

Landscape–stream interactions and habitat conservation for amphibians

GENTILE FRANCESCO FICETOLA,^{1,2,4} LAURA MARZIALI,^{2,3} BRUNO ROSSARO,² FIORENZA DE BERNARDI,²
AND EMILIO PADOA-SCHIOPPA¹

¹*Dipartimento di Scienze dell'Ambiente e del Territorio, Università degli Studi di Milano Bicocca,
Piazza della Scienza 1, 20126 Milano Italy*

²*Dipartimento di Biologia, Università degli Studi di Milano, Via Celoria 26, 20133 Milano Italy*

³*CNR - IRSA, Water Research Institute, U.O.S. Brugherio, Via del Mulino 19, 20047, Brugherio (MB) Italy*

Abstract. Semiaquatic organisms depend on the features of both water bodies and landscapes; the interplay between terrestrial and aquatic systems might influence the semiaquatic communities, determining the scale at which management would be more effective. However, the consequences of such interplay are not frequently quantified, particularly at the community level. We analyzed the distribution of amphibians to evaluate whether the influence of landscape features on freshwater ecosystems can have indirect consequences at both the species and community level. We surveyed 74 streams in northern Italy to obtain data on breeding amphibians, water, and microhabitat features; we also measured features of surrounding landscapes. We used an information-theoretic approach and structural equation models to compare hypotheses on causal relationships between species distribution and variables measured at multiple levels. We also used a constrained redundancy analyses to evaluate causal relationships between multivariate descriptors of habitat features and community composition. Distribution of *Salamandra salamandra* was related to landscape, hydrological, and water characteristics: salamanders were more frequent in permanent streams with low phosphate concentration within natural landscapes. Water characteristics were dependent on landscape: streams in natural landscapes had less phosphates. Landscape influenced the salamander both directly and indirectly through its influence on phosphates. Community structure was determined by both landscape and water characteristics. Several species were associated with natural landscapes, and with particular water characteristics. Landscape explained a significant proportion of variability of water characteristics; therefore it probably had indirect effects on community. Upland environments play key roles for amphibians, for example, as the habitat of adults, but upland environments also have indirect effects on the aquatic life stages, mediated through their influence on water characteristics. Synergistic effects can magnify the negative consequences of landscape alteration on amphibians; landscape management can be particularly effective, as it can also improve wetland features.

Key words: amphibian conservation; community structure; interaction chain; Lombardy, northern Italy; microhabitat; path analysis; semiaquatic species; spatial scale.

INTRODUCTION

In the last decade there has been increasing appreciation that freshwater habitats are strongly affected by the features of the surrounding terrestrial landscapes (Allan 2004, Jansson et al. 2007). Terrestrial ecosystems can provide a substantial subsidy of energy to freshwater habitats (Pace et al. 2004, Jansson et al. 2007), and the alteration of terrestrial landscapes can have profound consequences on their chemical and physical features, with important effects on communities of aquatic and semiaquatic organisms (Dunham and Rieman 1999, Kiffney et al. 2003, Allan 2004).

Semiaquatic organisms, requiring both terrestrial and aquatic ecosystems to complete their life cycles, constitute a substantial proportion of animal communities. Many amphibians and insects require freshwater habitats for breeding, while adults live in upland environments. Similarly, semiaquatic reptiles spend most of their lifetime in aquatic environments, but return to uplands for egg laying. Semiaquatic organisms can have a relevant functional role. For example, they allow a considerable flow of energy and biomass between terrestrial and aquatic ecosystems, and are sometimes considered keystone species (Davic and Welsh 2004, Gibbons et al. 2006, Whiles et al. 2006). Semiaquatic species often require multifaceted combinations of aquatic and terrestrial environments. Understanding of the relationships between semiaquatic species and the environment can therefore be complex, requiring inves-

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⁴ E-mail: francesco.ficetola@unimib.it

tigations at multiple spatial scales, and the integration between the approaches of freshwater and landscape ecology.

Amphibians are a major group of semiaquatic animals. Habitat loss is a principal cause of the amphibian decline observed at the global scale (Stuart et al. 2004, Gardner et al. 2007). Unravelling the complex relationships between species and habitat can help in identifying target habitats, improving management practices aimed at conservation. Habitat-suitability models are becoming a tool of choice to understand the relationships between species and their environment, to evaluate how environmental changes can affect species, and to inform management. In practice, habitat-suitability models for amphibians and other semiaquatic species usually focus on the direct effects of three major elements: water, wetland and landscape features on species distribution. *Chemical-physical water characteristics* describe key parameters of the quality of aquatic habitat, such as oxygen availability, and the presence of contaminants or other substances influencing the development and survival of aquatic life stages (Beebee 1985, Denoël et al. 2009). *Hydrological and morphological features of wetlands* describe the microhabitat available for the aquatic life stages, and can strongly influence their development and survival (Joly et al. 2001, Van Buskirk 2005, Denoël and Ficetola 2008). Finally, the *composition and configuration of landscape* describe both the availability of habitat for terrestrial life stages, and the possibility of dispersal/migration between terrestrial and aquatic habitats (“landscape complementation”) (Pope et al. 2000, Ficetola et al. 2009, Janin et al. 2009, Zanini et al. 2009).

Despite several studies considering the influence on amphibians of predictors at multiple scales (e.g., Joly et al. 2001, Stoddard and Hayes 2005, Denoël et al. 2009), in most cases only the direct, additive effects of environmental variables are considered, even if predictors at multiple scales are often included in the same analysis (Fig. 1A). However, there is growing evidence that the direct effects of environmental features on species are only one part of the story, as environmental modifications can have strong indirect effects on animal communities (e.g., Feeley and Terborgh 2008, Rohr et al. 2008, Ficetola and Padoa-Schioppa 2009). For example, Feeley and Terborgh (2008) showed that habitat loss can reduce bird communities both directly (through the decrease of available habitat) and indirectly (through its influence on the density of predators). Landscape features are important determinants of amphibian distribution. It has been recognized that landscape alteration can strongly influence wetland and water characteristics (Allan 2004, Jansson et al. 2007) that are in turn the aquatic habitats of amphibians; therefore landscape might also have indirect influence on the distribution of amphibians (Lowe and Bolger 2002, Stoddard and Hayes 2005, Semlitsch et al. 2009) (Fig.

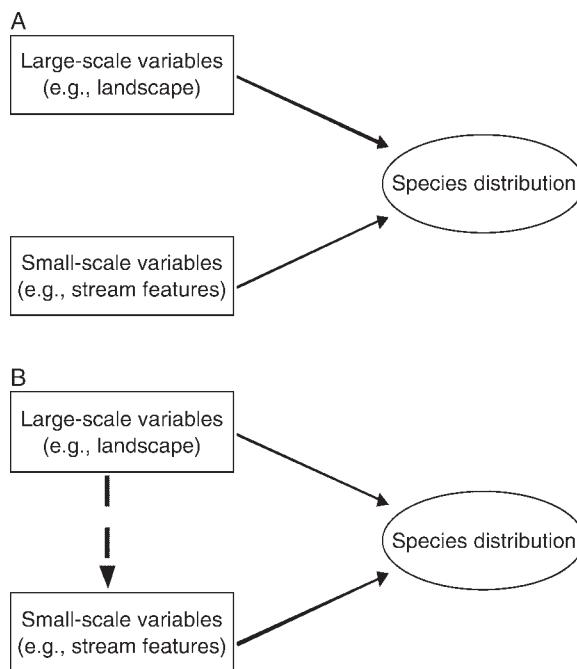


FIG. 1. Two approaches to the effects of multiscale variables on semiaquatic organisms. (A) Multiscale predictors are considered; environmental variables at different spatial scales are assumed to be independent, with additive effects (black arrows). (B) Environmental variables at different scales are not independent; large-scale variables can influence species distribution both directly (black arrows) and indirectly, with effects mediated through their influence on small-scale variables (dashed arrow).

1B). Nevertheless the potential indirect effects of landscape features on amphibians, mediated through their influence on small-scale features, are not always explicitly quantified (but see Rohr et al. 2008).

In this study we analyzed direct and indirect relationships between environmental features measured at multiple levels and amphibian communities, and we tested whether environmental features acting at relatively large (landscape) scale can affect amphibians also indirectly, by influencing key microhabitat features. Specifically, we tested whether the effects of multiscale variables on amphibians can be best described as a series of additive effects (Fig. 1A), or by more complex indirect dynamics (Fig. 1B). We built mechanistic hypotheses by integrating information on the possible consequences of landscape alteration on water characteristics (Kiffney et al. 2003, Allan 2004) with data on the relationships between amphibians and environmental variables. We performed our analyses both at the species level, and at the community level. Single-species analysis can provide specific information on some of the most representative species and an easier interpretation of patterns, while analyses performed on the whole community can provide more general results.

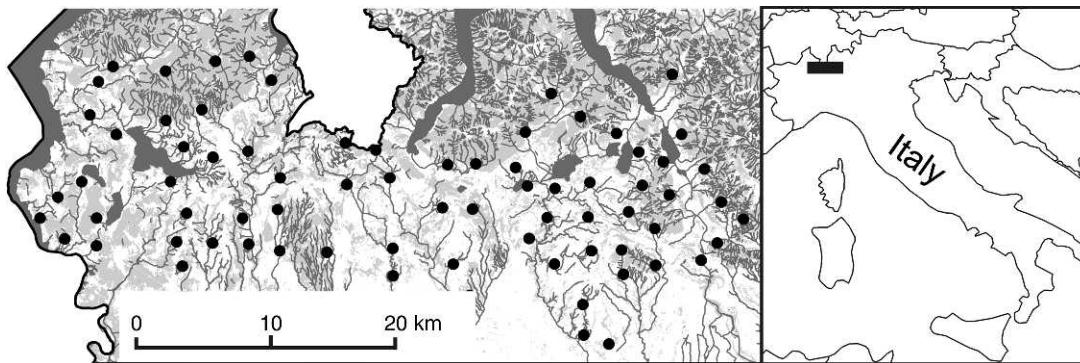


FIG. 2. The study area in Lombardy (northern Italy), and distribution of streams sampled for amphibians (solid circles). Pale gray denotes forested areas; dark gray, hydrographic network.

METHODS

Study area and design

We analyzed 74 systems composed of small watercourses (usually first- or second-order) and the surrounding landscape (hereafter, streams) in a hilly area of Lombardy, northern Italy (Fig. 2). The area is dominated by croplands with widespread urban settlements; about 23% of landscape was covered by forests, mostly in natural parks. All streams have similar altitude (200–570 m); we sampled 36 streams in 2004 and 38 streams in 2005. Forest cover was >50% in 43 streams. To ensure the independence among sampled systems (McGarigal and Cushman 2002), each surveyed stream was >3000 m from the nearest sampled locality. Due to the small size and depth, most streams were fishless, and

none harbored large predatory fish. For each stream, we recorded: (1) the amphibian community (both adults and larvae); (2) habitat features, measured at three levels: water, stream, and landscape features (Table 1).

Amphibian surveys

From April to early June we visited each stream at least 3 times to assess amphibian presence. In each survey two to three observers used multiple techniques to evaluate amphibian presence. At each visit, two observers performed a 100-m visual transect in the upper stretch of the streams, looking for amphibians in pools and under potential shelters. We performed dip netting of the stream bottom over the whole transect, and we placed three funnel traps (diameter, 6 cm) in different pools, facing the stream flow. During dip

TABLE 1. Habitat features recorded, grouped by type of environmental variable, and whether principal-component analysis was used in the analysis.

Environmental features	Summarized by PCA?
a) Water characteristics	
Conductivity (µS)	
Nitrate concentration (N-NO ₃ ; mg/L)†	
Orthophosphate concentration (P-PO ₄ ; µg/L)†	
Oxygen concentration (mg/L)	
b) Stream features	
Maximum width (m)†	PCA morphology
Maximum depth (cm)†	PCA morphology
Stream velocity (m/s)†	PCA morphology
Gravel % over the stream bottom	PCA morphology
Hydroperiod (see <i>Methods: Environmental features</i>)	
Canopy cover	
c) Landscape features	
Forest cover (%)‡§	Landcover 400 m or Landcover 100 m
Cropland cover (%)‡§	Landcover 400 m or Landcover 100 m
Urban cover (%)‡§	Landcover 400 m or Landcover 100 m
Road length (m; see <i>Methods: Environmental features</i>)	
Hydrographic network (m; see <i>Methods: Environmental features</i>)	

Note: Some groups of variables have been summarized by factors extracted through principal-component analyses (PCA) prior to performing single-species or community analyses.

† Log-transformed prior to analysis.

‡ Square-root arcsine-transformed prior to analysis.

§ Measured in a radius of 400 m for the analysis of *Salamandra salamandra*, and in a radius of 100 m for the community analysis (Ficetola et al. 2009).

netting, we placed a fourth funnel trap (diameter, 30 cm) downstream, to capture drifted individuals; sampling effort was standardized across streams and surveys (Heyer et al. 1994, Olson et al. 1997). We considered a species as present if we detected adults, egg masses, or larvae; for all species except pool frogs we detected reproduction in streams where we detected species presence. Preliminary detectability analyses showed that, for all species, per visit detection probability was >65% when using multiple sampling methods (Manenti et al. 2009; G. F. Ficetola, *unpublished data*), therefore with three surveys the cumulative detection probability is high and absence can be inferred with confidence > 95% (see Zanini et al. 2009, Sewell et al. 2010). Strong variation in detectability among sites may increase sampling error and affect analyses. Nevertheless, imperfect detection at moderate levels does not bias estimates of habitat-distribution models unless detection differed among habitat types (Gu and Swihart 2004). To reduce the risk that detection probability varies among sites, we used a protocol maximizing sampling homogeneity among streams (i.e., all streams sampled with the same methods, with the same efforts, and in the same season). Under these conditions, non-detection might more likely occur when abundance is low, in sub-optimal habitats; in that case, species-habitat relationships are expected to be estimated correctly (Van Buskirk 2005). Observed amphibian communities include both terrestrial species living in the surrounding landscape and using streams as breeding sites only (e.g., fire salamander, agile frogs) and semiaquatic species using streams also in part of their adult lifetime (e.g., pool frogs, newts).

Environmental features

Water characteristics.—In mid-May we collected a 0.5-L water sample for each stream from the central part of the stream about 15 cm below the surface. We measured chemical characteristics of samples (concentration of orthophosphates and nitrates) in the laboratory following standard protocols (APAT [Agenzia per la Protezione dell'Ambiente e servizi Tecnici; agency for the protection of environment and technical services] and IRSA-CNR [Water Research Institute–National Research Council]) (Capri et al. 2003). We measured the concentration of dissolved oxygen and water conductivity in the field, using a field multiprobe (Oxi 323; Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) (Table 1a). Nutrient concentrations can seasonally vary within sites (von Schiller et al. 2008), nevertheless land use and anthropogenic factors are major sources of variation among sites in human-dominated landscapes (Carpenter et al. 1998). Water samples from all sites were collected in a short time interval, as relative parameters to compare the features of different sites; the relationship between water and landscape characteristics (see *Results*, below) confirms

that the recorded characteristics provide useful information.

Stream features.—For each stream, we measured in the field several parameters used as standard characterization of features of streams and amphibian breeding wetlands: hydroperiod, maximum width, maximum depth, average stream velocity, canopy cover, and gravel cover over the stream bottom, measured as the average cover percentage visually estimated in three pools (Table 1b) (Barbour et al. 1999, Van Buskirk 2005, Urban et al. 2006). We measured hydroperiod using a rank scale: 1 = ephemeral stream, retaining water during one or two surveys; 2 = temporary, with strong oscillations in water level but retaining water during all surveys; and 3 = permanent and without major water-level oscillations during the sampling period.

Landscape features.—On the basis of the vector map of Lombardy (*available online*),⁵ we used the ArcView GIS (ESRI 1999) to measure five features describing the landscape surrounding each stream: land cover for three features (forest, crop, and urban cover), road length within a radius of 100 m, and length of the hydrographic network within a radius of 600 m (Table 1c). We measured land covers in a radius of 400 m from the stream for the analysis of *Salamandra salamandra*, and in a radius of 100 m for the analysis of amphibian communities. We chose these radii because previous analyses showed that these are the scales at which amphibians within the study area respond more strongly to habitat features (Ficetola et al. 2009).

Statistical analyses

Some measures of stream morphology (stream velocity, depth, width, percentage gravel) and of landscape composition were strongly correlated (Pearson's correlation: $|r|$ ranging from 0.4 to 0.7); strong multicollinearity among independent variables may bias regression analyses (Berry and Feldman 1985). In order to remove multicollinearity, and to reduce the number of candidate models, we used principal-component analyses (PCA) to reduce correlated variables to a smaller number of uncorrelated factors. We performed PCA using the correlation matrix and variable scaling, and we retained factors with eigenevalue >1; the components retained using the broken-stick rule (Jackson 1993) were the same. A first PCA run over the variables describing stream features extracted one variable explaining 52% of variance; this variable (hereafter, PCA morphology) was positively related to stream width ($r = 0.85$), depth ($r = 0.85$), velocity ($r = 0.65$), and gravel percentage ($r = 0.48$) ($P < 0.001$ for all correlations). Therefore, high values of PCA-morphology scores indicate large, deep streams with fast current and high gravel cover. Similarly, we ran PCA over the variables describing landscape composition (percentage

⁵ (<http://www.cartografia.regione.lombardia.it/>)

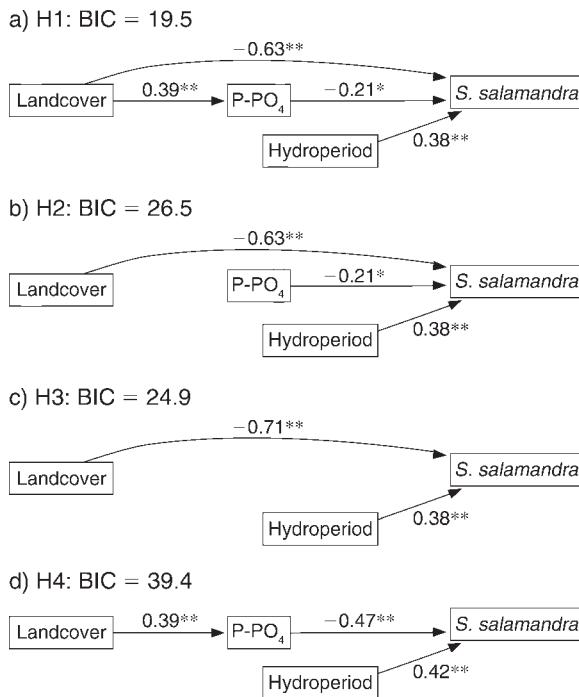


FIG. 3. Path diagrams describing four hypotheses (H1–H4) on the relationships between environmental variables and distribution of *Salamandra salamandra* (see *Results* for the description of hypotheses). BIC stands for Bayesian information criterion. Standardized coefficients are provided next to each path.

* $P < 0.05$; ** $P < 0.01$.

of wooded, urban and agricultural landscape); we repeated this analysis at two spatial scales (100 m radius and 400-m radius; see *Landscape features*, above). The component extracted by the PCA at the 400 m radius (Landcover 400 m) explained 66% of the variation of original variables measured at the 400 m radius, and was positively related to urban ($r = 0.69$) and agricultural cover ($r = 0.76$), and negatively related to wood cover ($r = -0.96$) at this spatial scale ($P < 0.001$ for all correlations). Similarly, the component extracted by the PCA at the 100 m radius (Landcover 100 m) explained 57% of the original variables at the 100 m radius, and was positively related to urban ($r = 0.59$) and agricultural cover ($r = 0.65$), and negatively related to wood cover ($r = -0.96$) at this spatial scale ($P < 0.001$ for all correlations). After the substitution of variables with PCA scores, we found no strong correlation among environmental variables (in all correlations between environmental variables, $|r| < 0.6$), suggesting that collinearity would not bias the results of our models (Berry and Feldman 1985).

Sampling was performed in two consecutive years. In our analyses, we pooled data of the two years in the same data set to increase power; preliminary analyses showed no major qualitative differences in the results of analyses among the two years (Ficetola et al. 2009). The occurrence of *S. salamandra* was similar in 2004 and

2005 (2004, occurrence = 64%; 2005, occurrence = 58%, likelihood-ratio test: $\chi^2_1 = 0.279$, $P = 0.597$); similarly, species composition was similar among the two years.

Single-species analysis

We focused the single-species analysis on *Salamandra salamandra*, which was the species observed in the largest number of streams (see *Results*, below) and is the amphibian more strongly related to stream environments in the study area. We used a two-step approach to evaluate the relationships between species distribution and environmental features. First, we used an information-theoretic approach, based on Akaike's information criterion (AIC; Burnham and Anderson 2002), to identify the combination of variables best describing the distribution of *S. salamandra*, following the procedure detailed in Denoël et al. (2009). We built generalized linear models (GLM) assuming binomial error including all possible combinations of environmental variables. For each model, we calculated AIC and Δ -AIC, which is the difference in AIC between a candidate and the model with lowest AIC (i.e., the best model). The use of AIC as the sole selection criterion may select overly complex models, thus we considered a complex model only if it had a Δ -AIC less than the Δ -AIC of all its simpler nested models (Richards 2008). For each candidate model, we also calculated Nagelkerke's R^2 (R^2_N) as a measure of variance explained. Spatial autocorrelation may affect the results of regression models; however, the residuals of our best models were not spatially autocorrelated (Monte Carlo permutation test following the procedure of Lichstein et al. 2002; $P > 0.1$) suggesting that spatial autocorrelation did not bias the estimates of our analysis (Lichstein et al. 2002). GLM assumes linear relationship between species occurrence and environmental variables. As ecological relationships may be nonlinear, in preliminary analyses we also looked for nonlinearity in the relationships, using generalized additive models and piecewise regression (see Ficetola and Denoël [2009] for details). However, we did not detect strong deviations from the linear pattern for any of the variables in the best candidate models.

Environmental variables can have both direct and indirect effects on species distribution (e.g., Feeley and Terborgh 2008, Rohr et al. 2008, Ficetola and Padoa-Schioppa 2009). The second step of our analyses was therefore using structural equation models (SEM) (Pugesek et al. 2003) to compare different models representing potential causal relationships between environmental variables and the distribution of *S. salamandra*. SEM is a theory-oriented method, and can therefore enhance our interpretation of relationships among variables and improve our interpretation of results (Pugesek et al. 2003, Grace and Bollen 2005). Following the approach of Rohr and co-workers (2008), we built models reflecting four alternative a priori hypotheses on the causal relationships among variables

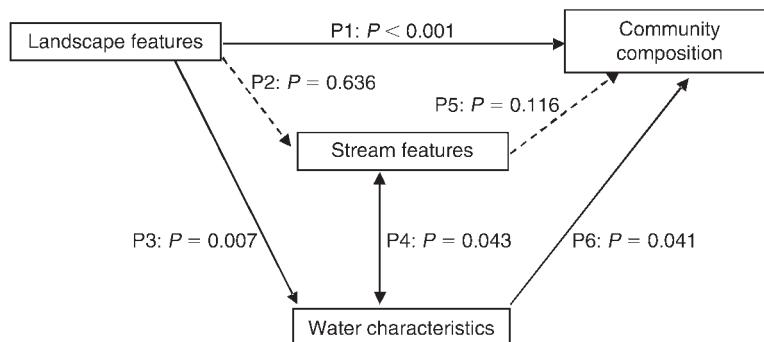


FIG. 4. Path diagram illustrating the relationships between matrices of environmental variables and amphibian communities, as analyzed using a series of constrained redundancy analyses. Dashed arrows represent nonsignificant relationships. P1 indicates path 1, and so on.

identified as potentially important by GLM and the distribution of *S. salamandra*, and comparing models with additive effects only with models considering the interplay among variables (Figs. 1 and 3; see the *Results* section, below, for details on the hypotheses underlying the models). We subsequently used the Bayesian information criterion (BIC) to evaluate the relative performance of the models. As for AIC, models with low BIC have the strongest support, given the data; BIC is the information criterion most frequently used in SEMs (Raftery 1993). We considered differences in $BIC > 5$ as “strong evidence” in favor of a model (Raftery 1993). SEM assumes normal error distribution, however our dependent variable was binomial (presence or absence). Therefore, if necessary we used polyserial correlation, as implemented in the statistical package POLYCOR (Fox 2009), to compute the input covariance matrix. Polyserial correlation is suitable in evaluating the relationships between quantitative and ordinal variables; we calculated polyserial correlations using maximum-likelihood (Drasgow 1986). Furthermore, we used bootstraps (1000 re-samplings) to estimate the standard errors of path coefficients and to assess significance. We performed SEMs using the sem package (Fox 2008).

Community analysis

We used a series of constrained redundancy analyses (RDA) to evaluate the direct and indirect effects of landscape, microhabitat, and water characteristics on the multivariate structure (i.e., species composition) of amphibian communities in streams. RDA is a canonical analysis, combining the properties of regression and ordination (e.g., PCA [principal-component analysis]) techniques, that allows one to evaluate how much of the variation of the structure of one data set (e.g., community composition in a locality) is explained by independent variables (e.g., habitat features) (Legendre and Legendre 1998, Quinn and Keough 2002). RDA may also be viewed as a simple type of SEM between two sets of variables, if the components of one data set (exogenous data set) influencing the endogenous data set are considered as latent variables (Takane and Hwang

2005). We considered three matrices of exogenous environmental features: water, stream, and landscape features (see Table 1 for the list of variables); we used the matrix of species composition across streams as endogenous. Similarly to the rules of the calculation of path coefficients (Sokal and Rohlf 1995, Quinn and Keough 2002), we estimated the variation explained along a path by performing a constrained RDA, controlling for the effect of other environmental features. For example, to estimate the variation explained along path P1 in Fig. 4, we performed RDA assuming species composition as dependent, landscape as constraining matrix, and microhabitat and water characteristics as conditioning matrix (the effect of which is partialled out). Our approach used a priori hypotheses on causal relationships among variables to build a model examining the suggested mechanism by which landscape, microhabitat, and water characteristics influence amphibian communities. Note that the percentage of variation explained along a path is not analogous to the coefficients of standard path analysis, but corresponds to the independent contribution of a set of independent variables, calculated using variance partitioning (Cushman and McGarigal 2002, Furlani et al. 2009). We calculated the significance of variance explained by RDAs by performing ANOVA-like permutation tests (10 000 permutations) (Legendre and Legendre 1998). We performed RDA using the vegan package (Oksanen et al. 2007); we performed all statistical analyses in the R 2.9 environment (R Development Core Team 2009).

RESULTS

We observed seven species of amphibians in the 74 streams in northern Italy (Fig. 2): fire salamander *Salamandra salamandra*, smooth newt *Lissotriton vulgaris*, green toad *Bufo viridis*, common frog *Rana temporaria*, agile frog *R. dalmatina*, Italian agile frog *R. latastei*, and pool frog *Pelophylax synklepton esculentus*. The fire salamander was the most frequent species (61% of streams; 102 larvae trapped and >1200 larvae observed). The frequency of other species was lower (*L. vulgaris*, frequency = 1% of sites; *B. viridis*,

TABLE 2. Candidate regression models relating the distribution of *Salamandra salamandra* to environmental features, ranked from best to least good.

Rank	No. variables in model K	Δ -AIC	Variables	$R_N^2 \dagger$
1	3	0.0	landcover 400 m (-); hydroperiod (+); phosphates (-)	0.51
2	2	0.1	landcover 400 m (-); hydroperiod (+)	0.49
3	1	1.4	landcover 400 m (-)	0.45
4	4	17.4	hydroperiod (+); phosphates (-); conductivity (-); hydrographic network (+)	0.31
5	3	17.6	hydroperiod (+); phosphates (-); hydrographic network (+)	0.28
6	3	19.3	hydroperiod (+); conductivity (-); hydrographic network (+)	0.26

Notes: Only models with Δ -AIC <20 are reported here. All models performed significantly better than the null model. For landcover, “-” indicates association to more natural landscapes (see *Methods*); for hydroperiod, “+” indicates association to more permanent streams. K is the number of variables in the model; $\dagger R_N^2$ is Nagelkerke’s R^2 .

frequency = 3%; *R. temporaria*, frequency = 3%; *R. dalmatina*, frequency = 12%; *R. latastei*, frequency = 3%; and *P. s. esculentus*, frequency = 12%).

Single-species analysis

The best-AIC (Akaike information criterion) model suggested that the fire salamander was associated with streams in natural landscapes, with more permanent hydroperiod and low concentration of phosphates; this model explained a substantial amount of variation ($R_N^2 = 0.51$) (Table 2). Simpler models, not including the effects of phosphates or hydroperiod, had slightly lower Δ -AIC (the difference in AIC between a candidate and the model with the lowest AIC [i.e., the best model]). Models not including landcover had much higher AIC (Table 2), suggesting that landcover was the variable most important to explain the distribution of the fire salamander.

On the basis of AIC analysis, landcover, phosphates, and hydroperiod were the only variables included in models with good support (Table 2). We formulated four a priori hypotheses, describing the causal relationships between these variables and the distribution of the fire salamander; these hypotheses determined the structure of models:

- H1) Landcover can influence the nutrients in streams, including phosphates (Kiffney et al. 2003). Therefore, the effect of landcover can be both direct and indirect, mediated through its influence on phosphates. Phosphates and hydroperiod have a direct effect on the quality of streams for larvae (Fig. 3a).
- H2) All environmental variables have direct effects only (Fig. 3b).
- H3) Landcover and hydroperiod have direct effects, while phosphates do not have any effect (Fig. 3c).
- H4) Landcover does not have direct effects; the effect of landcover is indirect only, mediated through phosphates. Phosphates and hydroperiod have direct effects (Fig. 3d).

SEM (structural-equation models) showed that the hypothesis H1 (landcover has both direct and indirect

effects) had the lowest BIC (Bayesian information criteria) score, and showed strong support, compared to the alternative ones (Fig. 3). Model H1 confirms the significant positive relationship between hydroperiod and salamanders, and the negative relationship with phosphates. Landcover had both direct and indirect effects. The occurrence of salamanders was highest in the most-natural landscapes. Furthermore, natural landscapes had streams with lower concentrations of phosphates, thereby further increasing the suitability for salamanders (Fig. 3a).

Community analysis

RDAs (redundancy analyses) showed strong relationships between environmental variables. Water characteristics were significantly related to landscape features (permutation test, $P = 0.007$, Fig. 4): streams in human-dominated landscapes, with high road density, showed high conductivity and high concentration of phosphates; landscape features explained 14% of the variation of water characteristics. Furthermore, there was a weak but significant relationship ($P = 0.043$) between stream and water characteristics. RDA did not detect significant relationships between landscape and stream features.

The series of RDA showed strong relationships between environmental features and the structure of amphibian communities. Overall, environmental features explained 33% of the variation of community structure. Landscape was the attribute with the largest independent contribution (Fig. 4: path P1); 42% of explained variance accounted for). Communities with *S. salamandra* and *R. dalmatina* were associated with streams in natural landscapes, with a dense hydrographic network; communities with *P. esculentus* were associated with the less natural landscapes (Fig. 5A). Furthermore, *R. dalmatina*, *R. temporaria*, and *P. esculentus* were associated with landscapes with a limited road network (Fig. 5A). The independent contribution of water characteristics (Fig. 4: path P6) accounted for 22% of explained variation. Communities dominated by *P. esculentus* and *B. viridis* were associated with streams with low oxygen concentration, high conductivity and high phosphate contents (i.e., relatively eutrophic

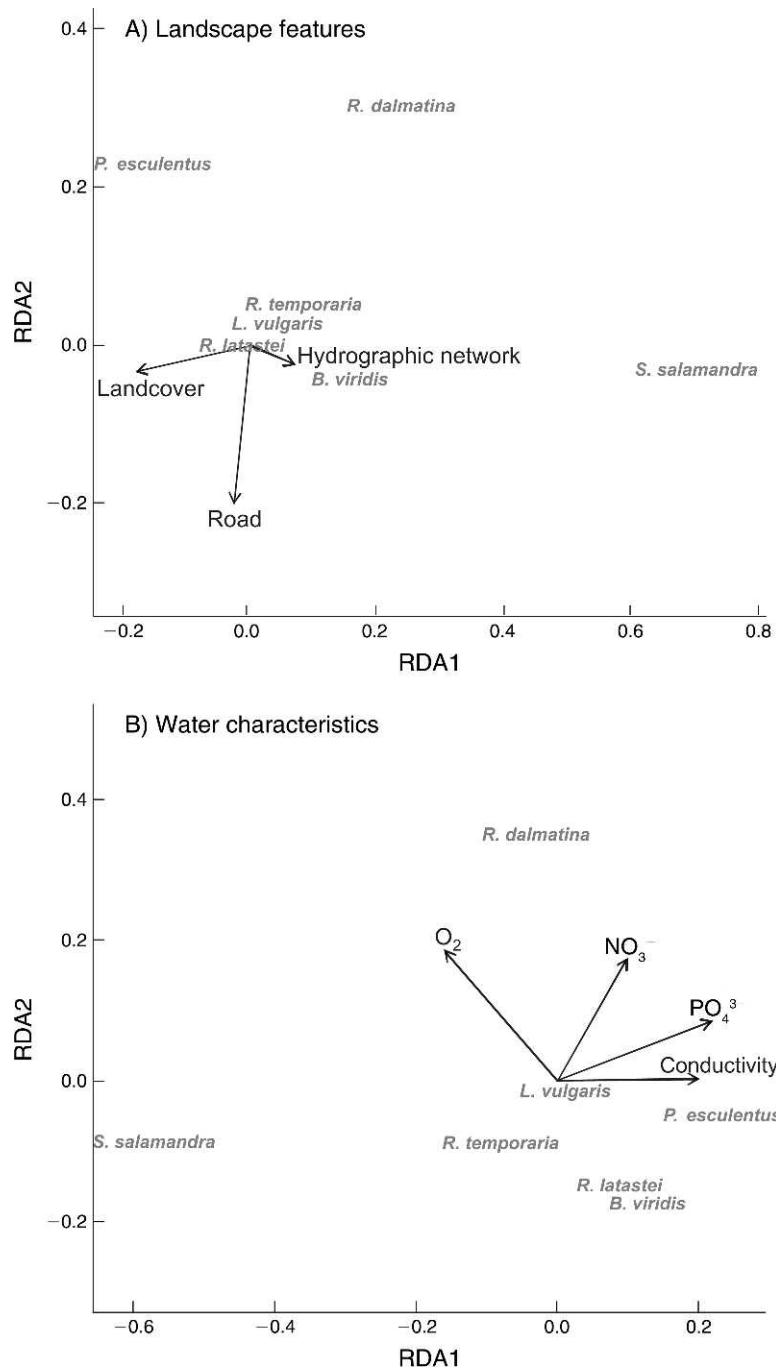


FIG. 5. Constrained redundancy analyses (RDAs) describing the relationships between amphibian communities and (A) landscape features and (B) water characteristics. Black labels and arrows represent environmental variables; gray italic labels represent species. See *Results* for full species names.

conditions), while communities dominated by *S. salamandra* were associated with more oligotrophic streams (Fig. 5B). Stream features (Fig. 4: path P5) did not explain a significant proportion of variation of community structure ($P = 0.116$; Fig. 4). The joint effect of landscape and water characteristics accounted for 14% of explained variation.

DISCUSSION

Our analysis showed that the distribution of amphibians is related to environmental features at multiple spatial scales (water, microhabitat, and landscape). Importantly, landscape features had both direct and indirect effects on amphibians; overall, direct and indirect effects of large-scale environmental features

explained most of the variation of community composition. Some species (*Salamandra salamandra*, *Rana dalmatina*) were strongly associated with the most-natural landscapes. Natural landscapes have a key role for amphibian populations, as they can constitute the habitat of adults, and are necessary for the long-term maintenance dispersal and metapopulation dynamics. This determines a strong, direct relationship between landscape features and amphibian distribution. Furthermore, landscape features can also have an extremely important, indirect role, as they can affect water characteristics (Kiffney et al. 2003, Allan 2004), and thereby influence the habitat of aquatic life stages (Stoddard and Hayes 2005, Semlitsch et al. 2009). In this study, streams with more eutrophic conditions were within the urban and agricultural landscapes, and altered water features can strongly affect amphibian larvae. Although it is well recognized that environmental features of wetlands and the surrounding uplands are not independent, the extent to which indirect relationships and covariation among environmental features mediate the relationship between environment and amphibian communities is rarely taken into account (Rohr et al. 2008).

Single-species analysis

Analyses performed at the species level for *S. salamandra* yielded results similar to the ones performed on the whole community: landscape features show both direct and indirect effects on amphibian distribution. In the study area, the fire salamander (*S. salamandra*) is the only amphibian breeding mostly in small streams, and was indeed the only species observed in a large proportion of sites. Our analysis suggests that environmental features occurring at multiple levels (landscape composition, hydroperiod, and phosphates) are important to explain species distribution. The three relationships depicted as “direct effects” in SEMs (structural equation models) (Fig. 3a) are not unexpected, and can be explained on the basis of knowledge of species ecology and life history. First, salamanders were associated with the most-natural landscapes, with high forest cover and limited extent of agricultural or urban areas. The importance of natural upland environments for the fire salamander is well recognized (e.g., Ficetola et al. 2009), as this species spends the post-metamorphic lifetime in terrestrial environments, and woodlands are the major habitat of adults in most of the species range. Furthermore, the fire salamander requires streams with intermediate to long hydroperiod, i.e., retaining water during the whole spring. Larvae of the fire salamander require several months for metamorphosis (Nöllert and Nöllert 1992); streams with relatively long hydroperiod are needed to complete larval development and metamorphosis. Finally, larvae were less frequent in waters with high phosphate content. The interpretation of phosphates as possible predictors of salamander distribution is less straightforward. It is possible that

phosphates have per se a negative effect on water quality for salamanders, but more complex, indirect relationships are also possible (Rohr et al. 2008). For instance, high phosphates can be related to high organic-matter content and to low oxygen levels, especially close to the bottom and during night time. Moreover, some macro-invertebrates do not live in streams with high nutrient contents (Rosenberg and Resh 1992), thus, it is possible that such streams do not host some taxa important for the feeding of salamanders (Weitere et al. 2004, Manenti et al. 2009). Phosphates can also increase the density of parasites (e.g., trematodes), with consequences on the survival of larvae (Rohr et al. 2008). Finally, high phosphate concentration is often the consequence of human activities; thus, it could be an indirect measure of the presence of other pollutants not analyzed in this study, such as pesticides or other chemicals.

Most importantly, landscape alteration also had an indirect effect on the distribution of *S. salamandra*, mediated through its influence on water characteristics: streams within less-natural landscapes had more eutrophic waters and more phosphates, probably as a consequence of urban waste and runoff over croplands (Allan 2004). In practice, salamanders in human-dominated areas have to withstand two joint negative effects acting on different life-history stages: the loss of terrestrial habitat has negative consequences on adult life, and unsuitable water conditions influence larvae. This synergistic, interactive effect can magnify the negative consequences of landscape alteration beyond what would be predicted if landscape and water characteristics would act independently (Didham et al. 2007).

Community-level analysis

The overall pattern obtained through the community-level analysis is consistent with the single-species analysis, suggesting that the indirect effects of landscape, mediated through its influence on water characteristics, are generally present within the study system, and are not idiosyncratic of just one species. Multiple species are associated the more-natural landscapes (Fig. 5A), and are related to water characteristics (Fig. 5B); in turn, water parameters are significantly related to landscape features (Fig. 4). Also in this case, the interplay between these two elements suggests strong synergistic effects (Fig. 4), with likely consequences at the community level (Semlitsch et al. 2009). Our analysis focused on amphibians. Nevertheless, these conclusion are probably valid for a wider spectrum of taxa because, in human-dominated areas, the effects of landscape features can also override microhabitat and water characteristics in other groups, such as aquatic insects (Urban et al. 2006).

Management implications

The presence of interactive, indirect effects between landscape and water parameters emphasizes the complexity of species–habitat relationships in semiaquatic species, and stresses the importance of integrating

multiple elements and spatial scales. Ecological changes are often determined by non-additive and chain effects; understanding these relationships is a challenge for both science and management (Didham et al. 2007). As landscape features can influence microhabitat, environmental modifications at this large spatial scale can also have cascade effects at smaller scales. Management focused on microhabitats can be effective only if processes occurring at large scales are taken into account. In practice, our analysis suggests that focusing on the conservation of landscape features can be more effective than considering wetland features only. Management performed at large scales can have positive consequences on key processes of populations, such as the survival of terrestrial life-history stages, and metapopulation dynamics (Cushman 2006, Roe and Georges 2007, Ficetola et al. 2009). Furthermore, the conservation of natural landscapes can also have positive consequences on the features of breeding wetlands, thereby enhancing the reproductive success. In Europe and North America, actions for the conservation of amphibians are often focused on breeding habitats, such as improving wetland features or creating/restoring wetlands (e.g., Gamble and Mitsch 2009, Silva et al. 2009). The rationale for these actions is that enhancing reproduction will allow population recovery. These management practices remain extremely important, particularly in areas where shifts in human activities are causing the loss or degradation of small water bodies (Moser et al. 1996). Nevertheless, performing management at a larger spatial scale, and considering that landscape features can also determine wetland features, can be a more effective long-term strategy. Of course, management at the landscape scale requires a complex coordination between environmental and development policies, and is more difficult and costly than the simple restoration of a pond or a small stream. However, the large-scale approach can be the most effective in the long term.

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