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Amphibians in a human-dominated landscape: the community structure is related to habitat features and isolation

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Abstract

We studied amphibian populations in a human-dominated landscape, in Northern Italy, to evaluate the effects of patch quality and isolation on each species distribution and community structure. We used logistic and linear multiple regression to relate amphibian presence during the breeding season in 84 wetlands to wetland features and isolation. Jackknife procedure was used to evaluate predictive capability of the models. Again, we tested the response of each species to habitat features related to the richest communities. Amphibian presence depends strongly on habitat quality and isolation: the richest communities live in fish-free, sunny wetlands near to occupied wetlands. The negative effects of isolation do not seem to be biased by spatial autocorrelation of habitat features. The system shows strong nestedness: amphibian persistence depends on the contemporary effects of species adaptability and mobility. The commonest species, the pool frog (*Rana* synklepton *esculenta*) and the Italian tree frog (*Hyla intermedia*), are able to move through the matrix using canals and hedgerows, and can maintain metapopulations across the landscape; the rarest species (newts and toads) are more sensitive to habitat alteration, and they are strongly affected by isolation effects. If human exploitation of the landscape continues, only few species, mobile and opportunistic, will persist in this landscape.

Keywords: Amphibian conservation; Community richness; Fish presence; Lowland; Patchy landscape; *Rana latastei*; *Triturus carnifex*

1. Introduction

The expansion of land use subsequent to human population growth results in the fragmentation of natural landscapes (Wilcox and Murphy, 1985). Therefore, in the human-dominated landscapes, natural habitats are reduced to discrete patches, surrounded by a matrix exploited by agriculture or urbanization. Selective extinction and colonization are two processes that forge community structure in patchy landscapes. Selective extinction causes the disappearance of some species from a habitat patch through different processes: a species may require a larger area for persistence and be incapable of using more than one patch (Schadt et al., 2002), habitat alteration due to human activities may make the patch environment unsuitable (Telleria and Santos, 1994; Marsh and Pearman, 1997), or a decreased immigration rate may reduce the probability of a rescue effect (Hanski et al., 1995). Moreover, genetic drift and inbreeding resulting from isolation may reduce fitness (Saccheri et al., 1998; Rowe et al., 1999). Also, stochastic processes may result in the disappearance of a population from a patch (Sjogren, 1991; Ficetola and Scali, 2002).

Successful colonization of patches within a fragmented landscape is generally shown by the more mobile species living in fragments, able to cross the matrix (Sarre et al., 1995; Johst et al., 2002; Pires et al., 2002), by species living in the matrix between patches and having high resilience to human alterations of ecosystems (Harrison et al., 2001) and by opportunistic species that are favoured by environmental alterations subsequent to human activities (Henein et al., 1998).

Community systems where selective extinction and/or colonization are active may show a nested pattern (e.g.,

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Wright et al., 1998). A community set shows nestedness if the species composition of a patch that is poorer in species number is a proper subset of the richest ones (Patterson and Atmar, 1986). In this case, poorer patches have similar communities, composed of the most resistant or best coloniser species (Telleria and Santos, 1994; Hecnar et al., 2002) and the conservation of poorer communities will not allow the biodiversity conservation in the landscape (Davidar et al., 2002). Nested patterns can be related to ecological features of species or habitats, allowing the formulation of hypothesis about the extinction and colonization processes active in the landscape (Patterson et al., 1996; Davidar et al., 2002; Hecnar et al., 2002).

Selective extinction and colonization cannot be considered independently in metapopulation theory (Hanski and Gilpin, 1997). For example, in a source-sink dynamic, species that are good colonisers can reach new suitable patches and recolonise fragments where they previously became extinct (den Boer, 1990). Moreover, species able to survive with large populations in each patch will also avoid genetic drift in the presence of a low number of migrants (Russel, 1996). The situation for species that are less mobile, or highly dependent on key habitat features, is more critical. Therefore, determination of environmental features that allow the survival of species, their dispersion pattern, and interactions between survival and dispersion can help to explain extinction/colonization dynamics. The results of such studies are useful for landscape planning directed to the conservation of biodiversity.

The investigation of amphibian responses to environmental alteration is an important issue in studies of biological conservation because of the global amphibian decline (Barinaga, 1990; Houlahan et al., 2000). Moreover, many amphibians have low mobility and strongly select habitat features. Thus they provide a useful test case with which study the effects of isolation and fragmentation on animal communities. Despite many studies about amphibian metapopulations considered each wetland a patch with its related population, this approach was recently criticised because many of the amphibian species that use the wetlands do so only in the reproductive season (Marsh and Trenham, 2001). Several studies have shown evidence of the importance of terrestrial habitats and landscape structure on these vertebrates (Vos and Stumpel, 1995; Vos and Chardon, 1998), suggesting that a larger scale approach could better explain the observed pattern (Marsh and Trenham, 2001).

Here we present the results of the response of amphibian communities to habitat features in the lowland surrounding the city of Milan, in Lombardy (Northern Italy). On the global scale, lowlands are some of the most human-dominated landscapes, and Lombardy lowland is one of the European areas with the largest agricultural and industrial development (Provincia di Milano, 2003). However, in this area there still exists a complex hydrographic network and some patch with relatively low disturbance, where some populations of endangered amphibians still survive. The aims of this study are to find the ecological requirements that allow amphibian presence in wetlands, to evaluate the effects of isolation on population distribution, and to examine how isolation and habitat quality interact with the species natural history. Our results shed light on regional forces that drive species abundance and community structure.

2. Study area

We investigated a surface area of 520 km^2 in the river Po floodplain (Lombardy region, Northern Italy), (Fig. 1). This area surrounds the city of Milan to the west, south and east and is comprised of the "Agricolo Sud Milano" and "Adda Sud" Regional Parks. The landscape is highly exploited by humans, dominated by the presence of urban suburbs and agriculture. Only a few small wooded fragments still exist and the wooded surface is less than 5% of the landscape. The altitude is 60–160 m and the geological features of this area cause the presence of numerous water springs used in the past for agriculture (Padoa-Schioppa, 2002). Agricultural irrigation of the region also causes the presence of thousands of channels that cross all the lowland. The web of hedgerows, traditionally used as boundaries between fields, covers 1.6% of the landscape's surface (Baietto et al., 2002). In the late 19th to early 20th century, nine amphibian species were living in this area: *Triturus vulgaris*, *Triturus carnifex*, *Pelobates fuscus insubricus*, *Bufo bufo*, *Bufo viridis*, *Hyla intermedia*, *Rana dalmatina*, *Rana latastei* and *Rana* synklepton *esculenta.* All of these, with only the exception of *T. vulgaris*, were very abundant near Milan (Campeggi, 1883; Vandoni, 1914).

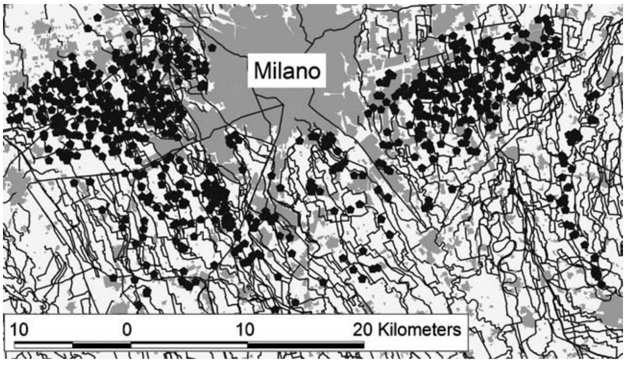

Fig. 1. Study area (Lombardy region, Northern Italy). The study area surrounds the city of Milan to the west, south and east. Grey: urban areas; black line: canals and ditches; pentagons: springs and wetlands.

3. Methods

We detected amphibian presence in 84 wetlands (ponds, temporary pools and ditches). The surface area of analysed wetlands is highly variable, ranging between 6 and 90,000 m^2 (median = 360 m^2; mean = 4861 m^2). We surveyed each wetland after dusk at least once every 3 weeks, during late winter, spring and early summer (February–June). The presence of calling males was recorded in 5-min point counts, following a 1-min pause between arrival and commencement of the survey. After the point count, we searched the entire perimeter of the wetland walking along the banks. We used a head-lamp to light up the wetland, recording all the adults, tadpoles or metamorphs seen. We also recorded the presence of egg masses of toads, Italian tree frog and Italian agile frog. Moreover, we deep-netted each wetland for tadpoles in May, sampling banks and every bottom type.

For each wetland, we recorded 15 environmental features (Table 1(A)) and five isolation features (Table 1(B)). We recorded morphology, soil and vegetation in the field during May. Surrounding vegetation was recorded as the % cover of a 30-m strip surrounding the wetland. Sun exposure was recorded as the % of wetland surface exposed to the direct sunlight. We measured sun exposure in May during sunny days, between 11.00 am and 1.00 pm (UTM). Animal presence was recorded during each survey and if fish or ducks were detected at least once, we recorded them as present. We recorded isolation features from the map (1:10,000 technical regional cartography), using a Geographic Information System. We used these 20 variables in multiple regression and in every logistic regression. Moreover, in each single species logistic regression we added three species-specific isolation features: distance from the nearest wetland occupied by the analysed species and the number of other occupied wetlands in the nearest 250 and 1500 m (Table 1(C)).

Since the "pond as patch" view of amphibian spatial dynamics may be an oversimplification (Marsh and Trenham, 2001), we repeated some analyses on two different spatial scales. We considered each wetland a single small-scale patch, and we clustered them in large scale patches. Large-scale patches are wetland groups without the presence of barriers (main routes, fences) between wetlands; in none of the large-scale patches the inter-wetland distance was higher than 3000 m, therefore we assumed that the more mobile amphibians species, like toads and pool frogs, are able to move across the overall patch (Sinsch, 1990; Tunner, 1992).

Table 1
Environmental and isolation features measured, and spatial autocorrelation of the environmental features. Expected Moran $I = -0.012 \pm 0.071$

	Code	Spatial autocorrelation (Moran *I*)
A. Environmental features		
Wetland surface (m^2)	Area[a]	0.134
Maximum water depth (cm)	Wat Depth[a]	0.163
Banks slope (°, mean of 4 measures on N, S, E, W banks)	B Slope	0.171
Water permanence between February and October (y/n)	Wat Perm	0.106
Sun exposure % (see text)	Sun Expos	−0.025
Soil granulometry (clay = 1, sand = 2, gravel = 3)	Soil	0.344*
Submerged deadwood abundance (n/m^2, along slopes)	Sub Wood[a]	−0.010
% of wetland covered by submerged vegetation	Sub Veg	0.170
% of wetland surface covered by floating vegetation	Float Veg	0.138
% of riparian vegetation along the shoreline	Rip Veg	0.018
Surrounding grass % (see text)	Grass	0.513*
Surrounding scrub % (see text)	Scrub	0.367*
Surrounding wood % (see text)	Wood	0.199*
Fish presence (y/n)	Fish Pres	0.144
Ducks presence (y/n)	Ducks Pres	0.053
B. Isolation features used for every species		
Distance from the nearest wetland (m)	Dist Wet	
Number of wetland on the nearest 250 m	N 250	
Number of wetlands on the nearest 1500 m	N 1500	
Total number of species on the wetlands nearest than 250 m	Sp[a] 250	
Total number of species on the wetlands nearest than 1500 m	Sp 1500	
C. Species-specific isolation features		
Distance from the nearest wetland occupied by the analysed species (m)	Dist Sp[b]	
Number of wetland on the nearest 250 m occupied by the analysed species	Sp[b] 250	
Number of wetland on the nearest 1500 m occupied by the analysed species	Sp[b] 1500	

[a] This variables were log transformed.

[b] Sp will be subsequently substituted by the species abbreviations: Tc, *Triturus carnifex;* Tv, *Triturus vulgaris*; Hi, *Hyla intermedia*; Rl, *Rana latastei*; Re, *Rana* synklepton *esculenta.*

* Significant spatial autocorrelation after the Bonferroni correction ($N = 15$, $\alpha' = 0.0033$).

All the large-scale patches are fully surrounded by routes and human-exploited matrix (agricultural field and suburbs). We considered 11 large-scale patches; each large-scale patch included 2–16 wetlands (Fig. 2).

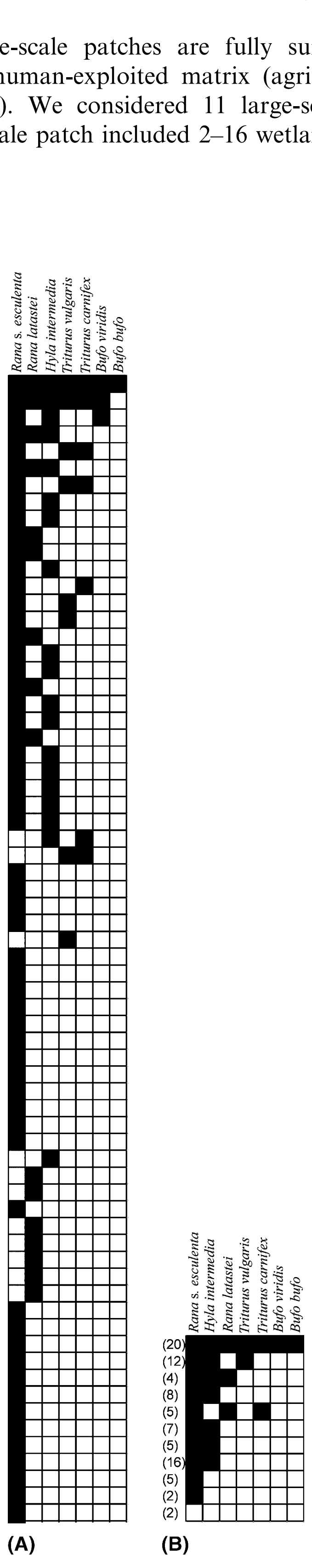

Fig. 2. Maximally nested matrix of species distribution. Each column represent the distribution of a species; each row represent a wetland (A, small scale patch), or a large scale patch (B). Between parentheses the number of wetlands per patch.

3.1. Statistical methods

We evaluated if the habitat features recorded were spatially autocorrelated using the Moran I statistic; this analysis was repeated for each variable using the CrymeStat®II software package (Ned Levin and Associates, Houston, TX). Since multiple tests were performed, we adjusted the significance level (α) of relationships for the number of tests performed (k) using the Bonferroni's method ($\alpha' = \alpha/k$) (Sokal and Rohlf, 1995).

We used logistic regression to relate species presence or absence to environmental and isolation features. A forward stepwise procedure was used to assess which variable should be added to the model: we used the likelihood ratio to select the variables that further reduced the log-likelihood of the model, until any new variable did not reduce it by any significant value. To distinguish the environmental from isolation features effect, we used a blockwise procedure. We first added the environmental variables and the first block of stepwise logistic regression ended when no other environmental variable explained a significant portion of the log-likelihood. Afterwards, we added the isolation variables explaining a significant portion of residual log-likelihood. This approach allow to test if isolation parameters are additional explanatory factors to the habitat quality factors (Laan and Verboom, 1990; Vos and Stumpel, 1995). Since we analysed the relationship between the isolation features and the residuals of the relationship between species presence and habitat, we measured the effects of isolation on the variance unexplained by habitat features. Therefore, the isolation effects observed can be considered independent by the habitat features. We added the habitat features in the first block, and the isolation features in the second block, to avoid increasing the likelihood of adding an "isolation factor" into our model. Because of the Wald's statistic use to assess the single variable significance may be unreliable (Menard, 1995), a variable was retained in the final model if remove P on the last step of each block were <0.05. We used Hosmer and Lemeshow $R^2(R^2_{\mathrm{L}})$ to estimate the proportional reduction in the absolute value of the log-likelihood measure. This measure is the proportion G_{m}/D_0, where G_{m} is the log-likelihood explained by the model and D_0 is the initial log-likelihood: R^2_{L} is a good analogue of the R^2 of linear regression for logistic regression (Menard, 1995). Similarly, we used multiple stepwise linear regression to relate the species richness of each community to the environmental and isolation features. In the first block, we added the environmental variables explaining a significant portion of variance; in the second one the isolation variables.

In the logistic and linear multiple regression models, we tested for multicollinearity using the correlation matrix between variables: if $r > 0.7$, then the regression may be biased (Berry and Feldman, 1985). We did not

Table 2
Pearson product–moment correlations among species-specific isolation features

T. carnifex	Tc 250	Tc 1500
Dist Tc	−0.848	−0.890
Tc 250		0.664
T. vulgaris	Tv 250	Tv 1500
Dist Tv	−0.375	−0.666
Tv 250		0.671
H. intermedia	Hi 250	Hi 1500
Dist Hi	−0.373	−0.578
Hi 250		0.685
R. latastei	Rl 250	Rl 1500
	−0.765	−0.789
Rl 250		0.761
R. s. esculenta	Re 250	Re 1500
Dist Re	−0.469	−0.207
Re 250		0.728

$N = 84$ for all correlations. Abbreviations are in Table 1.

find multicollinearity between the habitat features or between the isolation features used for every species (Appendix A: in all pairwise correlations $r < 0.7$). However, we detected strong correlation between species-specific isolation variables in *T. carnifex, R. latastei* and *R.* s. *esculenta* (Table 2). Since the intercorrelated variables are descriptors of the same factor, for these three species we used in the models only the variables with the higher partial correlation with the species presence (Dist Tc, Rl 1500, Dist Re and Re 250) (Bowerman and O'Connell, 1990). The inclusion of any of the excluded variables in lieu of the correlated variables would not change any model (data not shown).

We tested the effect of environmental variables selected by multiple regression on each species, to test if each species response show the same pattern of the full community, using one-tailed Fisher exact test and *t*-test. We used *G* test (after William's correction) to check for association between Wat Per and Fish and Mann–Whittney *U* test for univariate association between environmental features and species richness. If necessary, we log-transformed variables, to better meet the assumption of residual normality in multiple logistic and linear regression (Sokal and Rohlf, 1995).

In order to examine the degree of nestedness we used the method proposed by Atmar and Patterson (1993). We used the T°-value to evaluate the degree of nestedness. T° may vary between 0° and 100°, and it is an absolute disorder index, based on the differences between a theoretical perfectly nested set ($T^\circ = 0^\circ$) and the observed system (Atmar and Patterson, 1993). The significance of T° is assessed using Monte Carlo simulation. The null-model used by Atmar and Patterson Nested Calculator (1993; 1995) to evaluate the nestedness were recently criticised, since it can lead to overestimate the nestedness level (Fisher and Lindenmayer, 2002). Therefore, we repeated nestedness analysis using two null-models. The Atmar and Patterson null-model (thereafter *null-model 0*) assumes that each cell of presence/absence matrix has an equal probability of being occupied: it does not differentiate between species or patches, and only the total number of occupied cells in each simulated matrix is constant. The Fisher and Lindenmayer (2002) null-model (thereafter *null-model* 1) assumes that some species are more common in species assemblages, and cells in random matrices are occupied on the basis of the probability of encountering a given species in the landscape. The use of *null model* 2 yields a more conservative inference about the significance of nestedness. Our statistic was *P*, the probability of a random replicate being equally or more nested than the observed matrix. We generated random matrices based on *null-model* 0, and we calculated the related *P* using the program Nested Calculator (Atmar and Patterson, 1995); we generated random matrices based on *null-model* 1 using the program Random Matrix Generator (Colombo, 2003), and subsequently we loaded them into the Nested Calculator. The T° values for each matrix were recorded; then we calculated the mean and standard deviation of the resulting distribution of T° values. We calculated the associated *P* values using a two-tailed *z* test. For each null-model we performed 100 runs of Monte Carlo simulation. To detect nested patterns on different spatial scales, we repeated the analysis considering each wetland and each large-scale patch as a sampling unit.

4. Results

Data about soil granulometry and about vegetation surrounding the wetlands (grass, scrubs and wood) are spatially autocorrelated (Table 1): close wetlands have similar values for these four variables; spatial autocorrelation is lacking or weak for the remaining variables. We found seven amphibian species in the study area: two urodelans (Italian crested newt *T. carnifex* and smooth newt *T. vulgaris*) and five anurans (common toad *B. bufo*, green toad *B. viridis*, Italian tree frog *H. intermedia*, Italian agile frog *R. latastei* and pool frog *Rana* synklepton *esculenta*). The pool frog and Italian tree frog are the commonest species; the occurrence of each species in single wetlands and large-scale patches is shown in Table 3. Five species are protected because they are listed in the Habitat Directive of European Union (CEE 92/43; Table 3).

4.1. Species presence

Since we found the common toad and green toad in only one and three ponds respectively, it was not

Table 3
Species occurrence in the study area, and their protection status

Species	Occurrence		Protection status^A
	a	b	
Triturus carnifex	7	2	2, 4
Triturus vulgaris	8	3	–
Bufo bufo	1	1	–
Bufo viridis	3	1	4
Hyla intermedia	19	7	4
Rana latastei	16	2	2, 4
Rana s. *esculenta*	56	10	5
n wetlands/patches	84	11	

a, occurrence in wetlands.

b, occurrence in large-scale patches.

[A] Enclosures of Habitat Directive (CEE 92/43) were the species is listed. 2, species requiring the designation of special conservation areas; 4, species requiring rigorous protection; 5, species that could become object of management measures.

possible to perform logistic regression models for these two species; we built models for all the other species. Water depth, water permanence, sun exposure and fish presence seem the most important environmental features determining amphibian presence; we did not find any significant effects of vegetation. Environmental features and isolation features too are good predictors for presence in each species model; moreover, only the Italian tree frog presence seems not to depend on isolation from conspecific populations. For each species, environmental features explain 14–35% of total variance; isolation alone explain 15–25% of residual variance. The performance of the models was evaluated using a jackknife procedure. In turn, each of the 84 wetlands was removed from the dataset and the models of species occupancy were evaluated with the remaining data. Using the resulting model parameters, we calculated the predicted wetland occupancy of the removed wetland for each species. Therefore, we compared the predicted occupancy value with the observed one (e.g., Ciucci et al., 2003). This procedure was repeated for each of the 84 wetlands and for each of the five species. Correct classification % ranged between 79% and 94% (Table 4).

4.2. Species richness

The richest communities live in ponds without fish presence, with high sun exposure and near to species rich wetlands ($F_{4,79} = 1.649$, $P < 0.0001$, $r^2 = 0.371$, see Table 5). Environmental features alone explain only 17.5% of variance. Fish presence is an important limiting factor for newts and Italian tree frogs (Table 6); we found the green toad only in fish-free ponds too, despite it breeds in few wetlands to perform statistical analysis. The effect of fish presence on pool frog is marginally non significant. The pool frog and the Italian tree frog live in ponds with high sun exposure (Table 6). Only the presence of the Italian agile frog seems to be independent of sun exposure and fish presence. Fish presence is strongly associated with permanent water wetlands (*G*

Table 4
Logistic regression models for species presence

Species	Terms in model	Effect sign	Enter χ^2	Enter *P*	Remove *P*	Block *P*	lock R^2_L	Model *P*	Model R^2_L	% correct
T. carnifex	Wat Perm	–	9.994	<0.001	<0.0001					
	Wat Depth	+	7.081	<0.01	<0.01	<0.0001 (df = 2)	0.354			
	Dist Tc	–	11.035	<0.001	<0.001			<0.0001 (df = 3)	0.583	94
T. vulgaris	Fish Pres	–	7.211	<0.01	<0.01	<0.01	0.138			
	Tv 250 m	+	9.082	<0.001	<0.001			<0.001 (df = 2)	0.311	94
H. intermedia	Sun Expos	+	28.988	<0.0001	<0.0001					
	Fish Pres	–	5.618	<0.05	<0.05	<0.0001 (df=2)	0.390			
	Sp 250 m	+	12.828	<0.001	<0.001			<0.0001 (df = 3)	0.534	90
R. latastei	Sub Wood	+	13.468	<0.0001	<0.001					
	Wat Depth	+	5.051	<0.05	<0.05	<0.0001 (df = 2)	0.226			
	Rl 1500 m	+	21.604	<0.0001	<0.0001			<0.0001 (df = 3)	0.490	87
R. s. *esculenta*	Sun Expos	+	17.637	<0.0001	<0.0001	<0.0001	0.165			
	Dist Re	–	17.589	<0.0001	<0.0001			<0.0001 (df = 2)	0.329	79

Degree of freedom = 1 if not specified.

Correct classification % was calculated using a jackknife procedure.

Table 5
Multiple regression model: effect of wetland features (block 1) and isolation (block 2) on species richness

Block	Variable	β	SE	P
1	Sun Expos	+0.013	0.003	<0.0001
	Fish Pres	−0.445	0.209	<0.05
2	Sp 1500 m	+0.153	0.053	<0.01
	Sp 250 m	+0.589	0.283	<0.05
	constant	−0.292		

β, multiple regression coefficient; SE, standard error.

Table 6
Association of each species with high sun exposure and fish-free wetlands

	Sun Expos		Fish Pres
	t (df = 82)	P	Fisher exact test P
T. carnifex	0.941	>0.35	0.045[a]
T. vulgaris	0.703	>0.35	0.028[a]
H. intermedia	−6.098	<0.0001[a]	0.0097[a]
R. latastei	0.656	>0.35	0.125
R. s. *esculenta*	−4.346	<0.0001[a]	0.085

[a] Significant association between species presence and high sun exposure/fish absence.

Table 7
Community nestedness measured at small and large spatial scale

Spatial scale	System $T°$	*Null model* 0		*Null model* 1	
		$T°$ mean (SD)	P	$T°$ mean (SD)	P
Small	6.36°	45.34° (6.26)	<0.0001	11.08° (1.45)	0.0011
Large	3.10°	36.83° (10.19)	0.0003	19.34° (7.39)	0.028

test: $\chi^2 = 9.830$, df $= 1$, $P = 0.0017$): consequently, temporary water wetlands host the richest amphibian communities ($U_{26,58} = 490$, $P = 0.0065$).

4.3. Nestedness

Community system is ordered and shows a significant nested pattern at both spatial scale analysed using *null-model* 0 and using null model 1 (Table 7, Fig. 2). Neither *null model* 0, nor *null model* 1 generated matrices with $T°$ values lower that system $T°$. The rank of large-scale patches in the maximally packed matrix (Fig. 2(B)) is positively related to the number of wetlands in each patch ($r_s = 0.621$, $N = 11$, $P < 0.05$). The species richness is positively related to the number of wetlands in a patch ($r = 0.749$, $N = 11$, $P < 0.01$).

5. Discussion

The complex distributional pattern of Amphibians in Lombardy floodplain is strongly related to wetland features and isolation. Both features seem to have similar importance for each species and for community richness, and they likely interact in a complex mode. Nestedness analysis shows the presence of a significant gradient of environmental stress between fragments (see Worthen et al., 1998) at each spatial scale: this gradient reflects differences between fragments in environmental quality and isolation (Hecnar and M'Closkey, 1997a). Moreover, different species can have different response to habitat fragmentation since they have different movement capability and landscape connectivity can be different for different species. Our models do not show any relationship between wetland area and species richness. This is not surprising, since the biogeographic principle that larger areas support more species seems to have limitations in its application to wetlands. Several studies outlined the scarce importance of wetland surface for the richness of animal communities in wetlands (e.g., Pavignano et al., 1990; Hecnar and M'Closkey, 1998; Oertli et al., 2002; but see Lassen, 1975; Laan and Verboom, 1990), likely because other wetland features have a more direct effect on the species presence.

5.1. Habitat selection

Wetland features are very important as determinants of amphibian distribution, because each amphibian species living in this area needs water for breeding. Our results about habitat selection of analysed species are consistent with previous studies (e.g., Pavignano et al., 1990; Ildos and Ancona, 1994). Moreover, *T. vulgaris* avoids fish occupied wetlands (Tables 4 and 6): fish are some of the most important predators of newt larvae. *T. carnifex* prefers deep water: it is a nektonic species, and it needs well structured wetlands, with a well developed and structured vegetation (Pavignano et al., 1990). Fish absence in temporary wetlands can explain the preference of *T. carnifex* for these water bodies (Table 4). *H. intermedia* too avoids wetlands with fish, and it prefers sunny wetlands. *Rana* synklepton *esculenta* is a very adaptable species, and may colonize very heterogeneous habitats (Pavignano et al., 1990): the only significant preference is for high sun exposure (Tables 4 and 6). *R. latastei* shows very different ecological requirements from other analysed species. It breeds during late winter, in river washes with deep water surrounded by broadleaved lowland forest, and it fixes spawn to submerged deadwoods. Since the river washes can be connected with the main river stream during flooding, they are frequently inhabited by fish.

5.2. Effects of sun exposure and fish presence on community structure

The richest communities live in sunny, fish-free wetlands. Fish presence is a major threat for amphibians and it can drive amphibian community structure (Hecnar and M'Closkey, 1997b). Many fish predate amphibian larvae: newts, with nektonic active predator larvae, and tree frogs are some of the most endangered by fish presence (Bronmark and Edenhamn, 1994; Atkins, 1998). Species that can breed in fish inhabited wetlands have toxic/unpalatable tadpoles (i.e., toads: Semlitsch and Gavasso, 1992), or tadpoles acting anti predatory behaviour (i.e., pool frogs: Semlitsch and Reyer, 1992). Fish presence in many wetlands is an anthropogenic factor of amphibian decline: humans frequently introduce fish for sportive fishing and ornamental/exotic species in naturally fish-free ponds. In Lombardy lowland this problem seems to be so intense that the observed effects of hydroperiod on amphibian communities are the reverse than in more natural landscapes: usually, permanent wetlands support the most diverse communities, since amphibians with longer larval periods are restricted to relatively permanent waters (Skelly et al., 1999). Conversely, in the study area, temporary water wetlands host the richest amphibian communities, since no fish inhabit these wetlands. Fish abundance in permanent wetlands could be the cause of low number of species rich communities found (Fig. 2(A)) and of newts rarity: newt larvae require long time for development, but they do not live in fish occupied wetlands. Our results are consistent with those obtained in Britain (Beebee, 1997; Atkins, 1998): the largest, permanent ponds, that might host newt populations, are inhabited by fish, and newts, confined to temporary ponds, have declining populations.

Sun exposure is the second environmental features conditioning species abundance: shaded wetlands have lower light, lower dissolved oxygen, lower temperature and lower food resources (Werner and Glennemeier, 1999). Low temperature and food scarcity mean lower tadpoles growth rate and survivorship (Bachmann, 1969; Skelly et al., 1999; Werner and Glennemeier, 1999), and therefore low tadpoles fitness.

The Italian agile frog is the only analysed species that does not show any preference for fish-free or sunny wetlands and, on small-scale nestedness analysis, *R. latastei* is the only idiosyncratic species (see Atmar and Patterson, 1993). Two independent analysis showed strong differences on ecological requirement between *R. latastei* and other amphibian species. Since Italian agile frog is endemic of Northern Italy and it is an endangered species, recently it became object of a number of conservation projects (Scali et al., 2001). The results of this study show that conservation projects having *R. latastei* as target are of little help for many other species and conserving the richest communities can be unhelpful for conservation of *R. latastei*. Limitations associated with reliance upon species richness for conservation objectives are well recognised: when sub groups within a community exhibit opposite ecological requirements, the utility of species richness as a tool for reserve selection and habitat management is partially lost (Patterson et al., 1996; Pearman, 1997; Hazell et al., 2001). The limitation of species richness data for conservation is particularly strong when "idiosyncratic" species are the most endangered or endemic ones, like in this study.

5.3. Isolation

In this landscape the least isolated wetlands are the richest in species, and the distance from the nearest occupied wetland or number of occupied wetlands in the same patch are good predictors for the presence of either newts and frogs: many species distribution appears "clustered" in metapopulation systems with different dimensions. One cluster could be used by one "patchy population", with individuals regularly exchanging between close ponds, or could represent the habitat network of a metapopulation. The lack of spatial autocorrelation among the variables affecting amphibians distributions greatly strengthen the robustness of the observed relationship between wetland isolation and amphibians distribution: wetlands that are clustered do not have similar species composition because favourable habitat is clustered. This pattern can be interpreted as the effect of isolation on population persistence probability (Sjogren, 1991): metapopulation theory predicts that there are thresholds conditions of patch density for metapopulation persistence on the landscape, below which a metapopulation and related subpopulations may become extinct (Hanski and Ovaskainen, 2000; Wilson et al., 2002). Only the presence of the Italian tree frog does not seem to depend from distance to conspecific populations, but from community richness in the patch. Tree frogs can move across long distance using tree canopy (review in Stumpel and Tester, 1993), and it is possible that on the analysed spatial scale the effect of isolation on this specie is not evident; whereas, this species is more frequent in species-rich patches and it is possible that these patches have terrestrial habitats with better quality, because the quality of terrestrial habitat is very important for this strictly terrestrial species (Pavignano et al., 1990; Ildos and Ancona, 1994). Further studies are required to better evaluate the role of terrestrial habitat on structuring the amphibian communities of this landscape.

5.4. Landscape level persistence

The community system is significantly nested at each considered spatial scale. The pattern is very strong, as low T° values demonstrate. At small spatial scale, nestedness of communities is likely linked to nestedness of habitat suitability for species (see Davidar et al., 2002, for a similar interpretation of nestedness). The wetlands that are richest in species host both the common and the rare species: conversely, the poorest communities host only the common species. The significant nestedness of the community system show that the rare species are more common than expected in the richest community, and more rare than expected in the poorest communities. In other words, if a wetland is suitable for the rare species it is suitable also for the common species, and not the other way round. Thus, the wetland with low isolation and high suitability for the amphibians are rich in species and host both rare and common species; conversely, a more isolated wetland and/or with habitat features less suitable (for example, a fish inhabited wetland) can host only the species less sensitive to isolation and habitat alteration: the commonest species in the landscape, the pool frog and the Italian tree frog, seem to met these requirements for several reasons.

First, the commonest species (pool frog) is very adaptable, it does not require large terrestrial habitat and it can live also in polluted water (Bucci et al., 2000). Second, the pool frog and the Italian tree frog have a quick development, and they survive well in temporary wetlands. Third, these two species can easily cross the matrix of the landscape: pool frog can cross over long distances using water bodies (Tunner, 1992), and the very complex agricultural ditches web of Lombardy lowland allows this species to cross the landscape (Fig. 1). Moreover, since the pool frog is really abundant in this landscape, for this species the average distance between the occupied wetlands is lower: therefore, for the pool frog the landscape connectivity is intrinsically higher that for less abundant species. The Italian tree frog is an arboreal species, and it can move throughout the complex web of hedgerows existing in the Agricolo Sud Milano Park (Baietto et al., 2002). The capability of Italian tree frogs to move across the matrix is confirmed by the data about its occurrence. The Italian tree frog breed in only 19 out of 84 wetlands (22.6%), however, it is present in the 63.6% of large scale patches: this species breed in only few wetlands per large scale patch, but in almost all the patches. Therefore it seems that the Italian tree frog is able to move across the matrix to reach the suitable wetlands in each patch. Consequently, it is possible that for the pool frog and for the Italian tree frog a metapopulation system exists across the overall landscape: theoretical models and observational data demonstrate that long range dispersals have better persistence in a dynamic landscape, like cultural landscapes (Mader, 1990; Johst et al., 2002). A similar overall pattern, where Italian tree frog and pool frog can cross barriers that are insurmountable for more terrestrial amphibians, were found in Northern Italy in the Lambro Natural Park by Ficetola and Scali (2002).

Conversely, newts and toads are the rarest species. Them all are terrestrial during the post-breeding season: the effect of barriers on terrestrial amphibians is very strong, because they interrupt the metapopulation system (Hitchings and Beebee, 1997; Rowe et al., 2000), and hinder colonization of newly created ponds. It is remarkable that in our landscape isolation seem to be as important as environmental features for persistence of populations of these species, as high R^2_{L} values of the second blocks of the logistic regressions demonstrate. Moreover, newts can survive in very few wetlands, because of the scarcity of permanent wetlands without fish. The situation of *T. carnifex* seems to be very critical: the Italian crested newt development is slow, and larvae metamorphose on late summer (Griffiths, 1995), but in Lombardy lowland this species is confined to temporary, free-fish wetlands. In dry years *T. carnifex* reproduction could not be successful, and several consecutive reproductive failures can bring to extinction some newt populations (Atkins, 1998; Kupfer and Kneitz, 2000). Finally the common toad, the rarest species, requires large terrestrial, forested habitat patches (Romero and Real, 1996), and undergo to high mortality in landscapes with high road density (e.g., Cooke, 1995): therefore, because of small forested fragments and high road density in the study area, *B. bufo* is confined to the largest one of studied terrestrial patch.

Similar conclusion are suggested by the nested pattern revealed by analysing the large scale patches. The patches richest in species are those with more wetlands (Fig. 2(B)): therefore, the wetlands in these patches are less isolated, and the presence of a local network of populations is possible. Moreover, a patch holding a high number of wetlands most likely hold also some suitable wetlands for amphibians. The consistence of results obtained analysing the community system at both small and large spatial scale strengthen the hypothesis that nestedness of communities reflects a gradient of environmental stress caused by human disturbance between patches: the wetlands can be placed along a gradient of decreasing quality, from the high quality, low isolation wetlands, hosting all the species, to the low quality, high isolation wetlands, hosting none or few species. Analogously, the large scale patches can be placed along a gradient from the patches holding many high quality wetlands to the patches holding few, low quality wetlands (Fig. 2(B)).

5.5. Conclusion

The amphibian community assemblage on Lombardy lowland is related to two different forces, extinction and colonization, acting in a synergic mode on communities structure: degradation of wetland ecological features (like pollution and fish release) and landscape alteration (like increased wetland isolation) cause the extinction of

Table 8
Pearson product–moment correlations among environmental features and isolation features used for every species

		Area	Wat Depth	B Slope	Wat Perm	Sun Expos	Soil	Sub Wood	Sub veg	Float Veg	Rip Veg	Grass	Scrub	Wood	Fish Pres	Ducks Pres	Dist Wet	N 250	N 1500	Sp 250
Area	*r*	1																		
	P																			
Wat Depth	*r*	0.108	1																	
	P	0.326																		
B Slope	*r*	−0.202	−0.055	1																
	P	0.066	0.619																	
Wat Pem	*r*	−0.073	0.388	0.042	1															
	P	0.507	<0.001	0.704																
Sun Expos	*r*	0.622	−0.096	−0.156	−0.123	1														
	P	<0.001	0.385	0.156	0.267															
Soil	*r*	−0.058	0.124	−0.067	0.038	0.010	1													
	P	0.599	0.263	0.545	0.734	0.930														
Sub Wood	*r*	−0.210	0.280	0.064	0.278	−0.447	0.050	1												
	P	0.056	0.010	0.565	0.010	<0.001	0.650													
Sub Veg	*r*	0.153	−0.276	−0.166	−0.371	0.270	−0.078	−0.286	1											
	P	0.164	0.011	0.131	<0.001	0.013	0.482	0.008												
Float Veg	*r*	−0.057	−0.131	0.027	−0.012	0.156	0.214	0.143	0.026	1										
	P	0.609	0.234	0.808	0.917	0.156	0.050	0.194	0.814											
Rip Veg	*r*	0.026	−0.123	−0.095	−0.146	0.198	−0.109	−0.213	0.197	−0.239	1									
	P	0.816	0.265	0.389	0.186	0.071	0.323	0.052	0.072	0.028										
Grass	*r*	0.110	−0.345	0.322	−0.270	0.362	−0.243	−0.359	0.278	0.078	0.290	1								
	P	0.320	0.001	0.003	0.013	<0.001	0.026	<0.001	0.011	0.480	0.007									
Scrub	*r*	−0.079	0.361	−0.168	0.292	−0.338	−0.059	0.352	−0.108	−0.127	0.104	−0.502	1							
	P	0.477	0.002	0.127	0.007	0.002	0.592	0.001	0.329	0.250	0.347	<0.001								
Wood	*r*	−0.224	0.272	−0.076	0.138	−0.636	−0.147	0.345	−0.264	−0.172	−0.087	−0.371	0.436	1						
	P	0.041	0.012	0.489	0.209	<0.001	0.182	0.001	0.015	0.117	0.433	0.005	<0.001							
Fish Pres	*r*	−0.098	0.363	0.051	0.324	−0.116	−0.048	0.136	−0.057	0.080	−0.114	−0.216	0.259	0.225	1					
	P	0.375	0.007	0.646	0.003	0.294	0.665	0.217	0.608	0.471	0.301	0.048	0.017	0.040						
Ducks Pres	*r*	0.084	0.184	−0.090	0.149	−0.055	−0.256	−0.200	−0.091	−0.137	−0.160	−0.091	0.161	0.137	−0.009	1				
	P	0.446	0.094	0.413	0.177	0.622	0.019	0.068	0.413	0.213	0.145	0.409	0.145	0.212	0.938					
Dist Wet	*r*	0.109	0.169	−0.177	0.099	0.140	0.113	0.109	−0.067	−0.026	−0.067	−0.139	0.123	−0.100	0.066	−0.043	1			
	P	0.324	0.124	0.106	0.368	0.203	0.305	0.326	0.545	0.818	0.542	0.207	0.267	0.367	0.552	0.700				
N 250	*r*	−0.048	−0.237	0.264	0.016	0.071	0.342	−0.034	−0.209	0.284	−0.062	0.247	−0.270	−0.130	−0.225	−0.201	−0.184	1		
	P	0.667	0.030	0.015	0.884	0.520	0.001	0.760	0.057	0.009	0.576	0.023	0.013	0.240	0.040	0.067	0.093			
N 1500	*r*	−0.077	0.090	0.017	−0.031	0.021	0.467	0.095	0.041	0.185	−0.038	−0.055	−0.029	−0.028	0.004	−0.155	0.249	0.287	1	
	P	0.486	0.416	0.882	0.782	0.851	<0.001	0.391	0.710	0.092	0.729	0.619	0.794	0.803	0.968	0.160	0.022	0.008		
Sp 250	*r*	−0.195	0.118	−0.027	−0.149	−0.244	0.031	0.186	0.031	0.056	0.023	−0.129	0.237	0.243	−0.080	−0.076	−0.138	0.014	0.323	1
	P	0.075	0.287	0.808	0.176	0.025	0.782	0.091	0.777	0.610	0.836	0.241	0.030	0.026	0.467	0.494	0.209	0.897	0.003	
Sp 1500	*r*	−0.007	0.213	−0.195	0.003	−0.050	−0.096	0.239	0.018	−0.067	0.087	−0.202	0.279	0.114	−0.028	−0.022	0.325	−0.347	0.390	0.491
	P	0.948	0.052	0.076	0.975	0.650	0.385	0.029	0.873	0.543	0.433	0.066	0.010	0.303	0.803	0.843	0.003	0.001	<0.001	<0.001

$N = 84$ for all correlations. Abbreviations are in Table 1.

sensitive species from many patches. Moreover, only mobile and resilient species can cross the matrix, colonizing or re-colonizing the wetlands, preserving their metapopulation system and therefore allowing the long time species persistence in the landscape. If the process of patch degradation is not stopped, and if the landscape connectivity is not improved, the future will allow the persistence of very few species. Analogous extinction and colonization processes could be active worldwide in several other anthropogenic landscapes, driving the communities structure of vertebrates characterised by low mobility, like small mammals, reptiles and amphibians.

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Appendix A

See Table 8.

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