

Urban environment and reservoir host species are associated with *Batrachochytrium dendrobatidis* infection prevalence in the common toad

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ABSTRACT: Human-induced changes of the environment, including landscape alteration and habitat loss, may affect wildlife disease dynamics and have important ramifications for wildlife conservation. Amphibians are among the vertebrate taxa most threatened by anthropogenic habitat change. The emerging fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) has caused extinctions and population declines in hundreds of anuran species globally. We studied how the urban landscape is associated with the prevalence of *Bd* infections by sampling 655 anurans of 3 species (mainly the common toad *Bufo bufo*) in 42 ponds surrounded by different amounts of urban habitat (defined as towns, cities or villages). We also examined the association between *Bd* infections and a potential reservoir host species (the moor frog *Rana arvalis*). We found that 38% of the sites were positive for *Bd* with an infection prevalence of 4.4%. The extent of urban landscape was negatively correlated with *Bd* infection prevalence. However, the positive association of *Bd* with the presence of the possible reservoir species was substantially stronger than the urban effects. The body condition index of *B. bufo* was negatively associated with *Bd* infection. This *Bd* effect was stronger than the negative effect of urban landscape on body condition. Our results suggest that urban environments in Sweden have a negative impact on *Bd* infections, while the presence of the reservoir species has a positive impact on *Bd* prevalence. Our study also highlights the potential importance of *Bd* infection on host fitness, especially in rural landscapes.

KEY WORDS: Amphibians · Chytrid · Disease transmission · Body condition · Scandinavia

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1. INTRODUCTION

Wildlife disease dynamics can differ between urban and natural environments due to variation in biotic and abiotic conditions that impact the density, condition and behaviour of the host, and the persistence of the pathogen in the environment. Often this results in higher transmission probability in urban environments (e.g. Riley et al. 2004, Farnsworth et al. 2005, Bradley & Altizer 2007). However, urbanization does not necessarily increase disease occurrence. For example, low species diversity in urban landscapes may reduce the risk of infection due to a lack of reservoir hosts or vectors (Kilpatrick et al. 2006). With

regard to rapid global urbanization, increasing our knowledge of the associations between the urban environment and wildlife health is crucial for preventing further loss of biodiversity.

Chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) is an emerging infectious disease that poses a global threat to amphibian diversity. Although *Bd* is not harmful to all amphibian species or populations, it has caused extinctions and population declines of amphibians in all continents with amphibians (Stuart et al. 2004, Skerratt et al. 2007, Wake & Vredenburg 2008). While the global emergence of the highly virulent and pathogenic strain designated *Bd*GPL was probably caused by the

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commercial trade in amphibians from Eastern Asia (O’Hanlon et al. 2018), the mechanisms that drive small-scale transmission of *Bd* more broadly are less well understood. *Bd* transmission among habitats can be assisted by humans (St-Amour et al. 2008, Adams et al. 2010, Pauza et al. 2010, Spitzen-van der Sluijs et al. 2014), waterfowl and other amphibians (Padgett-Flohr & Hopkins 2010, Garmyn et al. 2012). Prevalence and infection load may vary strongly between neighboring waterbodies (Kriger & Hero 2007), which illustrates the complexity of understanding transmission mechanisms.

Environmental factors are also important for the occurrence of *Bd*. Previous investigations generally found an increased risk of *Bd* in habitats that (1) have large permanent breeding ponds (Kriger & Hero 2007, Spitzen-van der Sluijs et al. 2014); (2) have high amphibian species richness (Olson et al. 2013, but see Searle et al. 2011, Cohen et al. 2016); (3) are surrounded by forest (Pauza et al. 2010, Olson et al. 2013, Saenz et al. 2015, Scheele et al. 2015, but see Kärnvemo et al. 2018); (4) are associated with high elevations in tropical regions (Brem & Lips 2008, Catenazzi et al. 2011) or low elevations in temperate regions (Kriger et al. 2007, Chestnut et al. 2014); and (5) are in, or adjacent to, urban environments (St-Amour et al. 2008, Adams et al. 2010, Spitzen-van der Sluijs et al. 2014). Moreover, a higher amount of trees surrounding the ponds in rural areas may affect occurrence of *Bd* via changes in temperature (Becker et al. 2012) or pH in these environments (Stoler & Relyea 2016).

The main effects of *Bd* on individual amphibians are skin infections, which can disrupt the transport of water, oxygen and electrolytes, which impairs cardiac function (Voyles et al. 2009, Salla et al. 2018). In addition, experimental laboratory and field studies have shown that *Bd* infections can reduce body condition of the host (Retallick & Miera 2007, Deguise & Richardson 2009, Pearl et al. 2009, Voyles et al. 2012, Iglesias-Carrasco et al. 2017). An association between urban environments and poorer amphibian body condition has also been reported (Iglesias-Carrasco et al. 2017). As humans continue to modify natural habitats through urbanization, it is critical that we understand the impact that these changes could have on amphibian populations.

In Sweden, *Bd* was first detected in the southernmost part of the country in 2010 (Hallengren 2013). Further studies across southern and central Sweden (2015–2018) found *Bd* in 8 anuran species, with prevalence varying between 1.9 and 61.5% among species (Kärnvemo et al. 2018, S. Meurling et al.

unpubl. data). These studies have shown that while *Bd* prevalence was low (1.5–3.5%) in the common toad *Bufo bufo*, it was significantly higher in the moor frog *Rana arvalis* (12.9–13.9% in southern and 19.0% in central Sweden). In line with previous studies of *Bd*-induced mortality of *B. bufo* (Garner et al. 2009), experimental studies on Swedish populations showed that mortality of *B. bufo* was as high as 60% among juveniles infected with a Swedish *Bd*-strain, while there was no significant mortality among *R. arvalis* juveniles (Bengtsson 2018, S. Meurling et al. unpubl. obs.). As these 2 species commonly occur in the same ponds, these results suggest that *R. arvalis* may act as a *Bd* reservoir host species for *B. bufo*. Previous studies have reported that transmission of *Bd* can be promoted by the presence of a reservoir host species (Scheele et al. 2017) or, on the contrary, prohibited by a dilution effect in species-rich communities (Searle et al. 2011).

The aims of this study were to examine the association between urban environments and *Bd* prevalence in *B. bufo* in central Sweden. Although we concentrated on *B. bufo*, we opportunistically also sampled *R. arvalis* and *R. temporaria* (the only other anurans present in the area). We sampled breeding individuals in urban and rural environments with the following aims: (1) to examine correlations between urban habitats and the prevalence of *Bd* infections in populations at different spatial scales; (2) to determine if *R. arvalis* may act as reservoir species for *Bd*; and (3) to evaluate the relationship between *Bd* infection and body size and condition of male *B. bufo*.

2. MATERIALS AND METHODS

2.1. Study design and animal sampling

In April and May 2015–2018, we sampled 655 anurans from 3 species (*Bufo bufo*, *Rana arvalis* and *R. temporaria*) in nocturnal breeding aggregations in 42 ponds in central Sweden. In total, 22 of these ponds were located within Uppsala County and 20 were in adjacent Stockholm County, spanning a range of urban and rural habitats (see Section 2.2; Fig. 1 and Table S1 in Supplement 1 at www.int-res.com/articles/suppl/d134p033_supp1.xls). These areas have a relatively high human population density: 179 residents km⁻² (in comparison to 23 residents km⁻² in the whole country), with 3778 residents km⁻² in the urban areas (Swedish Bureau of Statistics; www.scb.se/en/). We focused on *B. bufo* (588 ind. sampled), but also sampled opportunistically (i.e. time

allowing) 11 *R. arvalis* and 56 individuals of the common frog *R. temporaria*. The selection of ponds sampled was based on previous knowledge and observations of *B. bufo* occurrence obtained from the national record database (Swedish Species Gateway; www.artportalen.se). In total, 5 ponds were sampled in 2015, 12 in 2016, 21 in 2017 and 7 in 2018. Three of the ponds were sampled in both 2015 and 2016. We exclusively captured adults with hand-nets and recorded sex, snout–vent length (SVL; in mm with a calliper) and body mass (to the nearest g with an electronic scale). Captured individuals were kept separately in plastic containers. The mean sample size was 16 ind. pond⁻¹ (median 15, min. 6, max. 32). We tested for the presence of *Bd* by using the standard swabbing protocol of Brem et al. (2007), changing gloves between animals and making 25 strokes on the ventral skin and feet of each sampled individual. Before analysis, the swabs were preserved in alcohol in 2015 and in Dryswab (MWE MW110) in 2016–2018.

2.1. Molecular methods

The presence of *Bd* was determined by methods previously described by Kärvemo et al. (2018). Briefly, DNA was extracted from the swabs, and the presence of *Bd* was assessed by quantitative PCR (qPCR) amplification of the internal transcribed spacer (ITS)-5.8S rRNA region of the fungus according to methods outlined in Kosch & Summers (2013). We used a Biorad CFX96 qPCR system and amplification conditions as described in Kosch & Summers (2013), with standards of 0.1, 1, 10 and 100 genomic equivalents (GE) for the qPCR assays. An individual was scored as *Bd*-positive if one or more of the triplicate samples exhibited a positive signal (i.e. a clear exponential amplification curve). If the qPCR showed signs of inhibition (no curve), such negative samples were amplified once more before being designated as impossible to score.

2.2. Model variables

Vector landscapes of urban areas were extracted from Urban Morphological Zones 2000 (UMZ2000) from the European Environmental Agency (www.eea.europa.eu). This layer, derived from the Corine land cover data, is defined as towns, cities, or villages with urban networks less than 200 m apart, including industrial and commercial units and green urban areas (i.e. vegetation-covered land within the urban fabric for recreational use, such as parks and pocket gardens). The remaining landscapes are defined as rural. To control for the effects of other landscape

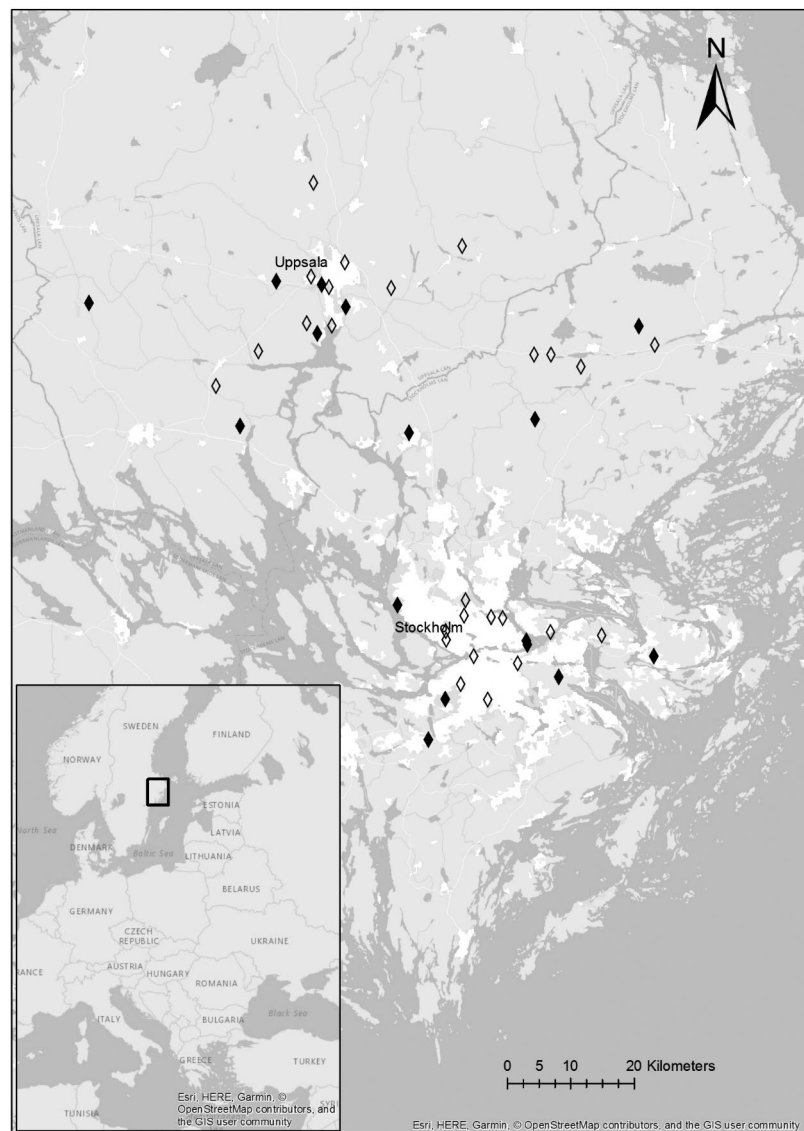


Fig. 1. Study sites and occurrence of *Batrachochytrium dendrobatidis* (*Bd*) infection in amphibians. White areas: urban zones (Urban Morphological Zones 2000; www.eea.europa.eu); black diamonds: *Bd*-positive sites; empty diamonds: no detection of *Bd*

variables, 'arable land' and 'mature forest' were added in additional models. Arable land (crop and fruit farms) was extracted using topographic vector maps from the Swedish National Land Survey, converted to raster format and quantified at 100 × 100 m resolution. Mature forest (timber volume > 200 m³ ha⁻¹) was quantified using *k*-nearest neighbor-raster data (kNN) obtained from the Swedish Forest Agency (Reese et al. 2003). It was originally quantified at 25 × 25 m resolution and then aggregated to 100 × 100 m by averaging, due to low accuracy at the original scale (Gjertsen 2007). The urban, arable and forested areas were determined in 3 nested circular buffer zones with a radius of 500, 2000 and 5000 m, respectively, around the main breeding location using the function 'spatial join' in ArcMap (ArcGIS, ESRI). The selection of buffer zone sizes was based on known general maximum movement distances of amphibians (Smith & Green 2005). Separate analyses were conducted at these 3 scales, as landscape variables at different scales could not be considered as being independent from each other.

Data on the presence of *R. arvalis* were based on our own field observations and records from the national database (Swedish Species Gateway; www.artportalen.se) between 2000 and 2018. Pond pH, available from 26 sites in 2017 and 2018, was recorded from water samples collected upon amphibian sampling (i.e. during the night) and measured within 12 h of the sampling occasion with a ThermoFisher[®] Orion 131S pH-meter. In 2017 and

2018, temperature loggers (Thermochron ibuttons[®]) were placed in all ponds (30–40 cm depth) at the location of the main breeding aggregation. However, due to pond desiccation, human disturbance, logger damage and other causes, we only received data from 15 ponds. The loggers recorded temperature every third hour from May to June, and mean values from these months were used in further analyses. We estimated a body condition index (BCI) of *B. bufo* males from the residuals of a linear regression of body length and mass. Females were excluded from these analyses as only a few had *Bd*, and they differ morphologically from males.

2.3. Statistical analyses

We conducted correlation analyses between pairs of all continuous model variables. None of the variable pairs had correlation coefficients >0.7. We ran 23 mixed models (Table 1) specifying site as a random effect in all models to attain the correct level of replication for the fixed effects. Effects of urban areas at 3 spatial scales and the presence of *R. arvalis* were analysed in generalized linear mixed models (GLMMs) with *Bd* prevalence (including all 3 species) as a binomially distributed response variable (Models 1–3). Area of mature forest and arable land and presence of large water bodies (if >20% of the buffer comprised large lakes or the sea) were included in additional GLMM models (3 scales) to control for poten-

Table 1. Response and explanatory variables (fixed and random) in each of the models. Model indicates model reference numbers and type (generalized linear mixed model, GLMM, and linear mixed models, LMM). The response variables include *Bd* infection prevalence (*Bd*), body condition index (BCI), body mass (weight), and body length (snout–vent length, SVL) of *Bufo bufo* males. The explanatory variables include presence of *Rana arvalis* (*Ra*), pond pH (pH), mean pond temperatures from May to June (temp), sampling day (day) and year 2015–2018 (year). The explanatory landscape variables (**bold**)—urban areas (urban), mature forest areas >200 m³ ha⁻¹ (forest), area of arable land (arable) and presence of large lakes or the sea (wetlands)—were analysed with 3 models at different spatial scales (500, 2000 and 5000 m). Sampling site (site) was included in all models as a random factor to attain the correct level of replications. N_{ind}: number of individuals included in each of the models. Tables S2–S8 are in Supplement 2 at www.int-res.com/articles/suppl/d134p033_supp2.pdf

Model(s)	Type	Response	Fixed variable(s)	N _{ind}	Table no.
1–3	GLMM	<i>Bd</i>	<i>Ra</i> , urban , day, year	655	2
4–6	GLMM	<i>Bd</i>	<i>Ra</i> , urban, forest, arable, wetland , day, year	655	S2
7–9	GLMM	<i>Ra</i>	Urban, forest, arable, wetland , year	655	S3
10	GLMM	<i>Bd</i>	pH	492	
11	GLMM	<i>Bd</i>	Temp	283	
12	LMM	BCI	<i>Bd</i> , day, year	577	3
13–15	LMM	BCI	Urban , day, year	577	S4
16	LMM	Weight	<i>Bd</i> , day, year	577	S5
17	LMM	SVL	<i>Bd</i> , day, year	577	S6
18–20	LMM	Weight	Urban , day, year	577	S7
21–23	LMM	SVL	Urban , day, year	577	S8

tial negative relationships with urban areas (Models 4–6). The effects of the same environmental factors were tested on the presence of *R. arvalis* (Models 7–9). Due to poor convergence, Models 4–9 were corrected by an optimization of the model algorithm via the ‘bobyqa’ function of the ‘glmerControl’ parameter (Powell 2009). As we only had data on pH and temperature for 26 and 15 of the 42 study sites, respectively, separate GLMM analyses were conducted for these potential effects on *Bd* prevalence (Models 10 and 11).

We used linear mixed models (LMMs) to test for effects of *Bd* infection prevalence on BCI (Model 12), body mass (Model 16) and snout–vent length (Model 20) for *B. bufo* males. We also tested the effects of urban areas on these 3 body size measurements (Models 13–15, and 18–23) to disentangle other potential confounding effects. Sampling day was included in these models as a covariate to control for potential changes in body mass over the mating period (Arak 1983). Year (2015–2018) was specified as fixed effect in all models to control for annual variation (Reading 2007), except for the models including pH and temperatures. The program R (v.3.5.0) and the package ‘lme4’ (Bates 2010) were used for mixed model analyses. The package ‘Multcomp’ (Hothorn et al. 2008) was used for post hoc analysis among the years. The package ‘effects’ (Fox 2003) was used for the predictive graphs. We approximated unconditional mean effects (estimates) and weights of important variables from multiple candidate models with a dredge function and model averaging from the package ‘MuMIn’ (Barton 2018). The statistical significance of differences in *Bd* infection prevalence between males and females of *B. bufo* and the presence of *R. arvalis* in strict urban and rural sites were analysed with χ^2 tests.

3. RESULTS

3.1. Urban areas and reservoir host

We found *Bd* in 16 (38%) of the 42 ponds studied. In total, 29 (4.4%) of 655 sampled individuals were infected. There were no significant differences in *Bd* infection prevalence among years (Table 2; multiple comparisons: $p > 0.371$). Infection prevalence was 4.4% (26/588 ind.) in *Bufo bufo*, 9.1% (1/11) in *Rana arvalis* and 3.6% (2/56) in *R. temporaria* (Table S1). Based on the Urban Morphological Zones 2000 (www.eea.europa.eu), *Bd* was found in 18.8% of the urban sites and 42.3% of the rural sites (Table S1). *Bd*

infection prevalence was significantly lower in urban areas at the 2000 m scale ($p = 0.02$), and almost significantly so at the 500 and 5000 m scale ($p \leq 0.06$; Table 2). The effect of urban areas was significant at all scales when all environmental variables

Table 2. Results of generalized linear mixed models on the effects of urban area, presence of *Rana arvalis* and sampling year on *Bd* infection prevalence at different spatial scales. The presence of large water bodies is included in the models as an additional factor. **Bold** p-values indicate significant effects ($p < 0.05$); AIC: Akaike’s information criterion

Model	Variable	Estimate	SE	z-value	p
500 m					
AIC	Urban areas	-0.621	0.332	-1.869	0.062
235.4	<i>Rana arvalis</i>	1.546	0.585	2.642	0.008
	Sampling day	-0.226	0.270	-0.837	0.402
	Year 2016	-0.214	0.657	-0.326	0.745
	Year 2017	-0.319	0.725	-0.440	0.660
	Year 2018	-1.114	0.912	-1.221	0.222
2000 m					
AIC	Urban areas	-0.736	0.321	-2.294	0.022
233.3	<i>Rana arvalis</i>	1.716	0.582	2.949	0.003
	Sampling day	-0.270	0.272	-0.995	0.320
	Year 2016	-0.222	0.639	-0.348	0.728
	Year 2017	-0.046	0.646	-0.072	0.943
	Year 2018	-1.188	0.861	-1.380	0.167
5000 m					
AIC	Urban areas	-0.589	0.306	-1.927	0.054
235.6	<i>Rana arvalis</i>	1.411	0.566	2.494	0.013
	Sampling day	-0.221	0.262	-0.844	0.399
	Year 2016	-0.121	0.640	-0.188	0.851
	Year 2017	-0.053	0.643	-0.083	0.934
	Year 2018	-1.004	0.850	-1.182	0.237

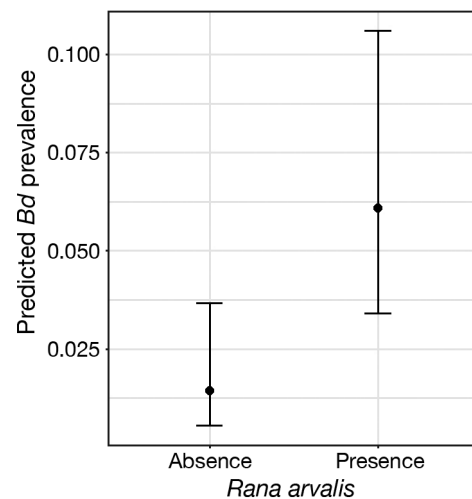


Fig. 2. Predicted association ($\pm 95\%$ CI), extracted from the best-fitting model (Model 2000 m; Table 2) between *Bd* infection prevalence and occurrence of *Rana arvalis*

were included (Table S2 in Supplement 2 at www.int-res.com/articles/suppl/d134p033_supp2.pdf, see below).

Bd infection prevalence was higher in the presence of *R. arvalis* at all scales ($p \leq 0.013$; Table 2). Individuals sympatric with *R. arvalis* had a 4- to 5-fold higher predictive risk of being infected (Fig. 2). Arable land and forest were negatively associated with *Bd* prevalence at the smallest and largest scales, respectively (Table S2). Large water bodies were positively associated with *Bd* prevalence at the 500 m scale. Including these variables did not change the significance of the presence of *R. arvalis*, but increased the significance of urban areas at the 500 and 5000 m scales (Table S2).

The presence of *R. arvalis* was not associated with urban areas or other environmental factors (mature forest, arable land and large water bodies) at any scale (Table S3 in Supplement 2), and there was no difference of the presence of *R. arvalis* in strict urban (50%) compared to rural sites (43%; $\chi^2 = 0.01$, $df = 1$, $p = 0.913$). Univariate GLMM models with pH (range: 6.9–9.1; estimate \pm SE: 0.394 ± 0.547 , $p = 0.471$) or mean May–June temperature (range: 12.8–18.4; estimate: 0.091 ± 0.485 , $p = 0.851$) were not associated with *Bd* prevalence. We found no differences in mean (\pm SE) pH between urban and rural sites (urban: 7.5 ± 0.2 ; rural: 7.8 ± 0.1 ; $t = -0.954$, $p = 0.409$) or in mean temperature (urban: $15.8 \pm 0.7^\circ\text{C}$; rural: $15.6 \pm 0.4^\circ\text{C}$; $t = 0.301$, $p = 0.793$). There was no difference in *Bd* prevalence between the sexes in *B. bufo* ($\chi^2 = 0.12$, $df = 1$, $p = 0.734$).

3.2. Body condition

BCI of *B. bufo* males was negatively associated with *Bd* (Table 3, Fig. 3) and the amount of urban landscapes at the 5000 m scale (Table S4). BCI in 2015 was significantly lower than in the other years (Fig. 4, Table 3). While the body mass of *B. bufo*

Table 3. Results of linear mixed models on the effects of *Bd*, sampling day and year on body condition of *Bufo bufo* males. **Bold** p-values indicate significant effects ($p < 0.05$)

Variable	Estimate	SE	t-value	p
<i>Bd</i>	-3.722	1.301	-2.860	0.004
Sampling day	0.531	0.558	0.951	0.342
Year 2016	12.324	1.654	7.450	<0.001
Year 2017	12.691	1.745	7.273	<0.001
Year 2018	15.405	2.089	7.373	<0.001

males was not significantly associated with *Bd* infection prevalence (Table S5), body length was positively associated with *Bd* infection prevalence (Table S6). Body mass and length were negatively associated with urban landscape across all spatial scales (Tables S7 & S8), except for body length at the largest urban scale.

Model averaging suggests that the positive effect of *R. arvalis* on *Bd* prevalence is stronger than the negative effect of urban environments, and that *Bd* prevalence has a stronger effect on BCI than urban environments (Fig. 5).

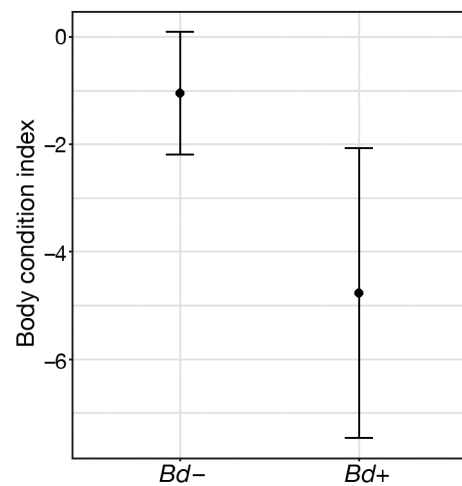


Fig. 3. Predicted association, extracted from the body condition model (Table 3) between body condition index and detection (Bd+) and non-detection (Bd-) of *Bd* in *Bufo bufo* ($\pm 95\%$ CI)

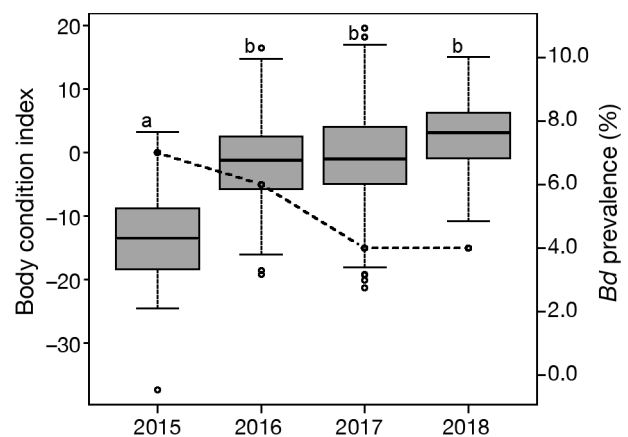


Fig. 4. Body condition index of *Bufo bufo* males in each study year. Differing letters denote groups that differ significantly in BCI ($\alpha = 0.05$ level). *Bd* prevalence for each year is denoted with a dashed line, but this did not differ significantly among years. Bar: median; box: interquartile range (IQR); whiskers: min./max. values $< 1.5 \times$ IQR below/above box; dots: outliers

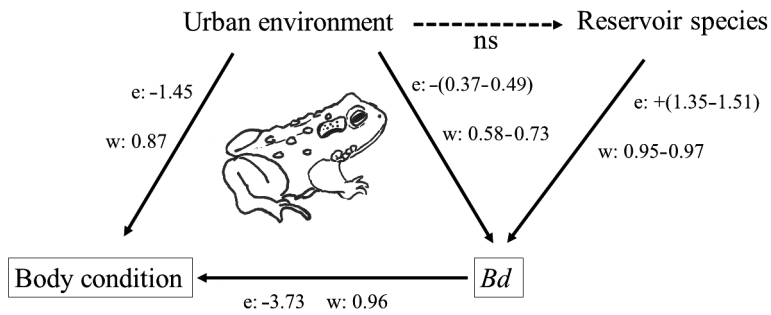


Fig. 5. Schematic description of factors associated with body condition and *Bd* prevalence from the main models; e: effects (estimate); w: (weight) relative importance of each variable from a model-averaging approach. Urban environment \rightarrow body condition indicate values from the 5000 m association in Model 15 (see Table 1), urban environment \rightarrow *Bd* indicate values (ranges) from urban associations in Model 4–6, reservoir species \rightarrow *Bd* indicate the range values of *Rana arvalis* associations in Model 4–6 and *Bd* \rightarrow body condition indicate values of the association in Model 12. ns: non-significant. Illustration by S. Kärvement

4. DISCUSSION

Our aims were to examine correlations between *Bd* prevalence and the extent of urban habitats at different spatial scales. We also wanted to determine if *Rana arvalis* may act as reservoir species for *Bd* and to evaluate the relationship between *Bd* infection and body size and condition of male *Bufo bufo*. Previous studies have found conflicting associations between *Bd* prevalence and surrounding land use as positive (St-Amour et al. 2008, Adams et al. 2010, Spitzen-van der Sluijs et al. 2014), negative (Saenz et al. 2015, Peralta-García et al. 2018) or non-significant (Duncan Pullen et al. 2010). In accordance with studies in the eastern USA (Saenz et al. 2015), we found a lower occurrence of *Bd* in urban sites compared to rural sites.

It should be noted that our results may have been affected by false negatives caused by a low sample size per pond and an overall low *Bd* infection prevalence (DiGiacomo & Koepsell 1986, Skerratt et al. 2010). Keeping these caveats in mind, our results seem to suggest that *Bd* prevalence was negatively affected by urban landscapes. This effect of urban landscapes was not influenced by the amount of forest, arable land or the presence of large water bodies. These environmental variables generally had negative or non-significant associations with *Bd* prevalence, with the exception of large water bodies, which were positively correlated with *Bd* prevalence only at the smallest scale (500 m). At this scale, the association with *Bd* and urban areas may be confounded by the proximity of large water bodies. Low *Bd* prevalence in urban habitats could be caused by

urban pollution due to chemicals and heavy metals that may clear fungal infections, or because *Bd* is negatively affected by the higher temperatures and lower amphibian diversity often found in urban environments (Saenz et al. 2015). Pollutants in urban waters can affect pH (Ford et al. 1992), which in turn may affect *Bd* growth (Piotrowski et al. 2004). However, in the present study, neither temperature nor pH were associated with either *Bd* infection prevalence or urban landscapes. It seems likely that temperatures in our system rarely reach the level at which they would limit *Bd* occurrence. The highest temperature measured in this study was 18.4°C, whereas a temperature between 17 and 25°C is considered to be optimal for *Bd* (Piotrowski et al. 2004). Lower *Bd* infection prevalence in urban areas may also be caused by lower dispersal capacity of amphibians in areas with many dispersal barriers (roads, buildings) and fewer forests (Homan et al. 2004), which may decrease *Bd* transmission. In this study, we conclude that urban areas were negatively associated with *Bd*, although the reasons for this remain unclear.

Based on occurrences of *R. arvalis* from a national database, we found a higher prevalence of *Bd* when *R. arvalis* was present, and this effect was much stronger (and had a higher model weight) than the urbanization effect at all spatial scales (Fig. 5). Neither urban areas, forests, arable land, nor the amount of large water bodies in the landscape had significant associations with the occurrence of *R. arvalis*. Interestingly, recent Swedish studies found that *R. arvalis* has a much higher risk of *Bd* infection (prevalence was 12.9–13.9% in southern Sweden and 19% within the present study area) than *Bufo bufo* (1.5–3.5%; Kärvement et al. 2018, S. Meurling et al. unpubl. data). Relatively high prevalence of *Bd* in *R. arvalis* has also been reported in Poland (28.6%; Czezugaga et al. 2014) and China (10.5%; Zhu et al. 2016), but not in Germany (2.2%; Ohst et al. 2013) or the Netherlands (0%; Spitzen-van der Sluijs et al. 2014). Infection experiments showed that while 60% of inoculated *B. bufo* individuals were killed by a Swedish *Bd* strain, the survival rate of infected *R. arvalis* individuals was close to 100% (Bengtsson 2018, S. Meurling et al. unpubl. data, see also Garner et al. 2009). Our findings, combined with the experimental studies, suggest that *R. arvalis* has the potential to act as a reservoir host and infect *B. bufo* with potential negative

effects on population size. In line with this hypothesis, Scheele et al. (2017) found that populations of the northern corroboree frog *Pseudophryne pengilleyi*—with low *Bd* prevalence—were declining when the eastern froglet *Crinia signifera*—a reservoir species with high prevalence—was present. However, neither Scheele et al. (2017) nor the present study can show any fundamental evidence that these transmission events have actually occurred (Garner 2018).

We found that adult *B. bufo* males with *Bd* infections had lower BCI than non-infected males. This is in accordance with the results of experimental studies that have shown that *Bd*-positive anurans have a reduced BCI (Retallick & Miera 2007, Ramsey et al. 2010), although the majority of field studies do not report such effects (e.g. Deguise & Richardson 2009, Richards-Zawacki 2010, Becker et al. 2012, Spitzen-van der Sluijs et al. 2017). Previous studies have linked low BCI with decreased survival and fitness in wild *B. bufo* (Reading 2007). Based on these results, as well as those from laboratory infection studies (Garner et al. 2009, Bengtsson 2018, S. Meurling et al. unpubl. obs.), it seems possible that *Bd* may negatively affect *B. bufo* populations. Consequently, *Bd* could be one of the factors behind population declines of *B. bufo* found across Europe (Carrier & Beebee 2003, Bosch & Martínez-Solano 2006, Petrovan & Schmidt 2016). An alternative explanation for the negative relationship between *Bd* and BCI is that individuals with low BCI are more likely to be infected, whereas individuals with high BCI may be able to fight the infection. Long-term population studies linking BCI and infection patterns to survival would provide important insights into the potential effects of *Bd* on survival and population dynamics of *B. bufo*.

We also found that body length and body mass of *B. bufo* were negatively associated with urban areas at almost all spatial scales, and that BCI was lower at the 5000 m scale. These relationships held even when we removed *Bd*-infected individuals from the data (results not shown). These correlations between urban areas and body mass are in accordance with many other studies on amphibians that have demonstrated negative effects of anthropogenic environments on amphibian body mass (Reading 1990, Egea-Serrano et al. 2012, but see Iglesias-Carrasco et al. 2017), including a study on *B. bufo* (Bókonyi et al. 2018). However, the link between urban areas and low BCI was only significant at the 5000 m scale, which may indicate that individuals living in core urban habitats are particularly affected by urbanization. It is worth noting that the negative effect of *Bd*

on BCI was stronger than the urban effect (Fig. 5), emphasizing the potential negative fitness effects of *Bd* on *B. bufo*.

Bd-positive *B. bufo* males had greater body length than *Bd*-negative males. Previous studies have usually found the opposite pattern (e.g. Beard & O'Neill 2005, Burrowes et al. 2007, Kriger et al. 2007), and we think that this result may be a confounding effect brought about by the lower *Bd* infection rates in urban environments, where individuals are also smaller. We also found that body length and to some degree body mass, but not BCI, were associated with sampling date. This is probably due to large toads arriving at breeding sites earlier than smaller toads (Gatz 1981). An early pond arrival of larger males may also be the reason why *Bd* prevalence correlates with body length. If larger males stay longer in the water, this may increase the risk of being exposed to *Bd*.

5. CONCLUSIONS

Our results suggest that *Bd* is less common in urban than rural landscapes in Sweden, possibly due to harsher environmental conditions for the fungus and thereby a reduced risk of transmission. The presence of *Rana arvalis*, a potential reservoir species, may considerably increase *Bd* infection risk for *Bufo bufo*. We also found that infected *B. bufo* males had a lower BCI than non-infected individuals, suggesting that *Bd* reduces individual fitness in *B. bufo*.

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