



Dependence of animal diversity on plant diversity and environmental factors in farmland ponds

E. Santi^{1,3}, E. Mari², S. Piazzini¹, M. Renzi², G. Bacaro¹ and S. Maccherini¹

¹ *BIOCONNET, Biodiversity and Conservation Network, Department of Environmental Science "G.Sarfatti", University of Siena, Via P.A. Mattioli 4, 53100 Siena, Italy*

² *Ecolab – Research Centre for Lagoon Ecology, Fisheries and Aquaculture, University of Siena, Polo Universitario Grossetano, via Lungolago dei Pescatori, 58015 Orbetello, Italy*

³ *Corresponding author. Phone: +39 0577232864, fax: +39 0577232896, e-mail: elisa.santi@unisi.it*

Keywords: Aquatic insect, Amphibians, Community composition, Conservation biology, Forward selection, Plant diversity, Variation partitioning.

Abstract: Farmland ponds represent habitats with a high conservation value that make a significant contribution to regional biodiversity. Understanding the influence of plant species composition and environmental variables in driving variations in animal species composition in ponds is an important issue in the fields of ecological research and conservation biology. Using variance partitioning techniques to quantify independent effects, we examined how plant species composition, local-landscape configuration and physicochemical variables interact in influencing aquatic insect and amphibian community composition. The ponds investigated in this study were located in the Site of Community Importance - Special Protected Area (Natura 2000 Network) "Monte Labbro - Alta Valle dell'Albegna" (Tuscany, central Italy). Our results showed that: (i) plant community composition (such as *Carex hirta*, *Glyceria fluitans*, *Potamogeton natans*, *Typha latifolia*) is a good predictor for amphibian but not for aquatic insect species composition; (ii) aquatic insect species composition was more strongly affected by the landscape context, whereas for amphibians the local characteristics of the ponds were determining; (iii) the physicochemical context is a poor predictor for these animal taxa; (iv) lastly, and notably, the explanatory variables explained a high proportion of the total variation in amphibian and aquatic insect species composition. Our results have important implications with respect to the creation of new ponds, which should preferentially take place close to semi-natural grasslands and other wetlands, in order to maintain greater connectivity, and away from urban areas. Moreover, larger ponds are preferable for the preservation of pond biodiversity. The management and conservation of ponds is necessary to ensure the protection of habitats, the survival of individual species and overall pond biodiversity.

Introduction

Farmland ponds are widely distributed in agricultural landscapes. Although created for agricultural activities (e.g., drinking water for cattle or irrigation), these habitats have a high conservation value and contribute significantly to regional biodiversity, as they support heterogeneous communities of aquatic organisms and often contain rare or unique species (Oertli et al. 2002, Nicolet et al. 2004, Williams et al. 2004). Despite this, their small size has made them highly vulnerable to threats related to the intensification of human activities and environmental changes, which in recent years have been responsible for a sharp decline in their number (Wood et al. 2003). The creation or restoration of ponds to mitigate the loss of biodiversity has become a common practice (Pechmann et al. 2001, Solimini et al. 2003, Declerck et al. 2006), and is a relatively simple and cost-effective form of management. In a previous paper (Mari et al. 2010), we confirmed that recent ponds were rapidly colonized by many pioneer plants and species of aquatic insects and amphibians.

Plants have repeatedly been shown to mediate trophic interactions between different groups of organisms (Jeppesen

et al. 1997) and are potentially important as a determinant of aquatic biodiversity (Scheffer 1998, Declerck et al. 2006). A high diversity of plants is critical to encourage a wide variety of animals in ponds. In fact, plants play an important role in freshwater ecosystems since they: (i) function as primary producers, (ii) provide a key habitat for many animal species, and (iii) provide sites for egg-laying, shelter and a source of food for macroinvertebrates and amphibians.

Many authors have shown pond community composition to be influenced by physicochemical environmental constraints and land-use in the catchment and surrounding area. Physical factors, such as pond size or depth, affect the number of species in the community and the proportion of predators within ponds (Spencer et al. 1999, Bilton et al. 2001). Individual ponds often differ in their physical and chemical characteristics even over small spatial scales, which can result in large variations in community composition within groups of adjacent water bodies (Spencer et al. 2002, Kiflawi et al. 2003).

Landscape characteristics are significant predictor of species presence and abundance for a wide variety of taxa and landscape types, corroborating the importance of land

use in pond conservation. A good understanding of the effects of land use on ponds may therefore be essential to mitigate the effects on existing ponds and develop efficient location selection strategies for the creation of new ponds (Davies et al. 2004). Ponds surrounded by semi-natural habitats, for example, are generally of higher conservation value than those in more intensively managed areas (Pond Action 1994).

Consequently, a good knowledge of the environmental constraints under which different processes take place is essential to better understand the impact of land use practices or physicochemical variables on the functioning and ecological characteristics of water bodies, and forms the basis for efficient integrated catchment management.

We know of no previous study that compares the predictive power of plant community composition and environmental variables on aquatic insect and amphibian communities in freshwater habitats. The most reliable explanation for cross-taxon congruence is probably the presence of similar responses to common environmental factors or to factors that are spatially covariant (Wolters et al. 2006). The aim of this study is to test the influence of plant community composition, local landscape configuration and physicochemical variables on both aquatic insect and amphibian communities in farmland ponds. More specifically, we aim to test whether the predictive power of plants is greater than that of environmental variables in structuring communities of animal species.

Materials and methods

Study area

The ponds investigated in this study were located in or close to the Natura 2000 SCI-SPA "Monte Labbro - Alta Valle dell'Albegna" (Tuscany, central Italy). This site covers 6127 ha and the altitude ranges from 260 to 1193 m a.s.l. The area lies in the Mediterranean region, with a mean annual rainfall of about 600 mm. The mean annual temperature is 11.8°C. In the study area, there are several hamlets and isolated farms, some of which have been abandoned; the main

human activities in the area are non-intensive agriculture and cattle and sheep grazing.

29 ponds (permanent or semi-permanent) were created on this site in 2003 and eight in 2007, through the European Commission's LIFE-Nature program (LIFE99NAT/IT/006229 and LIFE04NAT/IT/000173). The study area and surrounding landscape contains ponds that have existed for many years (minimum 20) and are still used for cattle watering.

Data species collection

To take into account the different ages of farmland ponds, 4 ponds created in 2003 and 4 created in 2007 were randomly selected, excluding any that were completely drained soon after creation or were of very small dimensions. Four ponds were selected from those that had already existed in the area for many years; in particular, we considered ponds with comparable characteristics (e.g., altitude, lithology) to those of the eight recently created ponds.

For each pond, between May and June 2007 we collected data on the number of larvae and adults of amphibians and aquatic insects (Coleoptera, Ephemeroptera, Odonata, Heteroptera), and the presence of plants (vascular plants and macroalgae). The collection period corresponded to the overlapping periods of vegetative growth and permanency for all the *taxa* considered. For each pond the presence and abundance of all the taxonomic groups were recorded along two belt transects of 50 cm in width; the first was positioned along the gradient of the inflow/outflow of water and the second was orthogonal to the first. Each transect was divided into two portions from the shore to the centre of pond, thus obtaining 4 sub-transects for each pond (the central plot was attributed to the sub-transect that started at the inflow of water). Along the sub-transects, square plots (50 × 50 cm) spaced 1 m apart were selected; the two extreme squares of each transect were placed at the land/water interface (Fig. 1). Amphibians and aquatic insects were sampled using hand-held nets of 40 cm in diameter and 15 stitches per cm², with intensive sweeping in the centre of the plot for 30 seconds, and all individuals were collected (see Oertli et al. 2005, Tri-



Figure 1. Location of the 4 sub-transects and the plots in the pond (the full plot was sampled). The sub-transects were represented by different colours (dark gray and white sub-transects were positioned along the gradient of the inflow/outflow of water, while black and light gray sub-transects were orthogonal to the first ones). Figure is in scale.

gal et al. 2007). The species collected were kept alive in a plastic bucket partly filled with water for counting and then identification. Taxa requiring microscopic identification were preserved in 70% ethanol solution. The nomenclature followed Pignatti (1982) for plants, Belfiore (2007) for Ephemeroptera, Utzeri and D'Antonio (2007) for Odonata, Bacchi and Rizzotti (2007) for aquatic Heteroptera, Rocchi (2007) for Coleoptera Hydroadephaga, and Balletto (2007) for amphibians. *Rana bergeri* and *Rana klepton hispanica* were grouped together as a single unit, *Rana skl hispanica*, because their extreme local similarity rendered identification in the field impossible.

Environmental variables

Factors influencing the composition of aquatic insect and amphibian communities were analysed using three sets of environmental variables to reflect the physicochemical, local and landscape characteristics of each pond. The physicochemical water characteristics were described by five variables and measured *in situ* in three replicates, located at the centre, near the outflow, and near the inflow of each pond, to check for internal variability within the pond. As reported by a recent paper, physicochemical variables in small shallow ponds show low spatial fluctuations due to their dimensions and, for this reason, two sampling stations are considered sufficient to describe the whole system variability (Fairchild et al. 2005). In this study, we decided to increase the sampling replicates in order to improve the representativity. pH (pH ± 0.01), conductivity (SpC ± 0.01 , mS cm⁻¹) and turbidity (Turb ± 0.1 , NTU) were measured *in situ* in a sub-superficial water layer (-50 cm) using a multiparameter field probe (Corr-Tek Hydrometria/Hydrolab, mod. Datasonde 5A). Superficial water samples were collected in pre-cleaned high density polyethylene (HDPE) bottles and transferred rapidly to the laboratory for further analysis. Analyses of dissolved

inorganic nutrients (nitrate, NO³⁻; total phosphorus, TP) were carried out according to Grasshoff et al. (1999).

The local characteristics of the ponds were described by three variables: altitude (m), total surface (m²) and maximum depth (m). The landscape features were analysed at two different scales: a 20 m radius comprised the immediate surroundings and a 200 m radius covered a more general pattern of the landscape characteristics around the ponds. Within the 20 m radius, we recorded the proportion of the area shaded by riparian vegetation, the trampling intensity (ordinal variable: low = 1; intermediate = 2; high = 3), and the presence/absence of stepping stones or refuges (considered as five different dummy variables, such as (i) the stone heaps with a minimum dimension of 3 m², (ii) the wood heaps with a minimum dimension of 3 m², (iii) the hedges with a minimum dimension of 5 m², (iv) the strips of trees or shrubs with a minimum dimension of 40 m², and (v) the presence of torrents or other wetlands, e.g., Feder and Burggren 1992, Wells 2007, Remsburg and Turner 2009).

In the 200 m radius, on the other hand, we estimated the percentage cover of principal land use types. Aerial photographs were subjected to an automatic segmentation technique which aggregates neighbouring pixels according to their spectral similarity by an agglomerative iteration (Fig. 2; Blaschke 2010). Segmentation was performed using the eCognition software (eCognition 2001). Finally, we manually attributed a land use type to each polygon created by segmentation, according to the physiognomic characteristics of the vegetation. For the definition of the land use, we adopted the second level CORINE Land Cover legend (European Environment Agency 2000) with some adaptations. We identified seven land uses: (i) arable land, (ii) pastures, (iii) semi-natural grasslands, (iv) shrublands, (v) forest and hygrophilous vegetation, (vi) urban areas, and (vii) other wetlands. Cover data for each land use class were obtained

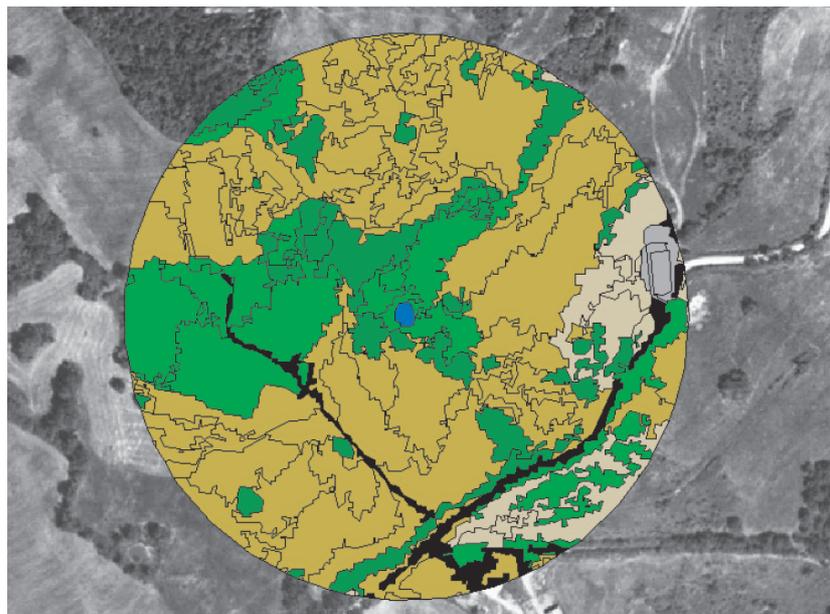


Figure 2. Automatic segmentation technique in a 200 m radius from the pond.

through the application of the GIS software ArcView GIS 3.2a (ESRI, Inc).

Data analysis

Variation partitioning analysis was performed to estimate the fraction of variation explained by plant communities and environmental variables for aquatic insect and amphibian communities. Variation partitioning is a quantitative statistical method by which the variation in response variables can be decomposed into independent components, representing the relative importance of different groups of predictors and their joint effects (Borcard et al. 1992).

The variation in the aquatic insect and amphibian communities was decomposed among the three groups of explanatory variables - plant community composition, physicochemical and local-landscape characteristics - using a series of (partial) regression analyses with redundancy analysis (RDA). The partial RDA is a residual analysis in which the relationship between responses and explanatory variables is analysed after the influence of the "co-variables" has been removed. This resulted in seven different non-overlapping fractions, apart from the unexplained variation: (i) the pure effect of plant community factors, (ii) the pure effect of physicochemical factors, (iii) pure effect of local-landscape factors, (iv) the joint effect of plant community and physicochemical factors, (v) the joint effect of plant community and local-landscape factors, (vi) the joint effect of physicochemical and local-landscape factors, (vii) the joint effect of the three groups of explanatory variables. In order to evaluate the relative importance of the two aspects of local-landscape variables for amphibian and aquatic insect community composition, we extended the variation partitioning to a second tier, considering these two sets of variables separately, according to Cushman and McGarigal (2002). Local-landscape variables were divided into two sets: local and landscape factors. Thus, the second-tier decomposition of local-landscape factors resulted in two variance components, which were obtained in the same way as those of the first tier.

The partitioning was based on an adjusted R^2 statistic, R^2_{adj} , as recommended by Peres-Neto et al. (2006). In order to test the significance of the first ordination axis and the significance of all axes together, 499 Monte Carlo permutations were performed.

Before variance partitioning analysis, all environmental variables were processed with a forward selection based upon permutations, and a parametric test (Blanchet et al. 2008) was used to identify significant environmental variables for amphibian and aquatic insect communities. Classic forward selection of ecological variables presents two well-known problems: (1) an inflated Type I error rate, and (2) an overestimation of the amount of variance explained. The forward selection procedure proposed by Blanchet et al. (2008) and adopted here overcomes these problems and the selection of predictors is done by applying a permutation of residuals under a reduced model. The forward selection proposed has to be carried out with two stopping criteria: (1) the

usual alpha significance level (significance level selected = 0.05), and (2) the adjusted coefficient of multiple determination (R^2_{adj}), calculated using all explanatory variables. When forward selection identifies a variable that takes one criterion or another over the fixed threshold, that variable is rejected and the procedure is stopped. The most important advantage of this method is that the selection of useless variables occurs less often. Moreover, this method is more conservative than classic forward selection and the fewer variables selected have a more realistic ecological meaning.

After the forward selection procedure, only environmental variables statistically significant at the $p < 0.05$ confidence level were selected and then divided into groups for the variation partitioning analysis. Concerning plant predictors, Principal Components Analysis (PCA) was used as a data reduction technique to obtain the main compositional patterns for plants, and the first two PCA axes (PC1 and PC2 site scores) were used in the variation partitioning analysis as variables explaining the compositional pattern of the aquatic insect and amphibian communities. We performed two different PCAs: one for insects (full dataset with plant species of all ponds) and one for amphibians (dataset with plant species of ponds created in 2003 and ponds that had already existed for 20 years, since no amphibians were present in the ponds created in 2007).

The analyses were performed with the abundance data for the three taxa in each sub-transect. Aquatic insect data (including larvae of Odonata, larvae and adults of Heteroptera, adults of Coleoptera and larvae of Ephemeroptera) and amphibian data for each sub-transect were obtained by summing the number of individuals in each plot, while plant data for each sub-transect were obtained from the mean percentage of cover, to derive the species abundance in each plot. Prior to the statistical analysis, aquatic insect and amphibian data were Hellinger-transformed, as this makes community composition data containing many zeros suitable for analysis (Legendre and Gallagher 2001) and provides unbiased estimates of the variation partitioning based on linear statistical methods, such as redundancy analysis (Peres-Neto et al. 2006). The abundance of plant species was $\log(x + 1)$ transformed, since this works well with percentage data (Ter Braak 1985). The environmental variables were standardized.

All analyses were performed using R for Windows software v. 2.9.2 (R Development Core Team, 2009). In particular, forward selection analysis was performed using the 'forward.sel' function available in the R 'packfor' library (Dray 2004); variation partitioning analysis was performed using the 'varpart' function in the 'vegan' library (Oksanen et al. 2005).

Results

A total of 56 plant species, 28 aquatic insect species, and 5 amphibian species were recorded in the 12 ponds. No amphibians were found in the ponds created in 2003. Data summarising all of the environmental variables for the 12 ponds

Table 1. Ranges and mean values (with standard deviation) of environmental variables measured at all 12 ponds.

Variables	Range	Mean \pm s.d
Physicochemical water variables		
pH	7.65-11.1	8.3 \pm 0.9
SpC (mS cm ⁻¹)	0.3-0.7	0.5 \pm 0.1
NO ³⁻ (μ M)	2-E4-0.2	0.1 \pm 0.1
TP (mg L ⁻¹)	1-E4-0.1	0.01 \pm 0.02
Turbidity (NTU)	0.6-272	27.9 \pm 74.5
Pond local variables		
Altitude (m a.s.l.)	660-105	938 \pm 105
Area (m ²)	50-189	97 \pm 53
Maximum depth (m)	0.4-1.8	1.3 \pm 0.4
Landscape variables		
Trampling intensity	-	-
Presence of shrubs (refuges)	0-30	9.6 \pm 11.2
Presence of torrents (refuges)	-	-
Presence of forests (refuges)	-	-
Shade cover from riparian vegetation (%)	-	-
Arable land (%)	0-58.3	19.3 \pm 21.9
Pastures (%)	0-54.3	27.7 \pm 20.8
Semi-natural grasslands (%)	0.9-63.5	15.8 \pm 17.1
Shrublands (%)	4.8-35.8	15.8 \pm 11.6
Forest and hygrophilous vegetation (%)	0-47.2	13.9 \pm 13.2
Other wetlands (%)	0-0.06	0.01 \pm 0.02
Urban areas (%)	0-15.9	4.5 \pm 5.4

Table 2. Environmental variables selected by the forward selection procedure ($p < 0.05$) for aquatic insect communities.

Selected variables	R ²	R ² Cum	AdjR ² Cum	F	P value
Altitude	0.126	0.126	0.107	6.638	0.001
NO ³⁻	0.092	0.218	0.183	5.279	0.001
Pastures	0.066	0.284	0.235	4.036	0.002
Presence of torrents (refuges)	0.075	0.358	0.298	4.996	0.001
Turbidity	0.046	0.403	0.333	3.218	0.003
Area	0.045	0.449	0.368	3.332	0.002
Semi-natural grasslands	0.043	0.491	0.402	3.352	0.005
Trampling intensity	0.027	0.518	0.419	2.170	0.022

Table 3. Environmental variables selected by the forward selection procedure ($p < 0.05$) for amphibian communities.

Selected variables	R ²	R ² Cum	AdjR ² Cum	F	P value
Shade cover from riparian vegetation	0.220	0.220	0.194	8.474	0.001
Area	0.128	0.348	0.303	5.675	0.002
Urban areas	0.089	0.437	0.376	4.405	0.004

are given in Table 1. From the forward selection procedure involving the whole set of environmental variables, 8 and 3 variables were retained for aquatic insect and amphibian communities, respectively (Tables 2, 3). In particular, no physicochemical variables were found to be significant for amphibian communities, while only two physicochemical variables were selected for aquatic insects. The first principal component (PC1) explained 48.7 and 52.6% of the total variance for the aquatic insect and amphibian communities, respectively. In general, considering both PCA results, we can see that the first principal component was positively correlated with the percentage cover of *Chara* sp. and *Potamogeton natans* and negatively correlated with *Arrhenatherum elatius* (Tables A1, A2 in Appendix). The second principal component was positively correlated with *Arrhenatherum elatius*, *Carex hirta*, *Cirsium arvense*, *Glyceria fluitans* and *Typha latifolia* (Tables A1, A2 in Appendix).

The variation partitioning analysis results indicated that the whole set of variables explained nearly 40% of variation in aquatic insect species composition (Fig. 3). The pure effect of the local-landscape variables explained the largest fraction of the variability (22.8%) in the aquatic insect species composition, while only 5% of the total variation was explained by the pure effect of physicochemical variables. The pure effect of plant community was very low and not statistically significant (Fig. 3). The joint effect that contributed most to explaining aquatic insect species composition was the combination of plant and local-landscape variables (9.4%). The negative value of the shared variation component, due to the joint effect of the three components for aquatic insects (-2.8%), indicated that the corresponding explanatory variables of the three sets had a synergistic effect (i.e., a function of the three factors together is able to explain more than the sum of the separate functions of these three factors; see Legendre and Legendre 1998). The second-tier decomposi-

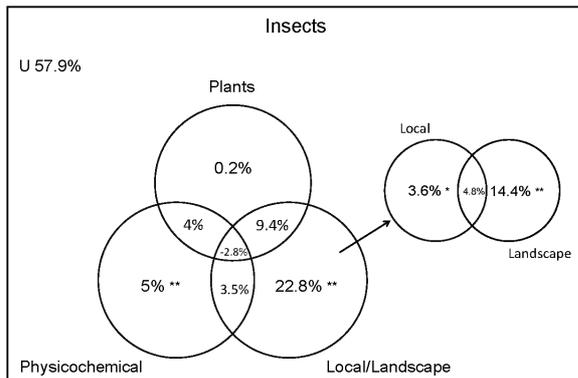


Figure 3. Results of variation partitioning. The first-tier decompositions of the influence of plant community composition, physicochemical and local-landscape variables, selected by forward selection, on aquatic insect community data; and the second-tier decompositions of the influence of local and landscape variables on aquatic insect community data.

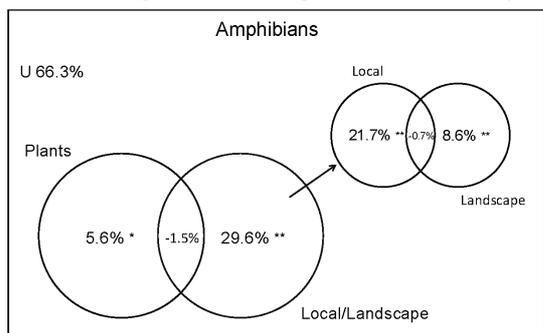


Figure 4. Results of variation partitioning. The first-tier decompositions of the influence of plant community composition and local-landscape variables, selected by forward selection, on amphibian community data; and the second-tier decompositions of the influence of local and landscape variables on amphibian community data.

tion of local-landscape factors showed that landscape metrics explained the highest portion of the variation (14.4%) for aquatic insect communities. The joint effect of the two variable sets was higher than the pure effect of local characteristics (4.8%, Fig. 3).

A partial RDA biplot is shown in Figure 5, to demonstrate the effect of the pure landscape component on aquatic insect species composition (only explanatory variables that were significant according to the variance partitioning are shown). *Pyrrhosoma nymphula* and *Anax imperator* were positively correlated with the pond area, whereas *Orthetrum brunneum* and *Notonecta glauca* were negatively correlated with this factor (Fig. 5). In this partial RDA, the second axis was mainly determined by the proportion of pastures, the trampling intensity and the proportion of semi-natural grassland. *Corixa punctata* and *Cloeon dipterum* occurred on sites with a high cover of pastures along the second axis. In contrast, *Ilyocoris cimicoides*, *Libellula depressa*, *Anax parthenope* and *Hyphydrus aubei* mainly occurred on sites situated at lower altitudes with a low cover of pastures. Ponds with higher frequencies of *Cloeon dipterum*, *Acilius sulcatus* and *Libellula quadrimaculata* were positively correlated with the presence of torrents (refuges). Finally, most of the aquatic insect species appear to be negatively correlated with high trampling intensity and high altitude.

Considering amphibians, the amount of variation captured by all the variables selected was over 30%. The largest fraction of the variation explained (29.6%) in the amphibian community was related to the pure effect of the local-landscape variables (Fig. 4). The pure effect of the plant community was higher (5.6%) than that of the aquatic insect community and statistically significant. The combined effect of the two groups of explanatory variables was negative. The

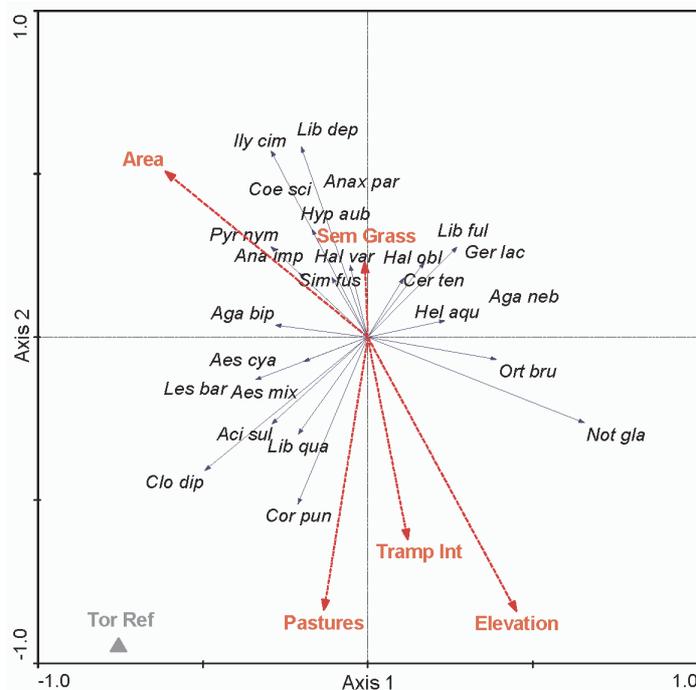


Figure 5. Ordination biplot of the first two axes of the pRDA for aquatic insect species composition, considering only the pure local-landscape component. Red dashed line arrows are the explanatory variables, the dummy variable (Tor ref) is represented in grey and the abbreviations are: Area: size of pond; Altitude: altitude of pond; Pastures: percentage cover of pastures; Sem Grass: percentage cover of semi-natural grasslands; Tor ref: presence of torrents (refuges); Tramp Int: trampling intensity (ordinal variable with values 1-3). Only species with scores > 0.20 are shown, for the sake of clarity. Species abbreviations are given as follows: Aci sul: *Acilius sulcatus*; Aes cya: *Aeschna cyanea*; Aes mix: *Aeschna mixta*; Aga bip: *Agabus bipustulatus*; Aga neb: *Agabus nebulosus*; Ana imp: *Anax imperator*; Coe sci: *Coenagrion scitulum*; Cer ten: *Ceragrion tenellum*; Clo dip: *Cloeon dipterum*; Cor pun: *Corixa punctata*; Ger lac: *Gerris lacustris*; Hal olb: *Haliphys obliquus*; Hal var: *Haliphys variegatus*; Hel aqu: *Helophorus aquaticus*; Hyp aub: *Hyphydrus aubei*; Ily cim: *Ilyocoris cimicoides*; Les bar: *Lestes barbarus*; Lib dep: *Libellula depressa*; Lib ful: *Libellula fulva*; Lib qua: *Libellula quadrimaculata*; Not gla: *Notonecta glauca*; Ort bru: *Orthetrum brunneum*; Pyr nym: *Pyrrhosoma nymphula*; Sim fus: *Simpeccma fusca*.

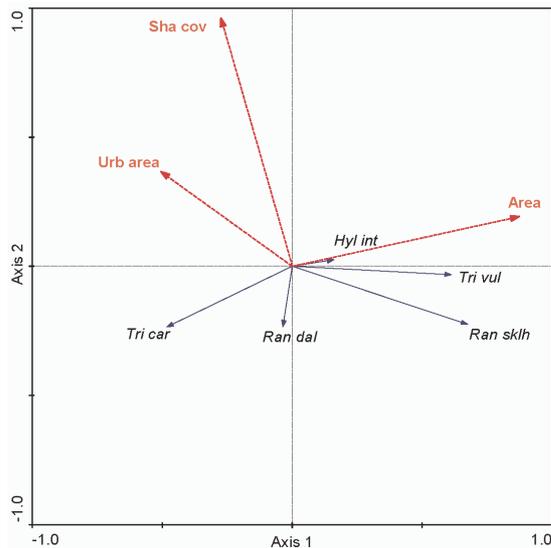


Figure 6. Ordination biplot of the first two axes of the pRDA for amphibian species composition, considering only the pure local-landscape component. Dashed-line arrows are the explanatory variables and the abbreviations are given as follows: Sha cov: the proportional shaded area; Area: size of the pond; Urb area: percentage cover of the urban area. The species abbreviations are: Hyl int: *Hyla intermedia*; Ran dal: *Rana dalmatina*; Ran sklh: *Rana skl hispanica*; Tri car: *Triturus carnifex*; Tri vul: *Triturus vulgaris*.

second-tier decomposition of local-landscape factors showed that the local characteristics of ponds explained the highest part of the variation (21.7%) for the amphibian community, compared to the pure effect of the landscape characteristics (8.6%). The joint effect of the two sets of variables was negative (Fig. 4). In Figure 6, a partial RDA biplot shows the effects of landscape variables on amphibian species composition. The first axis was determined principally by pond area and secondarily by the amount of land covered by urban areas. *Triturus vulgaris*, *Rana skl. hispanica* and *Hyla intermedia* occurred in sites with both a large pond size and lower presence of urban areas (these species have high scores with respect to the first axis). *Triturus carnifex* mainly occurred on sites situated at lower altitudes (see Fig. 6). *Rana dalmatina*, and to a lesser extent the other species, had a high score on the second axis and occurred in sites with a proportionately lower area shaded by riparian vegetation (Fig 6).

Discussion

Our results can be summarized as follows: (i) the plant community composition is a good predictor for the species composition of amphibians but not aquatic insects; (ii) the aquatic insect species composition was more strongly affected by landscape characteristics, whereas the local characteristics of the ponds were determinant for amphibians; (iii) the chemical context is a poor predictor for these animal taxa; (iv) lastly, and notably, the explanatory variables explained a high proportion of the total variation in the species composition of amphibians and aquatic insects. Nonetheless, there were still considerable amounts of unexplained variation

(42.1% for aquatic insects and 33.7% for amphibians), which might be associated with unmeasured local factors, landscape effects, biotic and stochastic processes, and sampling variability (Borcard et al. 1992).

In our study, plant community composition was not a determining factor for aquatic insect species composition, but the shared variance between plant community composition and local-landscape variables was very important, showing high collinear variation (e.g., plant community composition was closely related to landscape configuration). Other studies have shown macroinvertebrate richness to be affected by the richness of emergent plants (Biggs et al. 1994, de Szalay and Resh 2000, Kadoya et al. 2004, Carchini et al. 2005); in the majority of these studies, the highly vegetated ponds had a high taxon richness (such as Hemiptera, Coleoptera, Odonata, and Ephemeroptera). In Gioria et al. (2010), plant species composition displayed a positive predictive capacity for an aquatic insect community (e.g., water beetles), although environmental variables (e.g., maximum depth and substratum of pond) showed a higher predictive capacity.

In agreement with our results, a study carried out in Romanian ponds (Hartel et al. 2009) showed that the emergent vegetation cover was an important predictor for amphibians. In particular, in this paper wetland plants such as *Carex hirta*, *Glyceria fluitans*, *Potamogeton natans*, *Typha latifolia* and the algae *Chara* sp., which were highly correlated with the first PCA axis, determined part of the compositional variability of amphibians. These wetland plant species are mainly used by amphibians for oviposition and as a refuge for larvae, and provide habitats for aquatic species both above and below water, thus allowing more taxa to coexist (Lanza 1983, Pough et al. 2004).

In this study, the most important community-structuring factors for the aquatic insect communities were local-landscape characteristics such as the altitude, pond area, proportion of land covered by pastures and by semi-natural grasslands, the presence of torrents (refuges) and trampling intensity. From the RDA ordination diagram, it seems that a high altitude negatively affects the presence of aquatic insects, in agreement with previous results for insect richness (e.g., Odonata, Carchini et al. 2005). Pond size in this study was found to be an important variable for some dragonfly species, such as *Anax imperator* and *Pyrrhosoma nymphula*. These species prefer larger ponds than pioneer species such as *Notonecta glauca*, which quickly colonize small and ephemeral water bodies. In general, the species richness is greater for larger areas as they encompass a greater breadth of environmental conditions and contain more niches, so they can accommodate more taxonomic groups. In fact, the area of ponds has previously been identified as a factor determining species richness in major macroinvertebrate groups such as Hemiptera (Savage 1982), Coleoptera (Nilsson 1984), Odonata (Oertli et al. 2002, Kadoya et al. 2004) and macroinvertebrates in general (Gee et al. 1997). In contrast to these results, Friday (1987) and Jeffries (1991) did not find a significant effect of area on the richness of macroinvertebrates.

Semi-natural grasslands within a 200 m radius around each pond were determinant for various aquatic insect species, such as *Anax partenope*, *Hyphydrus aubei*, *Haliphys variegates* and *Coenagrion scitulum*; these species are associated with well-preserved environments and are considered to be rare or uncommon in Italy (Carchini 1983, Utzeri and D'Antonio 2007). Semi-natural grasslands could therefore be considered as suitable habitats for aquatic insects, in contrast to urban areas and pastures, the latter may cause an increase in nutrients and reducing the richness of macroinvertebrates (Steinman et al. 2003).

The presence of torrents (refuges) within a 20 m radius around the ponds was a key factor for pioneer or ubiquitous species, such as *Cloeon dipterum*, *Acilius sulcatus* and *Libellula quadrimaculata*, which actively disperse (Tamanini 1979, Belfiore 1983, Carchini 1983). In fact, these aquatic connective elements may constitute an important pathway for aquatic insect dispersal and allow the rapid colonization of newly created ponds (Van de Meutter et al. 2006, 2007). From our results, an elevated degree of trampling by cattle seems to have a high impact on the species composition of aquatic insects. In particular, cattle and trampling can compromise pond stability, causing the destruction of eggs and larvae of aquatic insects, and can directly increase the turbidity of ponds due to an increased nutrient input via defecation and urination (Biggs et al. 1994, Declerck et al. 2006).

The most important community-structuring factors for the amphibian community were the local-landscape characteristics, such as pond area, shade cover and the proportion of land covered by urban areas. In general, the pond area was a significant predictor for amphibian species composition (Laan and Verboom 1990); our results show that various amphibian species, such as *Hyla intermedia*, *Rana skl hispanica* and *Triturus vulgaris*, prefer larger ponds. However, other studies have failed to find a species-area relationship for amphibians (Lehtinen and Galatowitsch 2000, Snodgrass et al. 2000, Hartel et al. 2009).

The occurrence of most species seems to be negatively associated with the proportion of land area shaded by riparian vegetation, although this element is generally considered a useful foraging, hibernating, and traveling habitat for many amphibians (Guerry and Hunter 2002). This contrasting result is probably at least partially due to the short range variability in shade cover, which renders it difficult to understand its influence on amphibian communities.

In this study, the proportion of land covered by urban areas had a negative effect on the amphibian communities. Strong dispersal barriers (mainly roads) and isolation from source ponds probably explain why few species are able to colonize ponds (Lehtinen and Galatowitsch 2000, Rubbo et al. 2005, Pillsbury and Miller 2008); for example *Triturus vulgaris* and *Rana dalmatina* were negatively associated with the presence of high-traffic roads, as shown in Hartel et al. (2010). A lack of appropriate terrestrial habitats near a pond may also limit the establishment of some species. Habitat loss and fragmentation usually occur together and have

undoubtedly led to greater pressure on a number of pond species, due to the reduction of dispersal and colonization opportunities.

Several studies have examined the relationships between community freshwater systems from the point of view of species richness alone, whereas community composition has rarely been studied explicitly, particularly in ponds (Briers and Biggs 2003, Paavola et al. 2006). Species richness represents only one measure of species diversity (*sensu* Whittaker 1960), and does not represent the inter-relationships between different taxonomic groups. Indeed, additional data on the relationships between community assemblages are urgently needed to avoid making false conclusions regarding the management requirements of many freshwater habitat groups. This is particularly important for relatively neglected habitats such as ponds, and for taxonomically difficult yet species-rich macroinvertebrate groups.

In conclusion, our results could have important implications for the management of existing ponds and the creation of new ones. In fact, colonization by aquatic insects was found to be constrained by the characteristics of the landscape in which farmland ponds were created or restored. Specifically, much of the variation in the species composition of aquatic insect communities was attributed to the pond area, the presence of torrents (refuges), and the amount of semi-natural grasslands.

The local characteristics of ponds, such as area and altitude, were determinant factors for amphibians and hence could be a key to successful colonization by amphibians in pond restoration projects focusing on pond-breeding amphibians. Furthermore, plant community composition was relatively closely correlated with that of amphibians and therefore has important practical implications for pond conservation.

The creation of new ponds should preferentially take place close to semi-natural grasslands and other wetlands, in order to maintain greater connectivity, and away from urban areas. Moreover, larger ponds are preferable for the preservation of pond biodiversity.

Even though the traditional function of ponds in agriculture may have changed, they remain an important landscape feature. The management and conservation of ponds may be necessary to ensure the protection of habitats, the survival of individual species and overall pond biodiversity. It is therefore important to focus on studies at the local scale because conservation planning and management decisions are most likely to be made and be most effective at the level of habitats and landscapes (Prendergast et al. 1999, Kerr et al. 2000). Further research is required to increase our understanding of pond habitats and ecology, and the importance of maintaining connectivity within ponds at the local scale.

Acknowledgments: This study was funded by the European Commission's LIFE-Nature program (LIFE99NAT/IT/006229 and LIFE04NAT/IT/000173) and by the "Comunità Montana del Monte Amiata-Area Grossetana". We also wish

to acknowledge the Regional Agency for Innovation and Development in Agriculture for providing climatic data.

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Received April 30, 2010
 Revised July 23, 2010
 Accepted November 3, 2010

Appendix

Table A1. Species scores for aquatic insect communities. Table A2. Species scores for amphibian communities. The files may be downloaded from the web site of the publisher at www.akademai.com.