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Do human-induced habitat changes impact the morphology of a common amphibian, *Bufo bufo?*

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Abstract

Human activities induce habitat modifications and the intensification of land use including urbanisation and agriculture. Human-modified habitats are often fragmented, generating patches separated by dispersal barriers. In order to cross landscape obstacles, animals need to adapt or show plastic responses allowing effective dispersal and the pursuit of fitness-relevant activities. Due to their limited dispersal capacity and the need for terrestrial and aquatic habitats to complete their biphasic life cycle, amphibians can be expected to be particularly vulnerable to changes in the landscape. Yet data to test these ideas remain relatively scarce. To test whether human-induced habitat modifications impact the morphology of animals with low dispersal ability we measure 15 morphological traits on 137 common toads (*Bufo bufo*) across four habitat types represented by two or three independent sites across the Ile de France region. The results shown morphological differences among habitat types and sexes. Animals show habitat specific morphologies that may allow them to survive in human-modified habitats. Moreover, females have larger heads possibly to avoid trophic resource competition with males. Males, on the other hand show longer limbs possibly related to their greater movements when finding females during the reproductive season. Overall, our results that animals living in different habitat types are morphologically different with differences between natural and urban habitat being the greatest. Whether these differences are due to local adaptation or plastic responses remains to be investigated.

Keywords Land use · Urbanisation · Agriculture · Morphology · Toad · Sexual dimorphism

Introduction

By exerting selective pressures, environmental variation and biotic interactions are important drivers of phenotypic variation in organisms (Grenier et al. 2016). For example, interactions with prey, predators, and conspecifics impose selective pressures on phenotypes (Liao et al. 2022) and impact the fitness of organisms. Moreover, changes in landscape structure and microhabitats will affect the morphology of organisms through the link between morphology and performance (Arnold 1983). Selection on heritable variation or plastic responses may result, but survival will depend on the speed of the morphological change relative to the speed of environmental change (Ghalambor et al. 2007). Anthropogenic changes can be extremely rapid, making adaptation a real challenge (Jacobs et al. 2019). Human activity is transforming natural habitats into modified environments and intensifies land use at an ever-increasing rate. These land use changes include amongst others urbanisation and agriculture (Foley et al. 2005). Their consequences are numerous and have resulted in habitat fragmentation and deforestation ultimately leading to biodiversity loss (Defries et al. 2004). Fragmentation alters landscape connectivity - the degree of ease by which individuals can move through a landscape– by generating patches of habitat that differ in size and composition, separated by barriers (Fahrig et al. 2003). The ability of organisms to cross landscape obstacles depends on their behaviour and locomotor performance

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(Trochet et al. 2019), which is in turn dependent on the morphology of an organism.

Morphological traits often reflect adaptation to a particular habitat type (Melville & Swain 2000). The link between morphology and ecology has been observed many times and is particularly striking in organisms with low mobility like amphibians and reptiles (Garland and Losos 1994). Morphological adaptations may improve the performance of individuals in a given habitat (Herrel et al. 2000). Performance is defined as the ability of an organism to execute a function which is relevant in its ecological context (Huey and Stevenson 1979). Locomotor performance is fitness-relevant as it is linked to predator avoidance, territory defence, foraging, reproduction, and dispersal, needed to maintain gene flow between populations (Herrel et al. 2000; Trochet et al. 2016). However, different habitat types will exert different selection pressures on morphological traits due to the relationship between locomotion and habitat type.

Studies examining the effect of habitat modification on morphology in vertebrates have focused mainly on mammals and birds. For example, a recent study showed negative effects of urbanisation on species occupancy and diversity in warmer cities with less green space (Haight et al. 2023). Moreover, these authors showed that mostly large bodied species were impacted. Similarly, in birds more urbanised environments led to a reduction in species richness. However, in contrast to what has been observed for mammals in birds omnivorous large-bodied species thrived better (Pena et al. 2023). Amphibians are under-represented in research into habitat degradation and species declines (Tan et al. 2023). Yet, they are particularly threatened by global warming, habitat fragmentation and the spread of infectious diseases: 41% of amphibian species are threatened with extinction according to the IUCN Red List of Threatened Species (IUCN 2023). Moreover, the diversity of amphibian species decreases as the degree of urbanisation increases (Mazgajska and Mazgajski 2020). Amphibians are particularly vulnerable to changes in the landscape because they depend on connectivity between terrestrial and aquatic habitats due to the biphasic life cycle present in most species, resulting in the need to use water bodies for breeding and terrestrial habitats for the rest of their life cycle (Homola et al. 2019). Any barrier between terrestrial and aquatic habitats is thus likely to affect their survival (Konowalik et al. 2020) and may exert selection on morphology and locomotor performance.

Several studies have tested the relationship between the morphological traits of amphibians and their habitats (Gomes et al. 2009; Tejedo et al. 2010; Eterovick et al. 2016) and have shown differences in limb and body dimensions as well as differences in fluctuating asymmetry. Often, the relationship between habitat use and morphology can be better understood by exploring performance (Arnold 1983). From a bio-mechanical point of view, greater limb length is associated with better locomotor performance and allows for a greater jumping distance, a greater movement speed and therefore better dispersal (Trochet et al. 2016). However, this may strongly depend on habitat type as animals in cluttered habitats may benefit from shorter limbs. although this may depend on habitat type. A previous study showed that in agricultural habitats, classified as "open" habitats (sparse plant cover and soils more exposed to wind and light), adult male *Bufo bufo* had a greater size, greater body mass and longer hind limbs than individuals living in forests, described as "closed" habitats (denser plant cover, higher humidity, and less light penetration; Guillot et al. 2016). The authors of this study put forward two hypotheses: (1) open landscapes would be characterised by less competition, which favours access to resources and better growth; (2) these landscapes could have fewer resources and greater predation pressure (fewer refuges; Guillot et al. 2016). Larger males would be selected for their better locomotor abilities, enabling them to move around to find resources and avoid predators. A physiological reason was also suggested: as small individuals have a greater surface/volume ratio, they may be more sensitive to desiccation in an open environment and therefore counter-selected (Kovar et al. 2009; Guillot et al. 2016). In addition to the degree of habitat openness, proximity to roads appears to exert selection pressure on amphibian limb length (Trochet et al. 2016). In *Lissotriton helveticus*, individuals with shorter hind limbs were observed near habitats with dense road networks. This could be the result of a counter-selection of individuals with longer limbs that are more mobile near roads (Trochet et al. 2016). However, whether these responses to differences in habitat types are the result of natural selection and adaptation of rather plastic responses remains to be tested in most cases.

The adaptive responses of morphology to habitat use may also differ between sexes (Herrel et al. 2002). For example, greater variation in limb length was observed in the males of 30 species of lizards of Phrynosomatidae compared to females. Territory defence is carried out by males and is maximised by better locomotor performance (Herrel et al. 2002; Van Damme et al. 2008). Thus, the ability of males to move is essential for defending resources and finding mates. Males with poorer locomotor performance are less likely to mate and are therefore subject to both natural selection and sexual selection, which act together to maximise the locomotor performance of males (Van Damme et al. 2008). Consequently, we can expect males to be more strongly impacted by barriers in their habitat resulting in stronger selection on morphology.

In this study, we explore morphological variation in response difference in habitat type in an amphibian, the

Common toad, *Bufo bufo*. We hypothesised that toads living in habitats impacted by human land use would differ in morphology. We predict that individuals from urban habitats will be larger and show relatively longer limbs allowing them to overcome landscape barriers. Animals from more open, agricultural habitats, for example, could be expected to be larger for physiological reasons (desiccation avoidance) as suggested higher. We further predict that the responses of males and females will be different because of the difference in reproductive behaviour with males having longer forelimbs due to their role in amplexus and females having larger bodies due to their investment in eggs.

Methods

Study area

The Ile-de-France region is one of the France's smallest regions with 12 012 km^2 equalling only 2.2% of the total surface area. Yet, 20% of the territory is urbanised and houses 18% of the total French population. A further 53% of the territory is agricultural and 23% is made up of forests, including the major forests of Rambouillet and Fontainebleau (La préfecture et les services de l'État en région 2024; ONF 2024).

This study was conducted on 10 sites classified into 4 categories: urban (3 sites), mixed (2 sites), agricultural (2 sites), and natural (2 sites; see Fig. 1). Sites were situated more than 10 km apart except for two urban sites. The distance between sites largely exceeds the typical dispersal distance of *Bufo bufo* (2.5 Km) ensuring that each site is an independent replicate. The two urban sites that were close together were effectively isolated by urban areas and the lack of green space. Our habitat classification was based on the percentage of each habitat type present using the level 1 of the CORINE Land Cover 2018 geographical data within a radius of 1 km of where animals were captured using the QGIS software. (Version 3.34.5-1) (Table 1). The most dramatic land use changes occurred over the last 200 years fragmenting the natural landscape and the populations of the common toad therein. A comparison of habitat types

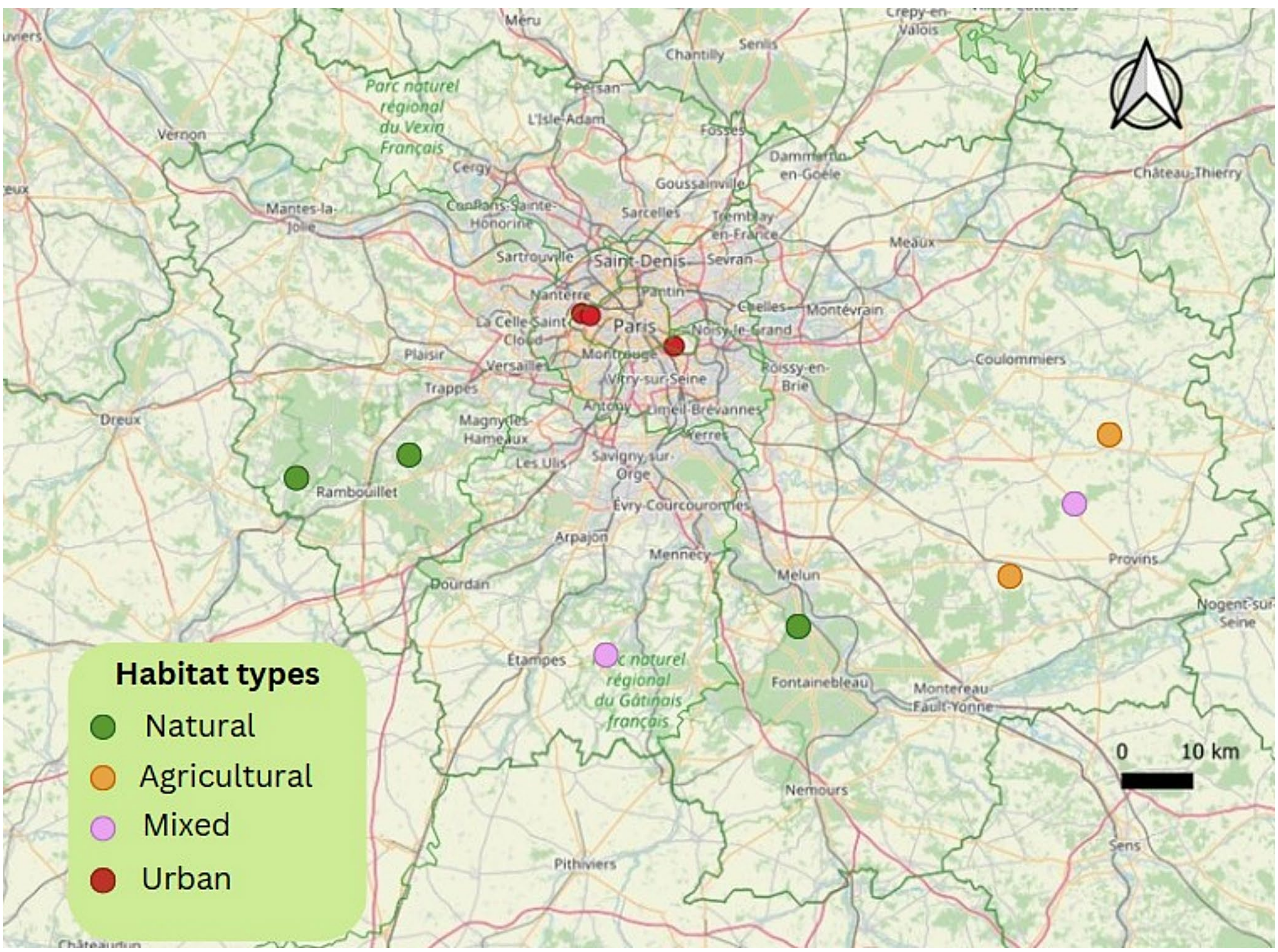

Fig. 1 Map showing sampling sites in the Ile de France region. Habitat types are represented by different colours

Table 1 Categorisation of the habitat type according to the percentage of land occupation in a 1 km radius around the point of capture of each site (Fig. 1). Ten sites were sampled throughout the Ile De France region

Habitat type	Artificial	Agricultural	Natural
Urban	≥95%	0%	≤5%
Mixed	≤25%	≤45%	≤55%
Agricultural	0%	>85%	<15%
Natural	<5%	<10%	>85%

Table 2 Number of common toads captured in each habitat type

Localisation	Natural (3)	Agricultural (2)	Mixed (2)	Urban (3)	Total (10)
Female	16	4	3	18	41
Male	25	26	27	18	96
Total	41	30	30	36	137

The number of sites for each habitat type is indicated between brackets

using Land Cover data from 2012 to 2018 shows that, at least for the past 10 years, this has been stable. Comon toads mature in less than two years and have a longevity of around 10 years (Hemelaar 1988).

Biological model

This study focuses on the Common toad, *Bufo bufo*, a generalist species, found in a great diversity of habitats (Guillot et al. 2016). It has a biphasic life cycle that involves seasonal migrations between terrestrial environments for hibernation and aquatic environments for reproduction (Duguet and Melki 2003). The migration distance between hibernation and breeding sites typically does not exceed 2.5 km (Nöllert and Böllert 2003; Kovar et al. 2009; Guillot et al. 2016) which makes it a relatively sedentary species with low mobility.

Morphological measurements

We captured 137 Common toads in early spring of 2023 and 2024 including 41 females and 97 males (Table 2). We measured 15 morphological traits (Fig. 2) including: snout-vent length, measured as the straight-line distance from the tip of the snout to the back of the urostyle; head length, measured as the straight-line distance from the tip of the premaxilla to the back of the parietal; head width, measured at the widest point of the head; head height, measured at the deepest part of the head; lower jaw length, measured as the straight-line distance from the tip of the mandible to the back of the jaw; ilium width, the widest distance measured at the cranial-most point of the ilium; femur length, measured on the ventral side of the animal from mid-body to the tip of the femur; tbiotarsus length, measured from the tip of the tibiotarsus at its articulation with the femur to its distalmost

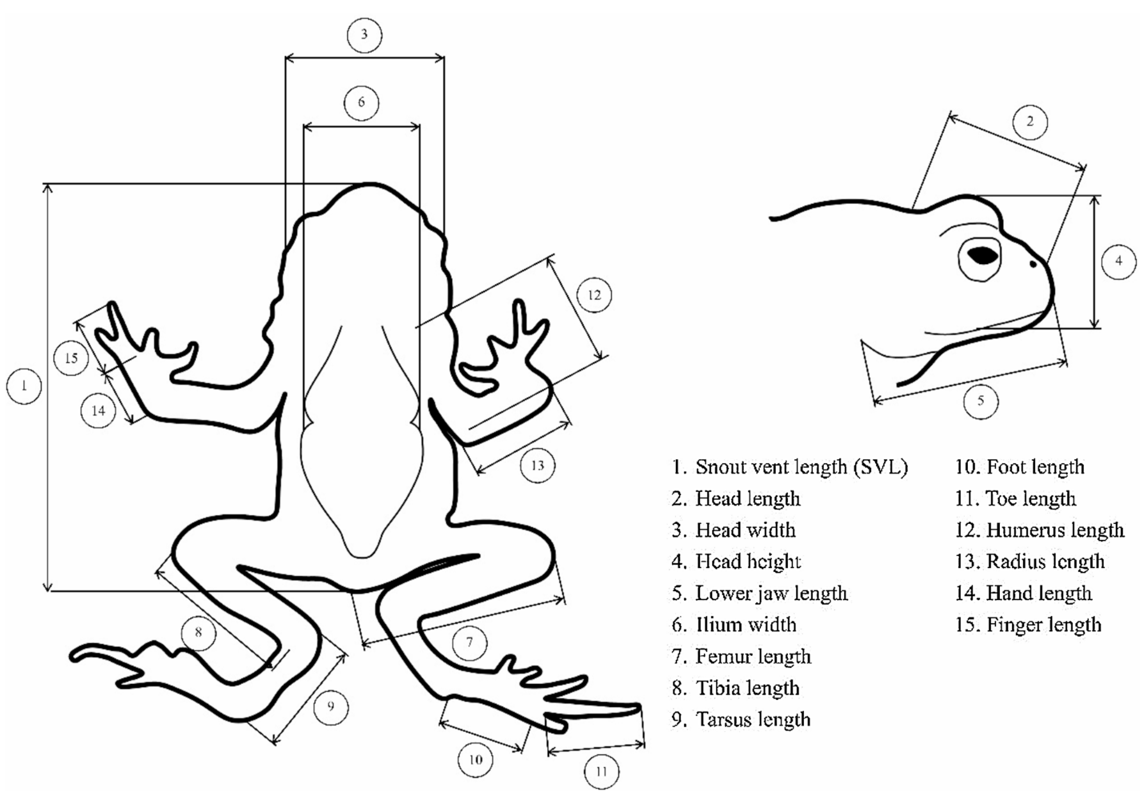

Fig. 2 Morphological measurements taken on *Bufo bufo*

point at the articulation with the foot (hereafter referred to as 'tibia' length); tarsus length measured from the articulation between tibiofibular to the beginning of the midfoot; foot length, measured from the beginning of the foot to the beginning of the toes; toe length, measured on the longest toe from its base to the tip; humerus length, measured from the articulation between humerus and the girdle (that can be sensed by palpation) to the tip of the humerus; radioulnar length, measured from the articulation between the humerus and the radioulnar to its tip (hereafter referred to as radius length); hand length from the distal end of the radioulnar to the base of the fingers; finger length measured on the longest finger from its base to the tip. All measurements were taken using digital callipers (Mitutoyo CD-15DC, ± 0.01 mm) on the right side of the individual. Sex and age class (juvenile, sub-adult, adult) were also recorded. Body mass (±0.1 g) was measured using a digital scale (Kubei-BM3). The measurements were taken directly in the field by a single operator (RA), after which the toads were released. Each site was visited only once and all toads were measured at the end of the visit before being released to avoid measuring the same animal twice.

Statistical analysis

All statistical tests were performed using IBMSPSS V.25 All data were Log_{10}-transformed before analyses. We first tested for differences in body size (Snout Vent Length - SVL) between sexes and habitat types using a two-way ANOVA with habitat and sex and the interaction between habitat and sex as our factors. A Bonferroni post-hoc test was used to explore which habitats were different from one another. As size significantly affected the morphological measurements, we used SVL as a covariate in all the following analyses. We ran a MANCOVA with sex, habitat, and the interaction between the two as our main effects and the morphological data as our independent variables. The MANCOVA was coupled with two-way ANCOVAs with sex and habitat type as well as their interaction for each trait to test for which traits differences were observed. Significance levels were adjusted using a sequential Bonferroni correction and Bonferroni post-hoc tests based on the marginal means were performed to quantify which habitats differed from one another.

Table 3 Results of post-hoc Bonferroni tests exploring the effect of habitat type on snout-vent length

Habitat types	Mixed	Agricultural	Urban
Natural	$\boldsymbol{P<0.001}$	$\boldsymbol{P<0.001}$	$\boldsymbol{P<0.001}$
Mixed		$\boldsymbol{P=0.010}$	$P=0.394$
Agricultural			$P=0.804$

Bold values indicate significant differences

Results

Snout-vent length differed between individuals of different sexes ($F_{1,129}$ = 72.750, $P<0.001$) and habitat types ($F_{3,129}$ = 22.783, $P<0.001$). However, no interaction between sex and habitat type was observed ($F_{3,129}$ = 2.190, $P=0.092$). Animals from natural habitats were larger than animals from other habitat types and females were bigger than males ($\bar{x}_{fem}$ = 72.6 ± 8.2 mm > $\bar{x}_{mal}$ = 62.8 ± 5.7 mm). Moreover, toads from mixed and agricultural habitats were larger than toads from urban habitats ($\bar{x}_{natural}$ = 71.1 ± 9.2 mm; $\bar{x}_{mixed}$ = 65.6 ± 5.1 mm; $\bar{x}_{urban}$ = 63.2 ± 6.0 mm; $\bar{x}_{agricultural}$ = 61.3 ± 6.3 mm; Table 3).

No interaction between habitat type and sex was detected (MANCOVA: Wilks' lambda = 0.636, $F_{45,339.5}$ = 1.243, $P=0.146$). However, both habitat type (MANCOVA: Wilks' lambda = 0.303, $F_{45,339.5}$ = 3.726, $P<0.001$) and sex (MANCOVA: Wilks' lambda = 0.422, $F_{15,114}$ = 10.412, $P<0.001$) had a significant influence on the morphology of toads irrespective of variation in size. Habitat type significantly impacted all the body dimensions except head width and hand length (Table 4; Fig. 3).

Natural habitats were most different from the other habitat types with both head, body, and limb dimensions being relatively larger in animals from natural habitats (Table 5; Supplementary Table 1). Toads from urban habitats stood out in having shorter humeri and toes compared to animals from the other three habitat types. Animals from mixed habitats stood out in having shorter finger lengths compared to animals from natural and urban habitats. Finally, animals from natural and agricultural habitats differed from animals from mixed and urban habitats in having a greater head length (Fig. 3; Supplementary Table 1).

Males and females differed in the relative dimensions of most traits except the relative femur length, the relative tibiofibular length, tarsus length, foot length, radioulnar length, and hand length (Table 4; Fig. 3). After correction for multiple testing differences in head length and lower jaw length were no longer significant. Females are heavier and have a greater head width and height, a greater ilia width, and longer toes. Males have relatively longer humeri (Fig. 4; Table 4).

Discussion

In a changing habitat, species survival depends on their capacity to respond by either adaptation or phenotypically plastic responses (Bock 1980). By understanding amphibian responses to land use modifications, it may be possible to predict future trends and derive biologically informed conservation actions (Lambert et al.

Table 4 Effects of snout-vent length (covariable), habitat type, sex, and the interaction between sex and habitat on the 15 morphological measurements taken for *Bufo bufo*

		F	*P*
Snout-vent length (d.f. = 1,128)	**Weight**	188.245	0.000
	Head length	39.859	0.000
	Head width	98.595	0.000
	Head height	29.405	0.000
	Lower jaw length	96.003	0.000
	Ilium width	32.431	0.000
	Femur length	39.881	0.000
	Tibiofibula length	65.859	0.000
	Tarsus length	45.349	0.000
	Foot length	19.020	0.000
	Longest toe length	39.001	0.000
	Humerus length	78.775	0.000
	Radioulna length	58.582	0.000
	Hand length	55.341	0.000
	Longest finger length	20.177	0.000
Habitat (d.f. = 3,128)	**Weight**	9.702	0.000
	Head length	20.112	0.000
	Head width	2.618	0.054
	Head height	9.325	0.000
	Lower jaw length	9.149	0.000
	Ilium width	6.555	0.000
	Femur length	4.201	0.007
	Tibiofibula length	11.856	0.000
	Tarsus length	4.768	0.003
	Foot length	8.599	0.000
	Longest toe length	5.406	0.002
	Humerus length	5.560	0.001
	Radioulna length	5.707	0.001
	Hand length	2.254	0.085
	Longest finger length	5.192	0.002
Sex (d.f. = 1,128)	**Weight**	16.638	0.000
	Head length	6.293	0.013
	Head width	21.853	0.000
	Head height	16.350	0.000
	Lower jaw length	4.274	0.041
	Ilium width	16.482	0.000
	Femur length	0.709	0.401
	Tibiofibula length	3.259	0.073
	Tarsus length	0.052	0.821
	Foot length	3.866	0.051
	Longest toe length	10.328	0.002
	Humerus length	10.393	0.002
	Radioulna length	3.433	0.066
	Hand length	2.547	0.113
	Longest finger length	20.680	0.000

Table 4 (continued)

		F	*P*
Habitat * Sex (d.f. = 3,128)	Weight	4.074	0.008
	Head length	2.289	0.082
	Head width	0.569	0.636
	Head height	1.018	0.387
	Lower jaw length	2.276	0.083
	Ilium width	0.782	0.506
	Femur length	1.115	0.346
	Tibiofibula length	1.158	0.328
	Tarsus length	0.266	0.850
	Foot length	0.434	0.729
	Longest toe length	0.444	0.722
	Humerus length	0.262	0.852
	Radioulna length	0.439	0.726
	Hand length	1.019	0.386
	Longest finger length	4.129	0.008

d.f. = degrees of freedom; variables in bold remain significant after sequential Bonferroni correction

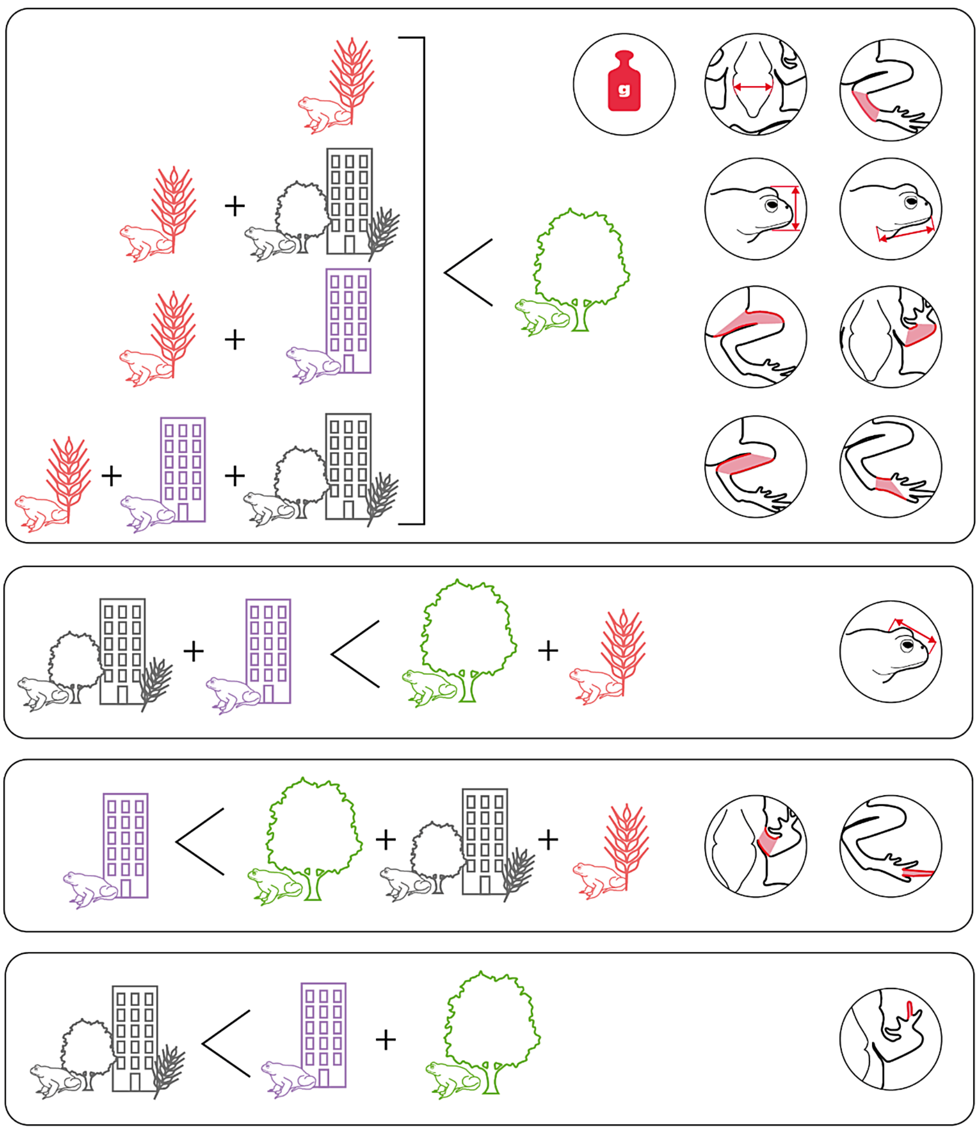

Fig. 3 Effects of habitat type (green = natural, orange = agricultural, grey = mixed and purple = urban) on the morphology of *Bufo bufo*. The pictogram of a weight with the letter 'g' refers to body mass

Table 5 Results of Bonferroni post-hoc test based on estimated marginal means

Variable			*P*
Body mass	Agricultural	Mixed	0.936
		Natural	**0.001**
		Urban	1.000
	Mixed	Agricultural	0.936
		Natural	0.163
		Urban	0.564
	Natural	Agricultural	**0.001**
		Mixed	0.163
		Urban	**0.000**
	Urban	Agricultural	1.000
		Mixed	0.564
		Natural	**0.000**
Head length	Agricultural	Mixed	**0.050**
		Natural	1.000
		Urban	**0.000**
	Mixed	Agricultural	**0.050**
		Natural	**0.002**
		Urban	0.513
	Natural	Agricultural	1.000
		Mixed	**0.002**
		Urban	**0.000**
	Urban	Agricultural	**0.000**
		Mixed	0.513
		Natural	**0.000**
Head width	Agricultural	Mixed	1.000
		Natural	0.238
		Urban	1.000
	Mixed	Agricultural	1.000
		Natural	1.000
		Urban	1.000
	Natural	Agricultural	0.238
		Mixed	1.000
		Urban	0.066
	Urban	Agricultural	1.000
		Mixed	1.000
		Natural	0.066
Head height	Agricultural	Mixed	1.000
		Natural	**0.002**
		Urban	1.000
	Mixed	Agricultural	1.000
		Natural	**0.003**
		Urban	1.000
	Natural	Agricultural	**0.002**
		Mixed	**0.003**
		Urban	**0.000**
	Urban	Agricultural	1.000
		Mixed	1.000
		Natural	**0.000**

Table 5 (continued)

Variable			P
Lower jaw length	Agricultural	Mixed	0.829
		Natural	**0.000**
		Urban	0.565
	Mixed	Agricultural	0.829
		Natural	**0.026**
		Urban	1.000
	Natural	Agricultural	**0.000**
		Mixed	**0.026**
		Urban	**0.001**
	Urban	Agricultural	0.565
		Mixed	1.000
		Natural	**0.001**
Ilium width	Agricultural	Mixed	0.974
		Natural	**0.003**
		Urban	1.000
	Mixed	Agricultural	0.974
		Natural	0.285
		Urban	1.000
	Natural	Agricultural	**0.003**
		Mixed	0.285
		Urban	**0.001**
	Urban	Agricultural	1.000
		Mixed	1.000
		Natural	**0.001**
Femur length	Agricultural	Mixed	1.000
		Natural	**0.038**
		Urban	1.000
	Mixed	Agricultural	1.000
		Natural	0.124
		Urban	1.000
	Natural	Agricultural	**0.038**
		Mixed	0.124
		Urban	**0.040**
	Urban	Agricultural	1.000
		Mixed	1.000
		Natural	**0.040**
Tibiofibula length	Agricultural	Mixed	1.000
		Natural	**0.000**
		Urban	0.221
	Mixed	Agricultural	1.000
		Natural	**0.000**
		Urban	1.000
	Natural	Agricultural	**0.000**
		Mixed	**0.000**
		Urban	**0.002**
	Urban	Agricultural	0.221
		Mixed	1.000
		Natural	**0.002**

Table 5 (continued)

Variable			*P*
Tarsus length	Agricultural	Mixed	0.774
		Natural	**0.005**
		Urban	1.000
	Mixed	Agricultural	0.774
		Natural	0.593
		Urban	1.000
	Natural	Agricultural	**0.005**
		Mixed	0.593
		Urban	**0.036**
	Urban	Agricultural	1.000
		Mixed	1.000
		Natural	**0.036**
Foot length	Agricultural	Mixed	1.000
		Natural	**0.001**
		Urban	0.895
	Mixed	Agricultural	1.000
		Natural	**0.001**
		Urban	1.000
	Natural	Agricultural	**0.001**
		Mixed	**0.001**
		Urban	**0.006**
	Urban	Agricultural	0.895
		Mixed	1.000
		Natural	**0.006**
Longest toe length	Agricultural	Mixed	1.000
		Natural	0.449
		Urban	1.000
	Mixed	Agricultural	1.000
		Natural	0.073
		Urban	1.000
	Natural	Agricultural	0.449
		Mixed	0.073
		Urban	**0.001**
	Urban	Agricultural	1.000
		Mixed	1.000
		Natural	**0.001**
Humerus length	Agricultural	Mixed	1.000
		Natural	1.000
		Urban	**0.005**
	Mixed	Agricultural	1.000
		Natural	1.000
		Urban	**0.022**
	Natural	Agricultural	1.000
		Mixed	1.000
		Urban	0.060
	Urban	Agricultural	**0.005**
		Mixed	**0.022**
		Natural	0.060

Table 5 (continued)

Variable			*P*
Radius length	Agricultural	Mixed	1.000
		Natural	**0.002**
		Urban	1.000
	Mixed	Agricultural	1.000
		Natural	0.141
		Urban	1.000
	Natural	Agricultural	**0.002**
		Mixed	0.141
		Urban	**0.019**
	Urban	Agricultural	1.000
		Mixed	1.000
		Natural	**0.019**
Hand length	Agricultural	Mixed	1.000
		Natural	1.000
		Urban	1.000
	Mixed	Agricultural	1.000
		Natural	0.558
		Urban	1.000
	Natural	Agricultural	1.000
		Mixed	0.558
		Urban	0.106
	Urban	Agricultural	1.000
		Mixed	1.000
		Natural	0.106
Finger length	Agricultural	Mixed	1.000
		Natural	**0.026**
		Urban	1.000
	Mixed	Agricultural	1.000
		Natural	**0.021**
		Urban	1.000
	Natural	Agricultural	**0.026**
		Mixed	**0.021**
		Urban	**0.029**
	Urban	Agricultural	1.000
		Mixed	1.000
		Natural	**0.029**

Bold values indicate significant differences. See Supplementary Table 1 for marginal means

2021). This study can be added to previous studies on the effect of land use on amphibian morphology and shows that *Bufo bufo* from natural habitats are larger, in contrast to observations of previous studies (Guillot et al. 2016). Toads from natural habitats manifest more massive heads and greater body and limb measures, even when controlling for variation in overall body size, again different from what was observed in previous studies (Guillot et al. 2016). Toad humeri and toes are particularly shorter in urban habitats and fingers are shorter in mixed habitats. The impact of the habitat type is similar for both sexes, but sexes differed from one another with females having larger heads compared to males which, in contrast have longer limbs, irrespective of variation in body size. Whether these differences are the result of selection on specific traits or rather reflect plastic differences remains to be investigated, however.

Influence of habitat types on morphology

The toads sampled in our study showed morphological differences among habitat types. Animals from natural habitats stand out from those from other habitats and have a relatively greater body mass, wider ilia and greater femur, tibia, radius as well as tarsus and foot lengths. The longer hind limbs of toads in natural habitats suggest that they may move more between reproduction and wintering sites. Indeed, in toads, limb length is a good proxy for jumping capacity (Mariotto et al. 2022) as for example demonstrated for cane toads at the edge of the invasive range in Australia (Phillips

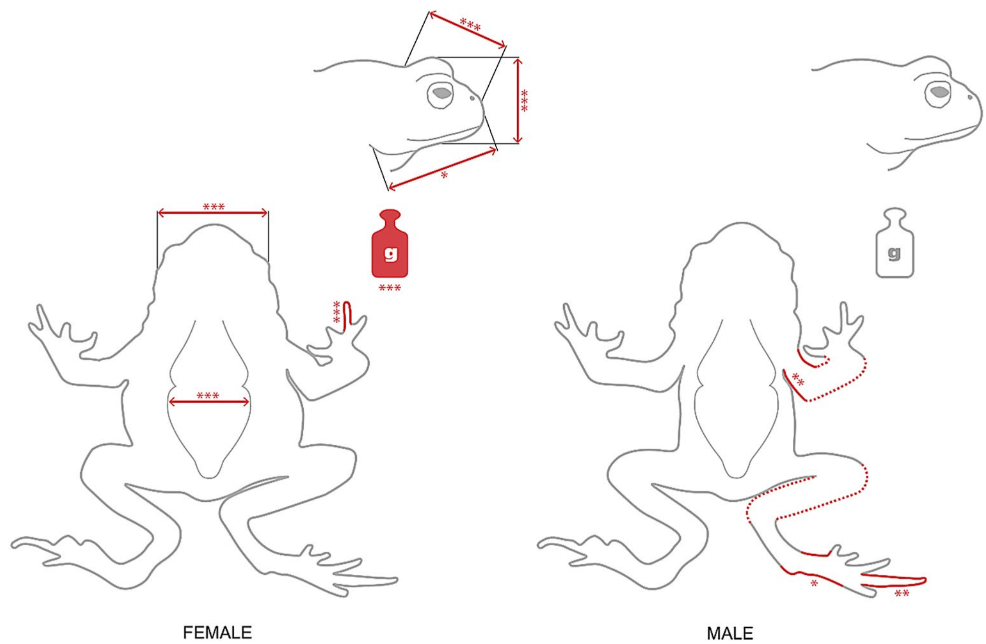

Fig. 4 Effects of sex on morphology of *Bufo bufo*. Grey line = no effect, red full line = significant: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$; see Table 4

et al. 2006; Hudson et al. 2020). Yet, these results stand in contrast to those from a previous study on the same species that suggested that more open habitats like agricultural ones would result in larger animals with longer limbs (Guillot et al. 2016). This suggests that the responses of amphibians like *B. bufo* may be strongly site-specific and illustrates the need for studies at the landscape level to better understand how habitat modification impact toad morphology. In addition, toads in natural habitats in our study had relatively taller heads and longer lower jaws. The ballistic tongue projection mechanism in toads is highly dependent on the acceleration of the jaw during mouth opening (Lappin et al. 2006), and relatively longer lower jaws will result in greater jaw tip and tongue velocities. Moreover, the relatively taller heads will result in longer depressor mandibulae muscles, the primary driver of mouth opening (Nishikawa and Gans 1996; Nishikawa 2000; Lappin et al. 2006). Overall, this suggests that animals in natural habitats may show greater tongue projection velocities allowing them to capture faster and possible more diverse prey.

In urban habitats toads also had generally shorter toes and humeri suggesting a more sedentary life-style. Similarly, in mixed habitats the fingers were relatively shorter suggesting that in human-modified habitats the forelimbs are generally shorter which may impact locomotion. The forelimbs in toads play an important role during landing (Cox and Gillis 2015) and longer hops are typically associated with greater humerus movements. Overall, this suggested that toads in urban habitats take shorter hops and consequently may move less. Not only may this be a consequence of the more fragmented habitats but this may also make animals less visible to predators. In urban environments common toads are known to have larger paratoid glands (Bókony et al. 2019), possibly in relation to the higher predation pressure. If this is indeed the case, then the shorter hops may make toads less visible to urban predators such as cats and dogs and result in shorter limbs. In addition to playing a role in locomotion, in toads the toes of the hind limb also play a role in what has been referred to as pedal luring (Sloggett and Zeilstra 2008). In cane toads toe twitching has been suggested to be related to cannibalism and is used by adults to lure metamorphs (Hagman and Shine 2008). Although it is unknown whether cannibalistic behaviour also occurs in the common toad studied here, shorter toes may reduce the effectiveness of pedal luring and thus reduce cannibalism. In small fragmented populations, typical of human-modified habitats, inbreeding is likely greater and the probability of consuming related individuals may be higher. This could possibly act as a counter-selection mechanism on toe length. This remains, however, speculative and needs to be investigated further.

Given our study design we cannot rule out non-adaptive origins for the observed variation such as population-level variation that existed before the land use changes took place, or founder effects with patches colonised by a small number of individuals. However, given that similar patterns were observed for independent sites (3 natural, 3 urban, 2 agricultural and 2 mixed sites) makes it unlikely that these differences are due to founder effects. Similarly, since the species is present across the entire region and used to occupy forest throughout the Ile de France region, pre-existing adaptations in local populations are unlikely. Again, the distance between sites of a similar habitat type across the sampled area suggests that it would be unlikely for animals from two sites that are now of a similar type be of the same pre-existing adapted morphotype. Genomic studies could provide further insights into geneflow and pre-existing population-level differences between sites and would be particularly insightful.

Sex specific differences

Female and male toads showed overall morphological differences. The female snout vent length (SVL) is greater than that of males as previously documented (Davies and Halliday 1977; Shine 1979). Independent of the overall variation in size, body and limb shape was also different between males and females. For example, females had relatively bigger heads overall. Previous studies have shown an impact of diet on head morphology (Herrel and Holanova 2008) suggesting that this may be related to diet. During the breeding season, females are more sedentary (Speybroeck et al. 2018) and exploit resources locally. The larger heads observed in females may allow them to eat larger and more diverse prey, providing them with the energy needed for reproduction. Stomach flushing could be useful to compare the diet composition between females and males. Female toes are also longer than those of males. The use of toes during feeding by wiggling is known to lure and attract preys (Parrish and Fischer 2024) and this may possible be one of the drivers explaining this difference. The ilium width of females is also relatively greater than in males. During reproduction, one female produces two threads of 5 m including 8000 eggs (Speybroeck et al. 2018). The enlargement between the ilia could be an adaptation to create more space for the eggs in the body of females. Males, on the other hand have longer feet, toes, and humeri. The radius and tibia follow the same trend albeit being not significantly different between sexes. During reproduction, males actively search for females which may explain selection on limb dimensions (Davies and Halliday 1979). Moreover, longer forelimbs could help males to hold on to females better during amplexus (Navas and James 2007).

Conclusion

In the common toad, *Bufo bufo*, we observed morphological variation in response to land use differences. The individuals from natural habitats were larger and had relatively longer limbs which may be due to longer migration distances between reproductive and wintering habitats. Animals in human-modified areas showed distinct morphologies that may be related to habitat fragmentation and differences in food availability and predation pressure. Moreover, females are larger and have larger heads, possibly allowing to avoid resource competition with males during reproduction. Males had relatively longer forelimbs, likely due to role during amplexus. Complementary studies into differences in diet, number of predators, movements, and microhabitat use during both the breeding and non-breeding season would be essential to better understand the differences described here. These results shed light on how animals with low mobility may adapt to human-modified habitats. Whether these responses are universal remains to be tested by comparison with sites across the distribution of the species. From a conservation perspective, our results suggest that this species may be able to survive even in strongly modified urban habitats. However, whether how population density and its dynamics are impacted in human-modified habitats remains to be explored.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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