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ORIGINAL PAPER

Bioacoustics survey revealed the occurrence of alien green frogs across an urban gradient

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Abstract The introduction and spread of alien amphibians pose significant threats to native biodiversity, particularly within vulnerable ecosystems. In Southern Europe, alien green frogs *Pelophylax ridibundus* represent a major conservation concern due to their ecological plasticity, competitive behaviour, and potential for genetic pollution. In this study, we employed bioacoustic surveys to assess the distribution and habitat associations of *P. ridibundus* across an urban gradient in the metropolitan area of Florence, Central Italy, where the species has been previously genetically identified. A total of 47 wetland sites were surveyed monthly during the breeding seasons of 2021–2022. We recorded spontaneous frog calls during nighttime surveys to model species occupancy using single-season occupancy models. Our results revealed that *P. ridibundus* occupied approximately 47% of surveyed wetlands. Occupancy was significantly influenced by the combination of aquatic vegetation cover and proximity to human-modified environments, indicating that the species may benefit from both habitat complexity and anthropogenic disturbance. Detection probability was relatively high ($p = 0.66$), and we also estimated that four nocturnal survey visits were sufficient to confidently confirm the species presence at a given site. These findings showed the high adaptability of *P. ridibundus* to urban environments, and suggest that suburban and urban wetlands with dense aquatic vegetation are particularly susceptible to invasion. Given the potential impacts of *P. ridibundus* on native amphibian communities, including competition and hybridization, early detection and regular monitoring are essential. Bioacoustic monitoring emerges as a cost-effective and reliable tool to monitor invasive anurans in human-dominated landscapes, supporting well-addressed conservation efforts to preserve urban biodiversity.

Keywords Amphibia · Invasive alien species · Mediterranean countries · *Pelophylax ridibundus* · Urban ecosystems

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Introduction

Alien green frogs belonging to the genus *Pelophylax* represent one of the most overlooked conservation issues in Southern Europe, despite representing a paramount example of genetic pollution due to human-mediated species introduction (Ficetola and Scali 2010; Denoël and Dufresnes 2025). Telling apart different species of green frogs can be challenging, given their morphological similarity and hybridogenetic reproductive system, most often requiring genetic analyses (Dufresnes et al. 2023). This made it complicated to detect non-native green frogs, which may be spreading unnoticed (Ficetola and Scali 2010; Denoël and Dufresnes 2025).

The first known introduction of alien frogs of the genus *Pelophylax* in Italy occurred in 1941, as an escape from frog farming in Liguria (Western Italy: Lanza 1962). Since then, they expanded their range throughout northern Italy, central regions, and subsequently also appeared in Aspromonte (Calabria, Southern Italy), as well as in Sicily and Sardinia (Andreone and Sindaco 1999; Bisconti et al. 2019; Di Nicola et al. 2021; Bellati et al. 2023). Until the early 1990 s, these introduced frogs were generally referred to as the "*Pelophylax ridibundus* group," but accurately identifying the species is challenging due to both taxonomic splitting and the complex mating system of these frogs, which limits the effectiveness of species delimitation methods (Tarkhnishvili et al. 2024). Recent genetic assessments have shown a quite complex picture of alien green frogs occurring in Italy, with particular reference to central regions, where *P. ridibundus, P. shqipericus, P. kurtmuelleri* (although this taxon is no longer considered as valid following Speybroeck et al. 2020) and *P. bedriagae* are known to occur as alien species (Domeneghetti et al. 2013; Bruni et al. 2020; Dufresnes et al. 2023). Alien green frogs are a threat to native amphibians by outcompeting them for food resources, by active predation, and through interbreeding which can pollute the genetics of native species (Ficetola and Scali 2010; Di Nicola and Ferrer 2022; Kolenda et al. 2024). Therefore, assessing their distribution is pivotal to protect native species, particularly where wetland ecosystems are mostly threatened by land use changes (see Ancillotto et al. 2024).

Genetic analyses require a large amount of funds and time, despite allowing an unequivocal species identification. Besides, acoustic communication such as advertisement calls produced by males during the breeding season, may allow identification of the different species complexes (Lukanov et al. 2014). Therefore, bioacoustic surveys may be used to successfully distinguish between calls of native (*P. lessonae* and the hybridogenetic hybrid *P. esculentus*) and alien green frogs of the *P. ridibundus* species group (Sinsch and Schneider 1996; Bisconti et al. 2019), although they cannot distinguish between different alien lineages of the same species complex (Di Nicola et al. 2021).

In the metropolitan area of Florence, previous genetic analyses identified the alien green frogs as belonging to the *P. ridibundus* clade, specifically to *P. r. ridibundus* (Bruni et al. 2020; Di Nicola et al. 2021). This alien taxon is well-adapted also to urban wetlands and may thrive in anthropogenic contexts (Korzh and Zahovalko 2015; Donmez and Şisman 2021), whereas the hybridogenetic hybrid *P. esculentus* complex seems to be less tolerant of anthropogenic environmental modifications (Konowalik et al. 2020). The aim of this work was to update and clarify the current distribution of alien green frog *P. ridibundus s.l.* in Florence and its suburban surroundings, by assessing which habitat drivers determine its probability of occupancy, and the minimum number of field days required to confidently declare its absence.

Materials and methods

We selected a total of 47 ponds, marshes, cattle troughs, lakes or river traits between the municipalities of Fucecchio and Pontassieve (Firenze province) along the Arno River (Fig. 1; Table S1 in Supplementary Material 1). All these areas were visited once per month between March and October 2021–2022, at night (22:00–24:00), i.e., when the probability to hear green frog calls was the highest (Di Nicola et al. 2021; Takahashi and Takeuchi 2021). No site was visited for the full project duration, i.e., we visited half of the sites in the first year and the other half in the second year.

A total of 47 listening points were placed across 29 wet areas, focusing on slow-flowing habitats such as ponds, marshes, and river backwaters, as these environments are preferentially used by green frogs (Di Nicola et al. 2021). The surveyed wet areas were

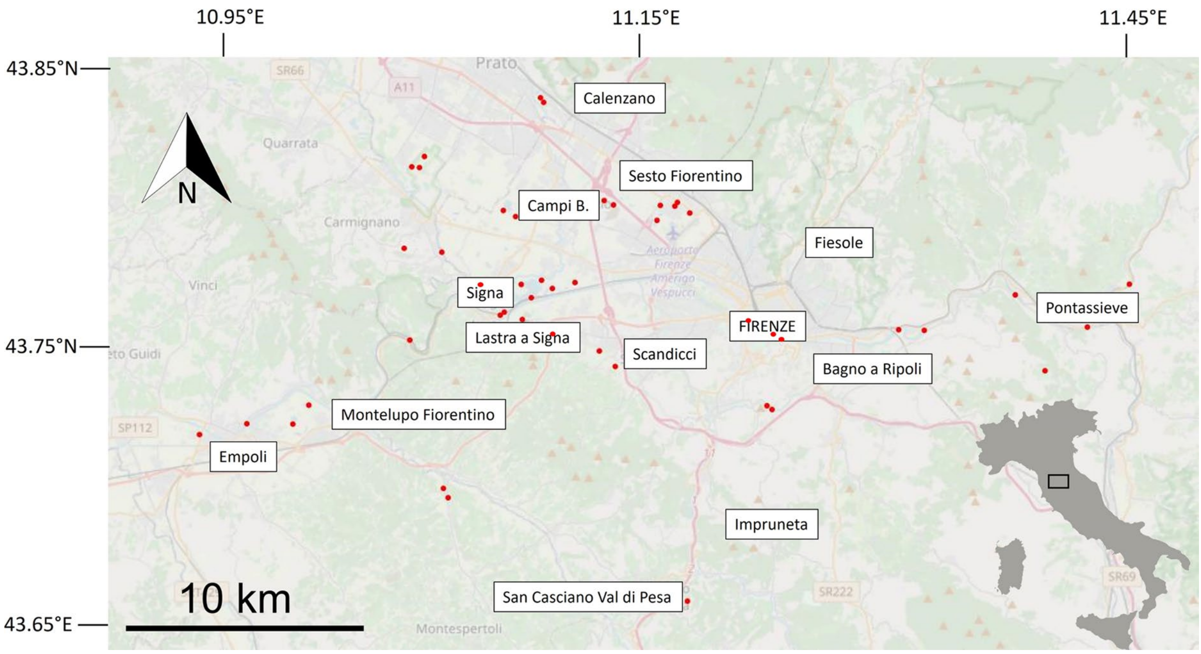

Fig. 1 Location of study site, with red dots referring to listening points. Main municipalities are highlighted

spaced approximately 500 m apart. This inter-area distance was chosen based on human ability to detect frog calls from adjacent wet areas and to avoid non-independent sampling, i.e. the same call being heard from more than one wet area. The wet areas varied in size, and the number of listening points in each area increased with their size, ranging from 1 to 5 (cfr. Mortelliti and Boitani 2008). When a wet area was smaller than 0.25 hectares, only one listening point was used. At each listening point, two observers, always in the same time slot, waited in complete silence for 15 min at a distance of 2–10 m from the water body, to record by ear on a notebook the spontaneous calls of green frogs of the genus *Pelophylax*, defining in situ the presence of one or more species belonging to this genus (i.e., native or alien taxon). No playback of either the native or alien species was used to avoid non-detection biases related to the potential competition between the two species (Bissattini et al. 2020). Playback might inhibit the calls of one or the other species (i.e., of the lower competitor).

A binary value of detection history (1, detection; 0, non-detection) was recorded for all the visits for each wetland (MacKenzie et al. 2006). Besides green frogs, anurans recorded within the study areas were: the Italian tree frog *Hyla intermedia,* the agile frog *Rana dalmatina,* the Apennine frog *Rana italica,* the Italian green toad *Bufotes viridis balearicus*, the common toad *Bufo bufo* and the American bullfrog *Lithobates catesbeianus* (Bruni et al. 2016; Di Nicola et al. 2021). In particular, *Rana dalmatina* and *Rana italica* mostly occurs in natural streams and ponds around urban areas, whereas the green toad and the tree frog mostly occurred in the northwestern area of the city of Florence, and the bullfrog was limited to the western part of the city of Florence (Vannini et al. 2015; Di Nicola et al. 2021). Two stochastic factors influence detectability: the probability of species presence at a site (ψ) and the probability of detection given the sampling effort (p). The probability of detection represents the likelihood that the event (detection) occurs during a visit. It is based on the assumption that these events are independent and that the population remains closed between visits (i.e., no migration of individuals out of or into the study area occurs). Occupancy analyses were run using the software R 4.5.0 (R Core Team 2024) and the package *unmarked* (Fiske and Chandler 2011). We selected single-season models, as our surveys were conducted during a single spring–summer period. We included the effects of the following covariates: [Cov1], location of the wet area; [Cov2], percentage of human settlement cover in a 1 × 1 km radius around the listening point (cf. Dondina et al. 2025); [Cov3], wet area size; [Cov4], percentage of wetland vegetation estimated by eye; and [Cov5], number of other coexisting anuran species. The optimal model was selected based on the minimum AIC (Akaike Information Criterion), a value calculated as $-2\log \text{Likelihood} + 2k$, where k is the number of parameters estimated by the model. Information theory, on which AIC is based, quantifies the information contained in the data and does not perform significance tests. This approach favours the most parsimonious model that best represents the data (MacKenzie et al. 2006), thereby minimizing the potential for error. ΔAIC is the difference between the AIC of a given model and that of the model with the lowest AIC (the best model). Models with an increasing number of variables with respect to the best model and/or with $\Delta\text{AIC} > 2$ are considered not to adequately represent the dataset (Burnham and Anderson 2002) and were therefore not considered in our results. The higher a model weight, the relatively better it is. The minimum number of repeated visits required to infer species absence was estimated using the formula $N = \ln(\text{a level})/\ln(1 - \text{p})$, where a represents the probability of a Type I error and was set at 0.05, and p represents the probability of detecting *P. ridibundus*.

Results

Pelophylax ridibundus was detected at 22 of the 47 surveyed listening points (47%). The naïve occupancy estimate (0.47) was only slightly lower—by about 1%—than the modeled site occupancy estimate ($\psi = 0.48$; Table 1). This suggests that approximately 1% of the sites may represent false absences, where *P. ridibundus* was present but not detected during the surveys. The best-performing model (Table 1) indicated that the probability of *P. ridibundus* presence was driven by two covariates: the percentage of aquatic vegetation and the percentage of human settlements. This model also had a high Akaike weight ($w_i = 81\%$). Although the second-best model had a $\Delta\text{AICc} < 2$, it was excluded due to its similarity to the top model, differing only by the inclusion of an additional covariate, which reduced its parsimony. Both the percentage of human settlement ($\beta \pm \text{SE} = -0.29 \pm 0.08$) and the percentage of aquatic vegetation had a positive effect ($\beta \pm \text{SE} = 0.18 \pm 0.07$) on *P. ridibundus* occurrence. These results indicate that wetlands closer to anthropized environments were more likely to be occupied by *P. ridibundus*, and that higher aquatic vegetation cover increased the likelihood of site occupancy by this alien green frog. The detection probability (p) was high in both selected models ($p = 0.66$). Based on our results, four repeated survey visits are sufficient to reliably detect the presence of *P. ridibundus* in a wet area.

Table 1 Summary of the estimated parameters for the occupancy models

Model	AIC	− 2LL	ΔAIC	w_i	Ψ (SE)	*p* (SE)	*N*
ψ(Cov2 + Cov4)p(.)	*386.78*	*365.38*	*0.00*	*0.81*	*0.48 (0.05)*	*0.66 (0.02)*	*4*
ψ(Cov2 + Cov4 + Cov1)p(Cov2)	*387.40*	*376.78*	*0.61*	*0.74*	*0.48 (0.05)*	*0.66 (0.03)*	*4*
ψ(Cov2 + Cov4)*p*(.)	389.92	381.92	3.14	0.63	0.42 (0.05)	0.52 (0.02)	4
ψ(Cov2 + Cov4 + Cov3)*p*(.)	391.82	381.82	5.03	0.54	0.42 (0.05)	0.52 (0.01)	5
ψ(Cov2 + Cov1)*p*(.)	403.51	395.50	16.73	0.26	0.41 (0.05)	0.48 (0.03)	5
ψ(Cov2 + Cov1 + Cov3)*p*(.)	405.48	395.48	18.69	0.23	0.41 (0.05)	0.55 (0.02)	5
ψ(Cov2)p(.)	411.17	405.16	24.38	0.12	0.42 (0.04)	0.46 (0.03)	5
ψ(Cov2 + Cov3)*p*(.)	412.94	404.94	26.16	1.0^{-6}	0.41 (0.05)	0.52 (0.02)	5
ψ(Cov1)p(.)	421.74	415.72	34.94	1.3^{-8}	0.42 (0.05)	0.52 (0.03)	5
ψ(Cov4 + Cov1)*p*(.)	422.73	419.26	35.94	7.7^{-9}	0.42 (0.07)	0.55 (0.03)	6
ψ(Cov4 + Cov1 + Cov3)*p*(.)	423.30	413.30	36.52	5.8^{-9}	0.36 (0.06)	0.55 (0.03)	5
ψ(Cov1 + Cov3)*p*(.)	423.71	415.72	36.93	4.7^{-9}	0.39 (0.05)	0.58 (0.04)	4
ψ(Cov4)*p*(.)	453.73	447.72	66.94	1.4^{-15}	0.35 (0.03)	0.51 (0.04)	6
ψ(.)*p*(.)	462.14	458.14	75.36	2.1^{-17}	0.37 (0.04)	0.50 (0.04)	6
ψ(Cov3)*p*(.)	463.34	456.34	76.56	1.2^{-17}	0.30 (0.06)	0.49 (0.07)	5

Models are ranked according to their AIC (Akaike Information Criterion) values: ψ, proportion of sites occupied; SE, standard error; *p*, detection probability; w_i, model weight; − 2LL, − 2 log-likelihood; *N*, estimated number of visits required to infer absence. Models with $\Delta\text{AIC} > 2$ are highlighted in italics. We only kept the best one, with the lowest number of variables

Discussion

Our study sheds new light on the distribution patterns of the alien green frog *P. ridibundus* across an urban gradient in the metropolitan area of Florence. Using a bioacoustic approach, we updated occupancy data efficiently, minimising costs compared to genetic and environmental DNA analyses (Cagnacci et al. 2012). Our findings suggest that *P. ridibundus* shows a remarkable adaptability to anthropogenic landscapes, reinforcing concerns regarding its invasive potential and impacts on native amphibian communities (Korzh and Zahovalko 2015; Donmez and Şisman 2021; Denoël and Dufresnes 2025).

One of the key outcomes of our survey was the detection of *P. ridibundus* in nearly half of the surveyed wetlands, with a modeled occupancy rate of 47% (cfr. Bruni et al. 2020). Interestingly, we observed that both the proximity to human settlements and the percentage of aquatic vegetation were the main drivers influencing site occupancy. This highlights a dual ecological strategy: on one side, *P. ridibundus* thrives near anthropized environments likely due to its tolerance of pollution and habitat modification; on the other, the availability of aquatic vegetation supports its breeding and foraging activities. Among the most widespread aquatic plants in the urban area of Florence, the invasive alligator weed *Alternanthera philoxeroides* (Mart.) Griseb. is particularly abundant (Brunel et al. 2010) and appears to serve as an important habitat facilitating the high occurrence of the invasive green frog observed in urban aquatic environments. Although we currently lack direct evidence regarding the drivers of *P. ridibundus* movements across the urban landscape, this represents an important suggestion for future research, and Passive Acoustic Monitoring could provide a valuable tool to investigate potential human-mediated and natural dispersal pathways of invasive green frogs.

Such findings align with previous research indicating that *P. ridibundus* can exploit a wide range of habitats, including those degraded or heavily influenced by human activity (Korzh and Zahovalko 2015; Donmez and Şisman 2021). The positive relationship between aquatic vegetation and frog presence emphasizes the importance of habitat structure in supporting alien frog populations. Aquatic vegetation likely provides shelter, breeding sites, and abundant prey resources for both alien and native anurans (Burrow and Maerz 2022; Denoël and Dufresnes 2025). Conversely, the preference for wet areas close to human settlements may reflect reduced predation pressure and the rapid exploitation of novel ecological niches created by urban water bodies (Vimercati et al. 2017; Callaghan et al. 2021; Denoël and Dufresnes 2025). Moreover, alien species often exhibit higher concentrations near their points of introduction, typically human settlements, particularly during the early stages of their invasion process. Despite the relatively high detection probability ($p = 0.66$), we recommend at least four survey visits to reliably infer absence of introduced frogs. This is a critical point for future monitoring programs, as it underscores the need for repeated sampling to limit false absences, mostly when dealing with cryptic or nocturnal species whose calling activity may fluctuate based on environmental conditions.

Our results raise significant conservation concerns. The successful colonization of urban wetlands by *P. ridibundus* poses a threat to native anuran species, not only through direct competition for resources but also via genetic pollution (Ficetola and Scali 2010; Bruni et al. 2020; Di Nicola and Ferrer 2022). In a context where urban wetlands are already highly vulnerable ecosystems (see Ancillotto et al. 2024), the establishment of invasive amphibians could exacerbate biodiversity loss.

Given that *P. ridibundus* may act as a driver of native species decline through predation, hybridization, and habitat alteration, proactive management strategies should be considered. These could include regular bioacoustic monitoring, habitat management to help native species (e.g., maintaining natural water areas with native aquatic vegetation), and public awareness campaigns to prevent further introductions.

Overall, our study confirms that bioacoustic surveys may represent a powerful tool for tracking the spread of invasive amphibians, particularly in fragmented and urbanized landscapes. A limitation of this study is that hybridogenetic hybrids between native and alien green frogs may produce intermediate calls between the alien and native species, potentially leading to an underestimation of the alien genotype distribution and furtherly confirming the importance of genetic analyses for accurate detection. Further research should integrate acoustic monitoring with genetic analyses to refine species identification further and assess the potential for hybridization events in invaded areas (Denoël and Dufresnes 2025). Moreover, investigating the ecological interactions between *P. ridibundus* and native amphibians across different habitat types may be pivotal to design effective conservation actions. Future studies should incorporate Passive Acoustic Monitoring (PAM) techniques to enhance detection probabilities of invasive frog populations, particularly in cases where traditional survey methods may underestimate presence due to low detectability or cryptic behaviour.

Author contributions EM and LA conceived the study design. EM and AV collected field data. EM, LA and OD analysed the data. All authors wrote the manuscript.

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