris, 2011; MacGregor-Fors et al., 2016; Oertli & Parris, 2019). However, the effect of urban greenspaces has never been examined at the intra-specific level, that is, if urban greenspaces influence the well-being or physical condition as an indicator for the viability, and hence the thriving of city populations of various species.

Frog sizes decreased until the beginning of the Second World War, but increased afterwards in Berlin. In 1959, the distribution of greenspaces was very unbalanced between the Berlin city center and the suburban districts. Based on the awareness that contact to nature is essential for human health, the "Hauptgrünflächenplan" (main greenspace plan) was released in 1960. The goal of this habitat management was to expand and connect existing greenspaces reaching into the urban residential parts of the city center (Senate Administration for Urban Development, 2002). Today Berlin is considered one of the most populated and simultaneously one of the greenest cities of Europe (Schewenius et al., 2014). Brandenburg, on the other hand, is still working on improving their greenspace network, villages are sprawling and remaining forest patches are fragmented through a high number of intensively managed agricultural fields (Kolk & Naaf, 2015; Ministry of Rural Development, Environment, & Agriculture of the Federal State of Brandenburg, 2014; Senate Administration for Urban Development & Ministry of Infrastructure and Agriculture, 2009). This might explain the continued presence of smaller body sizes in comparison to Berlin and the tendency for increasing CFA. Agricultural land use and fragmentation is associated with habitat loss and the introduction of contaminants to the environment, resulting in a negative impact on anuran
species’ abundances and individual fitness measures (Koumaris & Fahrig, 2016; Sievers et al., 2018; Trochet et al., 2016).

According to the EU water framework directive, in Germany, soils in areas with intense agricultural use are still heavily polluted, following the application of nitrate or ammonium fertilizers, despite the slowly declining nitrogen balance (the balance between nitrogen added to the soil and nitrogen removed from the soil in crops or through livestock grazing; Federal Ministry for the Environment, Nature Conservation and Nuclear Safety, & German Environment Agency (UBA), 2018; German Environment Agency (UBA), 2015, 2017). In accordance with the generally declining nitrogen balance in Germany, we detected decreasing δ15N values over time in both Berlin and Brandenburg. However, nitrogen enrichment was increased in the muscle tissue of frogs originating from agricultural sites (>2% arable land i.e., intensively/extensively cultivated fields and fallow arable). The enriched nitrogen stable isotopes signature reflects the use of nitrogenous fertilizers and the successive accumulation through the trophic network across ecosystem boundaries via tadpoles, which transport the nitrogen from the waterbodies to terrestrial ecosystems after metamorphosis (Jefferson & Russell, 2008). In contrast to agricultural soils, soils in human settlements are generally less nitrate enriched (Federal Ministry for the Environment, Nature Conservation and Nuclear Safety, & German Environment Agency (UBA), 2018). This fact is reflected in our result of the increased nitrogen enrichment in agricultural areas compared to non-agricultural areas, since most of the latter sites are characterized by a certain amount of built-up area and developed greenspace that is, settlement and public parks etc., respectively. Furthermore, variability of δ15N values in non-agricultural areas was higher. We did not analyze the carbon stable isotopes because we did not expect major differences in the origin of the carbon or in the general diet (i.e., trophic relationships) neither over time nor between land use types (Figure S3). However, the greater δ15N variation in non-agricultural areas indicates that the urbanized areas with their developed greenspaces offer more variable habitats with different nitrogen baselines and respective food resources than the agricultural areas. The loss of habitat heterogeneity as a consequence of agricultural intensification has been identified as a major cause driving biodiversity decline in agriculture-dominated landscapes (Benton et al., 2003). Opposed to that, urban landscapes including urban green infrastructure increasingly gain importance as they provide a variety of food resources, nesting sites, or hiding places and thus serve as refuges for several species that are expelled from their natural habitats, such as lizards, falcons, bees, and bumblebees (Becker & Buchholz, 2015; Hall et al., 2017; Kettel et al., 2018; Samuelson et al., 2018).

In conclusion, the study of frogs in the city of Berlin exemplifies that urbanized areas have the potential to serve as suitable refuges for environmentally sensitive species, such as amphibians. As indicated by the trait change over time and their association with environmental factors, habitats within cities can be restored whilst at the same time urbanization proceeds continuously. In this, and presumably many other examples, particular attention must be paid to the role of urban greenspaces in facilitating the function of urban ecosystems by providing high-quality habitat for wildlife. These findings underline the need of maintaining, establishing and connecting such green infrastructures by sufficiently large corridors and stepping stones within the urban matrix and considering their importance for future urban planning. However, areas with intense agriculture, such as predominantly but not exclusively found in Brandenburg, still seem to provide habitats of reduced quality. Management actions should further strive on minimizing and compensating for anthropogenic interventions in order to preserve and promote present biodiversity and associated ecosystem services in agricultural landscapes.

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CONFLICT OF INTEREST
All authors confirm that they have no conflict of interest to declare.

AUTHOR CONTRIBUTION
S.N., J.M., and M.O.R. designed the study; S.N. collected and analyzed the data; U.S. ran the isotope ratio mass spectrometry, S.N. led the writing of the manuscript. All authors contributed to the drafts and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are openly available in MN Data Repository at https://doi.naturkundemuseum.berlin/data/10.7479/5tm4-9r29.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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