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Habitat selection in the fossorial toad *Pelobates fuscus insubricus* (Amphibia: Pelobatidae): does the soil affect species occurrence?

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Abstract. For their rapid and alarming decline, the Italian populations of the Italian spadefoot toad (*Pelobates fuscus insubricus*) are of high conservation importance. In this study we examined habitat use by the spadefoot toads in north-west Italy, where one of the largest remaining populations lives. We used compositional analysis and logistic regression models to elaborate land-use and soil composition in order to determine the habitat preferences of the spadefoot toads. We found that the two main variables predicting the occurrence of spadefoot toads in the study area were the percentages of sandy-loam soil texture and the presence of Entisols. Our results showed that spadefoot toads preferred soils that keep soft and malleable structure: Entisols with sand texture, where sand represents the major component, and mature soils, with a high degree of pedogenesis and a relatively high natural fertility and humidity. Conversely, *Pelobates fuscus* avoids Inceptisols, probably too hard to be dug by the species.
This study demonstrates that, at least in the Italian range, the choice of areas in which to reintroduce *P. fuscus*, or where re-create favourable habitats for it, must take into account the soil types, which, in intensely cultivated areas, seems to be decisive for the possibility of survival of the species in the medium and long time.

Keywords. Spadefoot toad, terrestrial habitat, soil, compositional analysis, conservation.

INTRODUCTION

A central goal of conservation is to identify the main factors affecting the presence and abundance of species at large scales over long periods of time. Only when favourable and unfavourable factors are known, it is possible to take appropriate conservation measures, in order to reduce the negative impacts and improve the environmental conditions to the advantage of the species.

The spadefoot toad (*Pelobates fuscus*) is a fossorial amphibian widely distributed in Europe. In last century populations declined dramatically in the northern and western parts of its range, where several local extinctions were documented (France: Lescure, 1984; Parent, 1985; Dubois, 1998; Belgium: Rappè, 1982; Perczy, 1994; Netherlands: Pelt and Van Bree, 1965; Denmark: Fog et al., 1997; Gislen and Kauri, 1959; Sweden: Berglund, 1998).

In Italy, where the species was probably widespread in the whole Po Plain in the XIX century, it currently occurs in a few isolated populations (Andreone, 2006). Despite new breeding sites have been recently discovered (Mazzotti et al., 2002; Andreone et al., 2004; Mercurio and Li Vigni, 2007), the total number of known breeding sites has dramatically reduced since the end of the XX century (Andreone et al., 2004), and the species is considered one of the most threatened amphibians in Italy (Rondinini et al., 2013). For example, in Piedmont, out of 21 reproductive sites known in 1985, only 7 still exist despite several conservation projects (D. Seglie, pers. obs.).

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The decline of spadefoot toad in Italy is due to several concurrent causes, including the drastic reduction and alteration of suitable habitats, the increase of intensive agriculture, the change of agricultural practices in the ricefields, the worsening of water-quality, and the progressive fragmentation of the residual natural habitats. Additional threats are the introduction of fishes and allochthonous crayfishes (e.g., *Procambarus clarkii*), and possibly the introduction of allochthonous amphibians such as *Lithobathes catesbeianus* (Andreone, 2006; Andreone et al., 2007), which may convey amphibian pathogens like *Batrachochytrium dendrobatidis*. However, this fungus has not yet been found on *Pelobates fuscus* (Federici et al., 2008) in the areas of the Piedmont where it affects other amphibians (Adams et al., 2008).

The conservation of the Italian populations of the spadefoot toad is of particular interest, since they are considered as belonging to an endemic subspecies, *P. f. insubricus*, which is listed in the Annexes II and IV of the Habitat Directive 92/43/CEE as priority species. From the taxonomic point of view, this subspecies is separated from the other European populations on the base of genetic distinctiveness and some morphological and acoustic features (Héron-Royer, 1888; Andreone and Piazza, 1990; Andreone et al., 1993). However, while several authors recognize the Italian populations as a distinct subspecies (Nöllert and Nöllert, 1992; Andreone et al. 1993), its validity is still debated (Andreone et al., 2007; Crottini et al., 2007). The genetic study of Crottini et al. (2007) confirmed that Po Plain populations keep a very high genetic variability within the "western" lineage, and a high number of haplotypes not found elsewhere in Europe. This high genetic variability indicates that the Po Basin was one of the most important glacial refugia for the species during the Pleistocene.

As far as its ecology, in northern Europe the occurrence of *P. fuscus* is determined by the interaction between pond features and hydroperiod with the occurrence of large predators such as fishes and crayfishes. Breeding sites are large permanent ponds with high spring temperatures, high concentrations of phosphorus and oxygen, and a shoreline with a high proportion of steep banks (Strijbosch, 1979; Nyström et al., 2002). By contrast, composition of the terrestrial habitat close to the ponds and traffic has no or low effect on *P. fuscus* occurrence (Nyström et al., 2002).

In Italy *P. fuscus* occurs in lowlands up to 400 m a.s.l. (Andreone, 2006) and does not appear to be very selective for habitats with the exception of areas with substrates soft enough to allow burrowing, such as sandy soils (Lanza, 1983; Andreone et al., 1993; Gentilli and Scali, 2001). Indeed, it has been found in woods, meadows, poplar plantations, cereal crops and ricefields (Lanza, 1983; Andreone et al., 1993; Fortina and Andreone, 1999; Mazzotti et al., 2002; Scali and Gentilli, 2003). During the breeding season the species has been reported to use different types of wetlands, from ponds and marshes to ditches, almost all temporary sites, usually in open areas (Lanza, 1983; Scali and Gentilli, 2003; Andreone et al., 2007). However, no quantitative analyses on habitat preference have been carried out so far in Italy, probably because of species rarity, the low consistence of residual populations and the difficulty to locate the spadefoot toads in the field (Andreone et al., 2004). Similarly, few studies have been carried out on habitat use outside the breeding season (Eggert, 2002; Bosman and Van Den Munckhof, 2006).

In this paper, we analysed the habitat preference of spadefoot toads in the last population settled south the city of Turin; the main objective was to detect some of the habitat features surrounding breeding areas, with particular emphasis on soil, which could explain the presence/absence of the species in order to supply detailed information useful to develop management and conservation programs.

MATERIAL AND METHODS

Study area and collecting data

The study area (Fig. 1) includes a portion of the alluvial plain south the city of Turin and is delimited southwards by the towns of Carmagnola (44°50'N, 7°53'E), Poirino (44°55'N, 7°50'E) and Santena (44°56'N, 7°46'E). This area hosts one of the last populations of spadefoot toads of Piedmont, which breeds in several small and medium-large ponds that are included within the Site of Community Importance (hereafter SCI) "Stagni di Poirino-Favari" (IT111035), extended over an area of about 1,840 hectares (Sindaco et al., 2009).

Since the discovery of the species (by G. Boano in 1989), the area was thoroughly monitored by many researchers (see Acknowledgments), mostly during night transects conducted through low-speed driving on paved and gravel roads. The data were collected between 1988 and 1991, during March-May (corresponding to the activity period of the species in this area), and account for 70 individuals. The research effort has been constant over the entire area, as all the roads of the study area were covered with the same intensity. The position of each toad was accurately recorded on the topographic maps of the Italian Military Geographical Institute (I.G.M.) at the scale 1:25.000, and then mapped using a GIS. The localities of *P. fuscus* in the Turin area (Fig. 2) are derived from the available literature and the database of the regional herpetological atlas (Andreone and Sindaco, 1999).

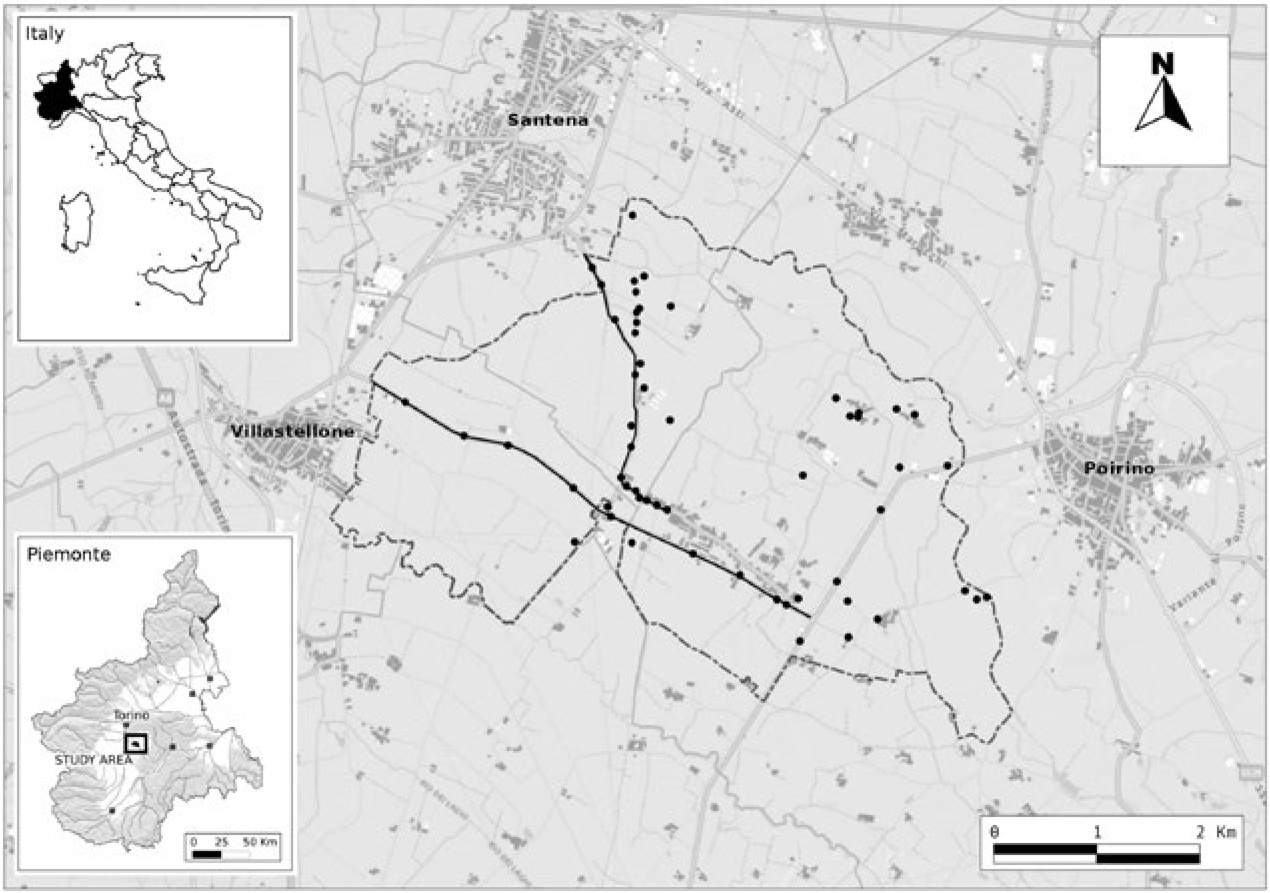

Fig. 1. Findings of *Pelobates fuscus insubricus* in the SCI IT1110035 "Stagni di Poirino - Favari" between 1988 and 1991.

Habitat variables

We investigated habitat selection by spadefoot toads by assessing the evolution and texture of soil and the land use in the 70 spots were *P. fuscus* was found (Fig. 1), and in 150 spots randomly selected within a 50 m buffer along the transects.

The habitat variables accounted for soil type, soil texture and landcover, and were measured using digital maps of the Regione Piemonte (surveyed at the scale scale 1 : 10.000, published at the scale 1:50.000) (http://www.regione.piemonte.it/agri/area_tecnico_scientifica/suoli/suoli1_50/carta_suoli.htm), reporting the degree of pedogenesis and soil texture according to the USDA classifications (http://soils.usda.gov/technical/classification/taxonomy/).

Our study area involved six main soil types, i.e., Alfisols with or without hydromorphisms (classes A1 and A3 respectively), Inceptisols with or without hydromorphisms (classes B1 and B2 respectively), and Entisols with or without hydromorphisms (classes C1 and C2 respectively). Since classes A1, B2, and C2 covered less than 5% of the study area, they were grouped in the same class. Therefore, in our analyses we used only four classes of soil evolution: Alfisols (A3), Inceptisols (B1), Entisols (C1), and other soils (A1, B2, C2 combined). Only five out of the twelve possible soil textures are present in the study area, with different percentages of sand and silt, from sandy soils (sand and loamy-sand classes, with more than 90% and 70% of sand respectively, in weight), intermediate soils (sandy-loam and loam classes, with 40-70% of sand) and silty soils (silt-loam class, with less than 40% of sand). In all these five classes clay occurs in no more than 20-25%. Although the soil data go back over about 15 years ago, these data are still valid, because in agricultural landscapes the pedogenesis will stop in the topsoil, or at least slow down significantly, while in depth it continues. However, the soil composition does not change in a few decades (I. Boni, pers. comm.).

Finally, habitat types were grouped in five main categories: urban areas (urban areas, parks and gardens within towns), woods (oak-hornbeam and locust woods), cultivated fields (maize, sunflower, and alfalfa), and poplar plantations. Remnant habitat types were combined in a single group, which cover less than 5% of the study area.

To compare the incidence of the aforementioned variables in places where *P. fuscus* individuals were detected, we considered 220 plots with a 50-m radius, centred on the point of observation of *P. fuscus* (70 plots), and on 150 random points (see above). Moreover, we calculated the distance (in meters) from each plot to the border of the nearest urbanized area and to the nearest pond.

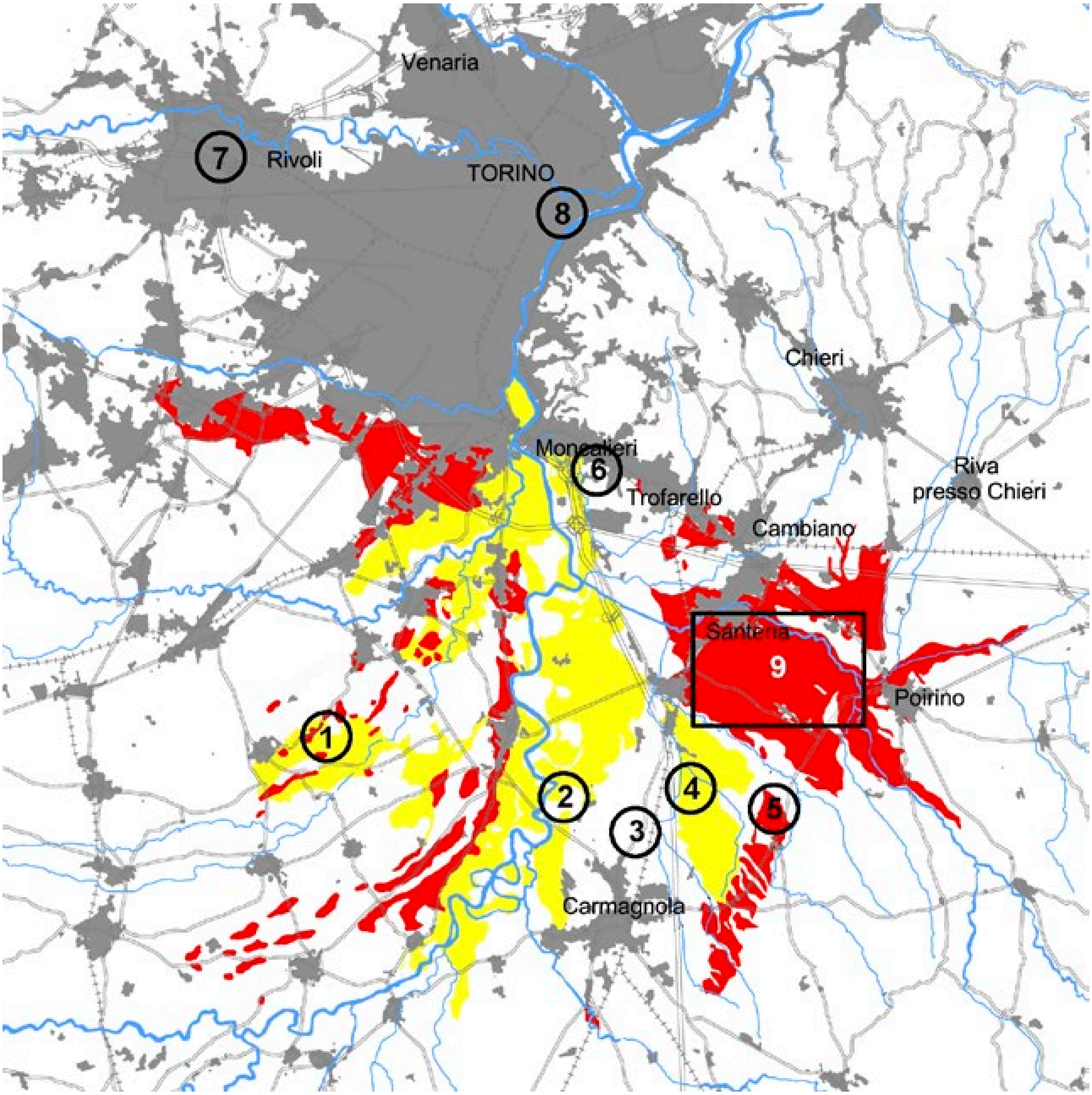

Fig. 2. Historical and current distribution of *Pelobates fuscus insubricus* in the plain south of Turin. Grey (intermediate gray): urbanized areas; red (dark gray): high suitable soils; yellow (light gray): suitable soils; white: unsuitable soils. Populations: 1) Piobesi T.se env., 2) right bank of Po river between Carignano and Carmagnola; 3) Carmagnola – San Michele; 4) Carmagnola – Cervirola and Tetti Grandi; 5) Carmagnola – Casanova and env.; 6) Moncalieri – Testona, 7) Rivoli, 8) Torino – Vanchiglia, 9) the study area. The species has been confirmed in recent times only in loc. 2 and 9. Colour legend between brackets refers to the printed version of the image (black and white).

Statistical analyses

We used a t-test to check for differences between species and random plots in mean distances from the nearest urban area and the nearest pond. Since soil variables were highly intercorrelated (Suppl. Mat. Table T1), we used the compositional analysis (Aebischer et al., 1993) to rank the soil evolution, soil texture and land-use variables according to their importance in promoting (or disfavouring) spadefoot toad occurrence. Habitat availability was estimated using the random plots, while the Wilk's lambda, determined by randomization tests, was used to assess the significance of the ranking matrix

(Aebischer et al., 1993). In order to assess the potential effects of soil and land use variables on the occurrence of spadefoot toads, logistic regression models were developed to identify the most important variables likely affecting toad presence. Since variables were intercorrelated, we developed all possible three-predictor models using one variable for soil evolution, one for soil texture and one for habitats respectively, in a way that every variable within each environmental group entered the same number of models. Following this procedure we built 100 models (4 texture soils × 5 soil evolution classes × 5 land-use variables). Inference from models was made according to the Information-theoretic approach (Anderson et al., 2000; Anderson and Burnham, 2002; Mazerolle, 2006): for each model, we computed the differences with the minimum AIC (ΔAIC) and Akaike weights (wAIC), and we ranked models according to this last index. The relative importance of predictor variables was measured by the sum of the models Akaike weights (Σw) where each variable appeared (Anderson and Burnham, 2002). To quantify the effects of the predictor variables the β (partial regression coefficients) were weighted and averaged on the models obtained β. The unconditional sampling variance (var β) and 95% confidence intervals were computed for all predictors in order to assess their statistical significance (Anderson and Burnham, 2002). All analyses were performed using the software R (R Development Core Team, 2010), and data reported are means and standard errors, unless otherwise stated. To meet the assumptions of the analyses, data in proportions were arcsine transformed prior to analysis.

RESULTS

The *P. fuscus* presence plots were significantly closer to the nearest pond (presence plots: 557.3 ± 43.7 m, random plots: 1014.2 ± 82.7 m; Welch two samples t-test: t = 7.581, df = 180.8, P < 0.001) and significantly apart from the nearest urban area (presence plots: 213.7 ± 26.9 m, random plots: 147.7 ± 14.0 m; Welch two samples t-test: t = 2.172, df = 107.7, P = 0.032) than randomly selected ones. The compositional analysis for the variables concerning the evolution of soil generated a highly significant ranking matrix (Λ = 0.752, P = 0.002), confirming that Alfisols and Entisols were preferred by the spadefoot toads, whereas Inceptisols were significantly avoided (Table 1). The ranking matrix was highly significant also as far as soil textures (Λ = 0.475, P = 0.002), and indicated that sandy-loam soils were clearly preferred by toads, followed by sand and silt-loam ones, whereas loamy-sand soils were overall avoided (Table 2). The ranking of habitat variables by compositional analysis was less clearly defined with respect to that obtained for the soil variables. Indeed, even if the ranking matrix was highly significant (Λ = 0.339, P = 0.002), it did not show a striking preference for a specific habitat but only a small preference for poplar plantations (Table 3). As expected, urbanized areas were avoided by the species.

Table 1. Ranking matrix of soil evolution variables comparing proportional soil use within plots of species occurrence with proportions of total available soil types evaluated in random plots. Each mean element in the matrix is represented by its sign. When the sign occurs three times it represents a significant deviation from random at P < 0.05.

	Alfisols	Entisols	Other soils	Inceptisols
Alfisols		+	+ + +	+ + +
Entisols	−		+ + +	+ + +
Other soils	− − −	− − −		+ + +
Inceptisols	− − −	− − −	− − −	

Table 2. Ranking matrix of soil texture variables comparing proportional texture use within plots of species occurrence with proportions of total available texture types evaluated in random plots. Symbols as in Table 1.

	Sandy-loam	Sand	Silt-loam	Loam	Loamy-sand
Sandy-loam		+ + +	+ + +	+ + +	+ + +
Sand	− − −		+	+ + +	+ + +
Silt-Loam	− − −	−			
Loam	− − −	− − −	− − −		+ + +
Loamy-sand	− − −	− − −	− − −	− − −	

Table 3. Ranking matrix of habitat variables comparing proportional habitat use within plots of species occurrence with proportions of total available habitat types evaluated in random plots. Symbols as in table 1.

	Poplar plantations	Woods	Cultivated fields	Other habitats	Urban areas
Poplar plantations		+	+	+ + +	+ + +
Woods	−		+	+ + +	+ + +
Cultivated fields	−	−			
Other habitats	− − −	− − −	− − −		+ + +
Urban areas	− − −	− − −	− − −	− − −	

The multi-model inference showed that *P. fuscus* occurrence was mainly influenced by sandy-loam soil texture, Entisols, poplar plantations, and urban areas (Table 4). The relatively major importance of the sandy-loam soil texture and Entisols was empirically supported by the high values of the sum of Akaike weights for the models where the variables appeared (sandy-loam soils: Σw = 0.98; Entisols: Σw = 0.96). Their effects

Table 4. Multi-model inference on models parameters and relative importance of the 14 soil and land-use variables used for analysing habitat selection by *P. fuscus*: Σw, Akaike weights; β, averaged weighted partial regression coefficient; SE(β), standard error of β; Lower CI95% and Higher CI95%, confidence interval at 95%.

Variabile	Σw	β	SE(β)	Lower CI95%	Higher CI95%
Sandy-loam	0.9810	3.5857	0.7998	2.0180	5.1534
Entisols	0.9619	2.2067	0.4346	1.3549	3.0585
Poplar plantations	0.5085	1.4634	0.1389	1.1912	1.7356
Urban areas	0.3583	-0.7623	0.0912	-0.9410	-0.5836
Woods	0.0820	0.2689	0.0619	0.1475	0.3902
Other habitats	0.0264	-0.0187	0.0128	-0.0437	0.0063
Cultivated fields	0.0248	0.0006	0.0038	-0.0068	0.0081
Loamy-sand	0.0190	-0.0601	0.0132	-0.0859	-0.0343
Inceptisols	0.0155	-0.0185	0.0039	-0.0261	-0.0109
Other soils	0.0126	-0.9625	11.5472	-23.5950	21.6700
Alfisols	0.0099	0.0143	0.0031	0.0082	0.0203
Sand	4.6×10^{-08}	1.1×10^{-07}	2.63×10^{-08}	6.2×10^{-08}	1.6×10^{-07}
Silt-loam	5.7×10^{-10}	-1.3×10^{-09}	3.6×10^{-10}	-2.0×10^{-09}	-6.0×10^{-10}
Loam	3.7×10^{-10}	-3.3×10^{-10}	9.1×10^{-11}	-5.0×10^{-10}	-1.5×10^{-10}

on the occurrence of *P. fuscus* were largely positive as expressed by estimating the regression coefficient using model averaging (sandy-loam soils: β = 3.59; Entisols: β = 2.21), even when models uncertainty was accounted for (β 95% confidence interval ranged from 2.02 to 5.15 for sandy-loam soils and from 1.35 to 3.06 for Entisols). Poplar plantations (Σw = 0.51) and urban areas (Σw = 0.36) were the third and the fourth predictors in order of importance. Percentage of poplar plantations had a positive effect on spadefoot toad occurrence (β = 1.46; 95% confidence interval ranged from 1.19 to 1.73), whereas the effect of urban areas was negative (β = -0.76; 95% confidence interval ranged from -0.94 to -0.58). All other variables had Σw less than 0.10, having a minor effect on *P. fuscus* settlement.

DISCUSSION

Habitat selection

In this paper we showed that the spadefoot toad has specific habitat requirements in our study area; its occurrence was primarily associated with the conditions of soils, and the two most important variables predicting its distribution were the percentage of sandy-loam soil texture and the presence of Entisols. Nonetheless, these two variable were not highly intercorrelated (Pearson's r_p = -0.18), but sandy-loam texture was mainly associated to Alfisols (r_p = 0.45), whereas Entisols related principally with sand soil texture (r_p = 0.79). Therefore, spadefoot toads occurs in two opposite soil conditions: on one hand the soils showing any profile development other than a small A horizon, basically unaltered from their parent material, where sand represents the major component (Entisols with sand texture); on the other hand the mature soils, with an high degree of pedogenesis, a well evident three-horizons profile, and a relatively high native fertility and humidity. In this last case, the percentage of sand remained high, but a relevant portion of silt and clay was also present. Despite their different composition, both types of soils keep a soft and malleable structure that can easily be dug.

Spadefoot toads in our study area avoided the Inceptisols, which are of relatively recent origin, having only a weak appearance of the horizons produced by pedogenesis. The humus does not accumulate in layers, but is mixed with the mineral matrix in a more thin texture (loam and silk-loam; Suppl. Mat. Table T1) with respect to the two other dominant soils. Consequently, the soil is denser and uneasy to be dug by the toads.

A second relevant result of this study was the negative impact of urban areas, which confirms that the disturbance by human presence and activities is among the main causes of the decline of the species. The negative effect of urban areas can be explained by several concomitant conditions: the progressive erosion of suitable habitats due to the expansion of the metropolitan area of Turin (inhabited by about 1,700,000 people), the increase of barriers (mainly roads) among breeding sites that segregates the population in smaller and more isolated subpopulations, and the direct killing due to traffic load.

Despite traffic load had not been found to be a relevant threat in northern Europe populations (Hels and Buchwald, 2001; Nyström et al. 2002), the proportion of toads killed might be relevant in our study area, since many of the observed specimens were found dead on road. These results agree with the past and current presence of the spadefoot toad in the area south of Turin (Fig. 2) where it is evident the presence of populations in areas with suitable soil and the extinction of some populations due to urbanization.

Finally, we also found that poplar plantations were among the preferred habitats of the spadefoot toads, as previously reported for other Italian populations (Andreone et al., 2007). The preference for this type of habitat is only marginally dependent on the quality of soils where poplars are planted: even though poplars are cultivated preferentially in sandy-loam soils ($r_p = 0.18$), the value of the correlation coefficient is too low to fully explain the positive selection of poplar plantations by toads as a simple correlation with soils texture. Two possible reasons leading spadefoot toads to prefer poplar plantations might be: 1) the fact that the large part of intensively cultivated areas of Piedmont, and in particular the corn-fields, are too disturbed by deep plowing and show a fauna of invertebrates very depleted compared to poplar plantations (Casale et al., 1993); 2) the milling, which farmers regularly do in order to keep the ground under the canopy free from weeds; this practice turns over and fragments the soil, which might become softer and suitable for spadefoot toads irrespective of its original structure and texture.

Conservation

Due to its rarity, several conservation actions have been carried out in Italy during the last decades to protect the spadefoot toad (Andreone, 1984, 2001; Andreone et al., 1993; Fortina et al., 2000; Scali et al. 2001; Ferri, 2002; but see Andreone et al., 2004). The initial projects had mainly dissemination and education purposes (Andreone et al., 1993; Andreone, 2001), whereas the more recent ones involved also habitat management actions (Sindaco et al., 2013). The latter gave priority to identification and conservation of the main breeding sites and the surrounding areas (Andreone et al., 2004), restoring of degraded ponds and habitats previously used for breeding (Scali et al., 2001), looking for new populations (Mazzotti et al., 2002, Mercurio and Li Vigni, 2007), digging of new ponds and tadpoles translocation (Scali et al., 2001).

The reintroduction attempts failed because some essential parameters were not considered, particularly the type of breeding sites and soil characteristics (e.g., in Vanzago - Lombardy, and in Bellinzago Novarese and Agrate Conturbia - Piedmont).

Despite based on a dataset dating back more than 20 years, the present paper provides soil parameters useful to identify areas suitable for the species on wide areas of the Po Valley, where the totality of the Italian populations of the spadefoot toad occurs.

As well as many areas of the Po Plain, the landscape of the plain south to Turin has been rapidly transformed from an area dominated by small scale farming and numerous fish-ponds, to an area with large scale agriculture, increasing urbanization and industry. Several original wetlands suitable for spadefoot toads went lost, and the long term survival of the species in most of its Italian range is strictly dependent from the conservation of the residual artificial ponds within a habitat matrix with features fitting its habitat requirements.

The information obtained in this study might help researchers to better identify and protect the areas characterized by the most suitable habitats for the species, to design actions of habitat restoring to improve the quality of the existing habitats, and to detect more efficiently where the digging of new ponds might provide the best results for the breeding performance of the species.

Lastly, combining the digital maps of soils now available with detailed land-use maps, large-scale models of habitat suitability for the species could be developed. These maps could help researchers to detect the areas where to concentrate the efforts to locate other yet unknown populations of spadefoot toads.

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SUPPLEMENTARY MATERIAL

Table T1. Pearson correlation matrix (r_p) among soil and land-use variables measured by the GIS in the 220 plots (70 with Spadefoot toad and 150 randomly selected).