The influence of habitat features on amphibian distribution in Northeastern Greece

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Environmental and isolation variables relating to abundance of breeding amphibians, species richness and community structure at different spatial scales were examined in the Dadia-Lefkimi-Soufli Forest National Park, Evros, Greece. Logistic regression and a generalized linear model were used to relate several habitat characteristics to species occurrence and species richness. The community structure responses to breeding-pond features were examined at four spatial scales using canonical correspondence analysis (CCA). The richest communities live in low-altitude ponds, with stony or clay bottoms, high solar exposure and abundant submerged and floating vegetation. The CCA models were significant ($p < 0.005$) and revealed the influence of altitude, percentage of field and broadleaf forest coverage, and number of water bodies on amphibian species assemblages at all four spatial scales. There is a specific need for holistic management of amphibians that will consider habitat connectivity, particularly between aquatic and terrestrial habitats, at a larger, more interconnected scale.

Keywords: amphibians; aquatic and terrestrial habitats; spatial scales; community structure

Introduction

Populations of amphibians are declining globally and there is a growing urgency to detect causative factors, including habitat loss, environmental pollution and climate change, all of which are becoming more pressing (Alford and Richards 1999; Dodd and Smith 2003; McCallum 2007; Dodd 2009; Blaustein et al. 2011). Increasing degradation of crucial habitats due to human activities, such as the replacement of natural habitats with cultivated land, industrial sites and road network development, contributes significantly to the decline of amphibian populations. Consequently, there is an urgent need to create and implement effective strategies that maximize conservation impacts (Gascon et al. 2007), and take into consideration amphibian distribution, which clearly depends on habitat characteristics (Bosch et al. 2004; Burne and Griffin 2005; Dayton and Fitzgerald 2006). Despite the pressing call to investigate patterns of distribution and abundance of amphibian populations (Cushman 2006), some obstacles exist to an improved understanding of amphibian ecology and the identification of key habitat requirements (Herrmann et al. 2005). According to Beebee and Griffiths (2005) a non-trivial issue is how to define a population. It has been common practice
to treat a breeding assemblage as a population, which may be unsafe for instance in habitats where multiple temporary ponds occur in close proximity.

Many amphibian species move between different types of habitats for reproduction, feeding and hibernation using habitat corridors, which interconnect aquatic and terrestrial sites. Maintenance of such wildlife corridors is crucial for the long-term conservation of amphibian populations through the process of metapopulation dynamics (Semlitsch 1998; Gibbs 2000; Semlitsch 2002). Water requirements influence the patchy distribution of amphibians, in particular during the breeding period (Dodd 2009). Hence, amphibians can survive in isolated patches for years only if these patches constitute suitable breeding habitats that are also surrounded by suitable terrestrial habitats. Patches of suitable habitats exist on several scales, depending mostly on the stage in the life cycle of the amphibian. Recent studies have shown the significance of ecological and landscape features at multiple spatial scales for this taxonomic group (Ficetola and De Bernardi 2004; Price et al. 2004; Fortuna et al. 2006; Ficetola et al. 2011). Amphibians depend on a variety of habitats, so they are especially at risk from habitat degradation, in particular when the degradation of one or more of their required habitat types occurs (Wells 2007). However, habitat features significantly related to amphibian abundance at one spatial scale might be of little importance at another (Levin 1992). As a result, deforestation and other forms of habitat degradation may eliminate aquatic-breeding amphibians even if the aquatic environment appears unaltered (Wells 2007). Similarly, if wetland management plans also fail to provide protection to surrounding terrestrial habitats via interconnected habitat corridors, then amphibian populations of the managed wetland may decline.

It is essential to acknowledge the complexities of ecosystem functions and the conditions that favour species richness in order to put into place a high-quality environmental management scheme (Helmer and Scholte 1985; Mazaris et al. 2008). Management plans of protected areas have to deal with the habitat requirements of specific amphibian species, as some species exhibit a notable capacity to adapt to human-altered habitats while others appear to be strictly correlated with unaltered environments (Pafilis and Valakos 2012). Amphibian conservation goals should be based on comprehensive frameworks for the protection and improvement of their wetland habitats (Bousbouras and Ioannidis 1997).

We studied amphibian abundance and community structure (at different spatial scales) associated with environmental and isolation characteristics in the Dadia-Lefkimi-Soufli Forest National Park (DNP), Evros, Greece. This part of the province of Evros is considered to be one of the richest and most varied ecosystems in Greece (Helmer and Scholte 1985; Kati et al. 2007; Bakaloudis 2010). However, to date amphibians are one of the least studied groups of vertebrates in this area (Schindler et al. 2011). To contribute to efforts for amphibian conservation in Greece the main objectives of our study were: (a) to identify the environmental and isolation–disturbance variables relating to breeding amphibian abundance and species richness; (b) to define the pattern of community structure at different spatial scales; (c) to demonstrate the primary role of habitat diversity in amphibian conservation.

Study area and methods

Study area

The Dadia-Lefkimi-Soufli National Park is situated in Evros Prefecture, in northeastern Greece (Figure 1). It consists of a forest complex that extends over 43,000 ha
Figure 1. Map of the study area showing habitat types and locations of water body samples.

(hereafter, study area) including two zones of strict protection (core areas), which cover a total of 7250 ha. The study area is characterized by steep valleys covered by extensive oak (*Quercus frainetto, Quercus cerris, Quercus pubescens*) and pine (*Pinus brutia* and *Pinus nigra*) forests, and includes a variety of other habitats, such as cultivations,
fields, pastures, torrents and stony hills (Adamakopoulos et al. 1995). Dadia-Lefkimi-Soufli National Park is acknowledged for its high ornithological interest, as it is used for nesting, wintering and passage by birds of prey (Poirazidis et al. 1996, 2011; Skartsi et al. 2008). The area also hosts other valuable taxonomic groups of animals such as amphibians and reptiles (Denis 1989; Bakaloudis 2010), mammals, fish and insects (Kati et al. 2003). In particular, the herpetofauna of the Evros province is extremely rich holding a total of 39 species, which might be difficult to find in any other area of Europe (Helmer and Scholte 1985).

**Amphibian surveys**

Within the study area, 57 water bodies (streams, main rivers, temporary pools, artificial dams) were surveyed. Each water body was visited once per month during the reproductive period, from February to July 2007. Amphibian species richness was estimated according to the existence of breeding evidence. Sampling was based upon breeding phenologies of species occurring in eastern Greece (Helmer and Scholte 1985; Arnold and Ovenden 2002). During diurnal transect surveys (late morning until dusk), by the banks of water bodies, the presence of amphibians was detected using a combination of visual encounters and surveys of male calling. The calling males were recorded 5 minutes after approaching the study area for a duration of 10 minutes.

**Environmental and isolation–disturbance characteristics**

The habitat preferences of the amphibians were analysed at a multi-scale level. Each water body was characterized by several environmental and isolation–disturbance features (Table 1). We analysed amphibian breeding habitats at an individual water body level using multiple variables, including: geomorphological and water body environmental characteristics as well as habitat isolation–disturbance features. At the landscape level, to assess terrestrial preferences, we analysed the type of surrounding habitat with geomorphological variables and water body isolation characteristics at four spatial scales 250 m, 750 m, 1250 m and 2000 m. Water body characteristics were collected during fieldwork in May and July. Soil type was recorded in four classes (1 = clay, 2 = sand, 3 = gravel, 4 = stones). Aquatic vegetation and tree canopy coverage were recorded as the percentage cover of all sample surfaces (1 = 0–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, 5 = 76–100%). To estimate solar exposure, we measured the percentage of water body surface exposed directly to daylight during sunny days in May and July, at around 12 o’clock.

Geomorphology, surrounding habitat type, disturbance and isolation variables were measured using a Geographic Information System (GIS). The SPATIAL ANALYST extension of ARCgis® software (Environmental Science Research Institute Inc., Redlands, CA) was used to generate geomorphological variables of altitude and aspect of slope (Poirazidis et al. 2004). Vegetation and anthropogenic features (habitations, roads etc.) were digitized from detailed maps of the study area, based on ICONOS satellite imagery (1-m pixel resolution) taken in July 2001.

**Statistical analyses**

Breeding amphibian abundance was analysed at the water body level, while community structure was examined at four spatial scales.
Table 1. Environmental isolation–disturbance variables used to characterize amphibians’ presence and community structure.

| Variable description                                                                 | Abbreviation |
|-------------------------------------------------------------------------------------|--------------|
| **A: Environmental at water body level:**                                           |              |
| 1. Elevation (m)                                                                     | ELEV         |
| 2. Aspect – eastness (sine transformation from $-1$ to $+1$)                         | EAST         |
| 3. Water body’s surface (m$^2$)                                                     | SURF         |
| 4. Proportion of surface with aquatic vegetation (submerged and floating) (%)       |              |
|   1. 0–5%                                                                            | VEGET1       |
|   2. 6–25%                                                                           | VEGET2       |
|   3. 26–50%                                                                          | VEGET3       |
|   4. 51–75%                                                                          | VEGET4       |
|   5. 76–100%                                                                         | VEGET5       |
| 5. Proportion of surface covered with tree canopies (%)                             |              |
|   1. 0–5%                                                                            | CANOP1       |
|   2. 6–25%                                                                           | CANOP2       |
|   3. 26–50%                                                                          | CANOP3       |
|   4. 51–75%                                                                          | CANOP4       |
|   5. 76–100%                                                                         | CANOP5       |
| 6. Water body hydroperiod classification                                            |              |
|   1. ephemeral                                                                       | HYDROPER1    |
|   2. permanent                                                                       | HYDROPER2    |
| 7. Type of soil                                                                       |              |
|   1. clay                                                                            | SOIL1        |
|   2. sand                                                                            | SOIL2        |
|   3. gravel                                                                          | SOIL3        |
|   4. stones                                                                          | SOIL4        |
| 8. Solar exposure (%)                                                                |              |
|   1. 0–5%                                                                            | SUN1         |
|   2. 6–25%                                                                           | SUN2         |
|   3. 26–50%                                                                          | SUN3         |
|   4. 51–75%                                                                          | SUN4         |
|   5. 76–100%                                                                         | SUN5         |
| 9. Fish presence (y/n)                                                               |              |
| **B: Isolation and disturbance features (m) at water body level:**                  | DST_WATER    |
| 10. Distance from the nearest water body (wetland, pool, river)                      |              |
| 11. Distance from the nearest field                                                  | DST_FIELD    |
| 12. Distance from the nearest road                                                   | DST_PAVED    |
| 13. Distance from the nearest village                                                | DST_VILLAGE  |
| **C: Type of surrounding habitat (%) at four spatial scales:**                      |              |
| 250 m, 750 m, 1250 m, 2000 m:                                                       |              |
| 14. Mixed (pine and broadleaf) forest                                                | MIXED 250, 750, 1250, 2000 |
| 15. Pure pine                                                                        | PINUS 250, 750, 1250, 2000 |
| 16. Pure broadleaf forest                                                            | BROAD 250, 750, 1250, 2000 |

(Continued)
Table 1. (Continued).

| Variable description                              | Abbreviation                                                                 |
|---------------------------------------------------|------------------------------------------------------------------------------|
| 17. Fields                                        | FIELD 250, 750, 1250, 2000                                                    |
| 18. Openings                                      | OPEN 250, 750, 1250, 2000                                                    |

D: Geomorphologic variables at four spatial scales: 250 m, 750 m, 1250 m, 2000 m:

19. Elevation (m)                                   | ELEV                                                                        |
20. Aspect – eastness (sine transformation from −1 to +1) | EAST                                                                        |

E: Isolation features (m) at four spatial scales: 250 m, 750 m, 1250 m, 2000 m:

21. Number of water bodies in a radius of 250, 750, 1250 and 2000 m | N_WET_250, 750, 1250, 2000 |
22. Total number of species on the water bodies in a radius of 250, 750, 1250 and 2000 m | N_SP_250, 750, 1250, 2000 |

Kleinbaum et al. (1998) in his study suggests a ratio of $n \geq 5k$ (where $n$ is number of observation and $k$ is predictor variables) to decrease the effects of collinearity and autocorrelation in regression analyses. For this study, the suggested number of predictor variables was 13.

Initially, all variables were analysed for normality using the Kolmogorov–Smirnov test. When necessary to meet assumption of normality, arc and log-transformations were used. Pearson correlation analysis was used to identify independent variables that exposed correlations ($r \geq 0.7$) to consequently eliminate them from the models. None of the independent variables used in the final analyses were significantly correlated with one another ($p > 0.05$).

**Analysis at the water body level**

Logistic regression was used to relate species occurrence to chosen environmental and isolation–disturbance characteristics at the water body site level. A forward stepwise method was used to assess which characteristic should be added to the final model ($p$ to remove = 0.10). Hosmer and Lemeshow $R^2 (R^2_L)$ procedure was used to estimate the proportional reduction in the absolute value of the log-likelihood measure. We used the likelihood ratio method to select the variables that further reduced the log-likelihood of the model, until no new variable could reduce it by any significant value (Ficetola and De Bernardi 2004).

We used generalized linear models with a Poisson error and log-link function to identify the relationship between species richness and water body environmental characteristics as well as isolation–disturbance characteristics. We performed two model combinations: an “environmental” model and an “isolation–disturbance” model. We used Akaike’s information criterion (AIC) to compare the suitability of the models to describe species richness. Among different statistical models the one with the smaller AIC values is considered to be more appropriate (Drakou et al. 2011).
Analysis at the landscape level

After performing a de-trended correspondence analysis, the longest gradients (value > 4.0) revealed that the data are too heterogeneous and that too many species deviated from the assumed model of linear response (Leps and Smilauer 2005). Because of this result we decided to use the unimodal method of canonical correspondence analysis (CCA) to examine the relationship between amphibian community structure and both habitat and isolation features at four spatial scales (250, 750, 1250 and 2000 m) (Pillsbury and Miller 2008). The method is designed to extract synthetic environmental gradients from ecological datasets (ter Braak and Verdonschot 1995). It is a constrained ordination technique whose axes are a linear combination of environmental variables that best explains variation in a matrix of species abundances (ter Braak 1986; Argyroudi et al. 2010). The final diagram shows the position of species related to the characteristics that are considered to be the ecological optimum for our studied species (Kati et al. 2008). The variables were log-transformed as necessary to achieve normality. In the second step, a Monte Carlo permutation test with 1000 iterations was used to select and present in the CCA plots only the significant (p < 0.05) habitat variables that explained variation in community structure. Variance inflation factors were examined during the CANOCO method procedure and did not indicate a problem for any of our variables. When the variance inflation factors were > 10, they indicated an increase in the variance of coefficients. All analyses were performed using the software CANOCO 4.5 for Windows (ter Braak and Smilauer 2002).

Results

A total of 11 amphibian species was detected in 55 of the 57 examined water bodies of the study area. The maximum number of species in one water body was eight (n = 2) with most water bodies having four (n = 12) or five (n = 15) species. We did not detect an amphibian in just two of our studied water bodies (3.5%). We detected an average of 3.93 ± 2.186 (mean ± SE) species per water body surveyed. The frog species the marsh frog *Pelophylax ridibundus*, the stream frog *Rana graeca* and the agile frog *Rana dalmatina*, occurred in 48, 29 and 18 water bodies, respectively. The smooth newt *Lissotriton vulgaris*, the common toad *Bufo bufo*, the green toad *Pseudepidalea viridis* and the common tree frog *Hyla arborea* occurred in 28, 32, 25 and 23 water bodies, respectively. In contrast, the fire salamander *Salamandra salamandra*, the southern crested newt *Triturus karelinii*, the eastern spadefoot *Pelobates syriacus* and the yellow-bellied toad *Bombina variegata* occurred infrequently in our study water bodies (<15).

Species presence at the water body level

We modelled environmental parameters relevant to all amphibian species. Environmental as well as isolation and disturbance characteristics were good predictors for species occurrence at the water body level. Based on a forward stepwise logistic regression, the most important environmental features influencing the presence of breeding amphibians were altitude, solar exposure and clay type of soil. The most important isolation–disturbance features were distance from the nearest field and village (DST_FIELD, DST_VILLAGE). No significant effect was found due
Table 2. Logistic regression independent models for all amphibian species.

| Species                  | Coefficient | SE     | Exp (B) | Sig.   | R²   | % correct |
|--------------------------|-------------|--------|---------|--------|------|-----------|
| Bufo bufo                |             |        |         |        |      |           |
| ELEV                     | -0.014      | 0.005  | 0.986   | 0.004  | 0.36 | 71.9      |
| DST_VILLAGE              | -0.001      | 0.0001 | 0.999   | 0.003  | 0.36 | 71.9      |
| Pseudemidalea viridis    |             |        |         |        |      |           |
| ELEV                     | -0.019      | 0.006  | 0.981   | 0.001  | 0.43 | 78.9      |
| DST_VILLAGE              | -0.001      | 0.0001 | 0.999   | 0.002  | 0.43 | 78.9      |
| Bombina variegata        |             |        |         |        |      |           |
| DST_FIELD                | 0.34        | 0.064  | 2.31    | 0.02   | 0.38 | 91.2      |
| Hyla arborea             |             |        |         |        |      |           |
| DST_FIELD                | -0.001      | 0.0001 | 6.105   | 0.013  | 0.36 | 75.4      |
| SOIL1                    | 1.583       | 0.829  | 0.056   | 0.032  | 0.36 | 75.4      |
| Salamandra salamandra    |             |        |         |        |      |           |
| DST_FIELD                | 0.001       | 0.0001 | 1.001   | 0.02   | 0.31 | 86.0      |
| DST_VILLAGE              | 0.004       | 0.0001 | 0.992   | 0.011  | 0.31 | 86.0      |
| Triturus karelinii       |             |        |         |        |      |           |
| ELEV                     | 0.014       | 0.006  | 1.014   | 0.023  | 0.1  | 89.5      |
| Lissotriton vulgaris     |             |        |         |        |      |           |
| SOIL1                    | -2.59       | 0.917  | 0.075   | 0.005  | 0.24 | 75.4      |
| SUN5                     | 2.12        | 0.671  | 8.33    | 0.002  | 0.24 | 75.4      |
| Pelobates syriacus       |             |        |         |        |      |           |
| SUN5                     | 2.303       | 1.08   | 8.75    | 0.034  | 0.12 | 77.2      |
| SOIL1                    | 1.23        | 0.47   | 3.88    | 0.02   | 0.12 | 73.7      |
| Rana dalmatina           |             |        |         |        |      |           |
| DST_FIELD                | 0.1         | 0.002  | 1.075   | 0.029  | 0.31 | 80.7      |
| SUN2                     | 2.03        | 0.844  | 7.64    | 0.016  | 0.31 | 80.7      |
| Rana graeca              |             |        |         |        |      |           |
| SUN5                     | 1.761       | 0.764  | 5.816   | 0.021  | 0.42 | 78.9      |
| ELEV                     | -0.015      | 0.005  | 8.76    | 0.003  | 0.42 | 78.9      |
| VEGET3                   | 1.68        | 0.794  | 0.19    | 0.034  | 0.42 | 78.9      |
| DST_OPEN                 | -0.005      | 0.002  | 0.99    | 0.021  | 0.42 | 78.9      |
| Pelophylax ridibundus    |             |        |         |        |      |           |
| ELEV                     | -0.006      | 0.002  | 0.99    | 0.017  | 0.26 | 84.2      |
| DST_FIELD                | -0.018      | 0.007  | 0.98    | 0.009  | 0.26 | 84.2      |

to the aspect of slope, the water body surface and fish presence (Table 2). The results showed that for three species, the smooth newt, the southern crested newt and the eastern spadefoot, their presence does not depend on isolation variables. In contrast the fire salamander and the yellow-bellied toad are dependent only on isolation features. Correct classification (% correct predictions by the model) ranged from 71.9 for the common toad to 91.2 for the yellow-bellied toad.
Table 3. Species richness associated with environmental variables.

| Terms in model | Coefficient | SE  | Wald $\chi^2$ | Sig.   |
|----------------|-------------|-----|---------------|--------|
| VEGET4        | 1.305       | 0.66| 3.95          | 0.047  |
| ELEV          | -0.011      | 0.0031| 12.48  | $p < 0.001$ |
| SOIL1         | 2.20        | 0.6  | 3.26          | $p < 0.001$ |
| SOIL4         | 2.88        | 0.67 | 18.18         | $p < 0.001$ |
| SUN5          | 1.72        | 0.83 | 4.3           | 0.038  |

**Species richness**

The next objective of the paper was to assess both the environmental and isolation–disturbance variables that could influence species richness. According to the “environmental” model results, the richest communities live in water bodies at low altitudes, with high solar exposure, high submergent and floating vegetation and with stony or clay bottoms (Table 3, only statistically significant variables are presented). The water bodies with high species presence (seven or eight species) were situated 5–100 m above sea level, while no species was recorded at the highest situated water body (260 m). Aquatic vegetation was detected in only one of 12 water bodies with fewer than three amphibians. High solar exposure was identified as significant in almost all water bodies with more than five breeding species (eight water bodies), while high tree canopy coverage of water body surface was not found to be significant.

The results of the “isolation–disturbance” model did not reveal any influence on amphibian abundance.

The estimated AIC value for the “environmental” model ($AIC = 213.03$) was smaller than the “isolation–disturbance” model ($AIC = 225.12$), indicating this to be the more appropriate model describing species richness.

**Community structure and typical species**

The CCA models were significant ($p < 0.005$), showing that environmental variables and habitat isolation variables influenced amphibian species assemblages at all four spatial scales. Important environmental variables were altitude and the per cent of surrounding habitat as fields or mixed and broad-leaved forest. An important habitat isolation variable was the number of water bodies present at different spatial scales. Data variability and species–environment relationships differed across spatial scales (Table 4). At all scales, the first CCA axis (in the right part of the CCA diagrams) predicted the structure of the amphibian communities at high altitude, in forest-dominated landscapes, both with mixed (pine and broad-leaved trees) and pure broad-leaved forest. Four species typical of these communities, the fire salamander, the yellow-bellied toad, the southern crested newt and the agile frog, were placed along the first axis. It was discovered that at the 250-m spatial scale a combination of mixed and pure broad-leaved forest influenced the fire salamander’s and the yellow-bellied toad’s community structure, while at the larger spatial scales the only effect was that of the broad-leaved forest (Figures 2 and 3). In addition, the agile frog and the southern crested newt at the largest scale were dependent only on mixed forest. As the eastern spadefoot appeared to represent mixed and pure pine forest species at the 250-m scale
Table 4. Data variability and species–environment relationship at the four spatial scales.

| Spatial Scale | Axis 1 Eigenvalues | Axis 2 Eigenvalues | Axis 3 Eigenvalues | Axis 4 Eigenvalues | Total Inertia |
|---------------|--------------------|--------------------|--------------------|--------------------|---------------|
| 250 m         | 0.130              | 0.095              | 0.048              | 0.030              | 1.692         |
| Eigenvalues:  |                    |                    |                    |                    |               |
| Species–environment correlations: | 0.624 | 0.613 | 0.398 | 0.420 | 1.692 |
| Cumulative percentage variance of species data: | 7.7 | 13.3 | 16.1 | 17.9 | 1.692 |
| Cumulative percentage variance of species–environment relation: | 41.4 | 71.7 | 87.1 | 96.6 | 1.692 |
| Sum of all eigenvalues | | | | | 1.692 |
| Sum of all canonical eigenvalues | | | | | 0.313 |
| 750 m         | 0.166              | 0.085              | 0.034              | 0.016              | 1.692         |
| Eigenvalues:  |                    |                    |                    |                    |               |
| Species–environment correlations: | 0.654 | 0.575 | 0.427 | 0.385 | 1.692 |
| Cumulative percentage variance of species data: | 9.8 | 14.8 | 16.8 | 17.8 | 1.692 |
| Cumulative percentage variance of species–environment relation: | 54.3 | 82.1 | 93.2 | 98.5 | 1.692 |
| Sum of all eigenvalues | | | | | 1.692 |
| Sum of all canonical eigenvalues | | | | | 0.306 |
| 1250 m        | 0.222              | 0.104              | 0.034              | 0.022              | 1.692         |
| Eigenvalues:  |                    |                    |                    |                    |               |
| Species–environment correlations: | 0.723 | 0.652 | 0.407 | 0.403 | 1.692 |
| Cumulative percentage variance of species data: | 13.1 | 19.3 | 21.3 | 22.6 | 1.692 |
| Cumulative percentage variance of species–environment relation: | 57.7 | 84.8 | 93.7 | 99.3 | 1.692 |
| Sum of all eigenvalues | | | | | 1.692 |
| Sum of all canonical eigenvalues | | | | | 0.385 |
| 2000 m        | 0.190              | 0.110              | 0.042              | 0.022              | 1.692         |
| Eigenvalues:  |                    |                    |                    |                    |               |
| Species–environment correlations: | 0.671 | 0.664 | 0.441 | 0.356 | 1.692 |
| Cumulative percentage variance of species data: | 11.2 | 17.7 | 20.2 | 21.5 | 1.692 |
| Cumulative percentage variance of species–environment relation: | 51.7 | 81.4 | 92.9 | 98.9 | 1.692 |
| Sum of all eigenvalues | | | | | 1.692 |
| Sum of all canonical eigenvalues | | | | | 0.368 |

it was situated on the positive side of Axis 1, but it fell on the negative side of Axis 1, because at the largest scale (2000 m) it occurred in human-dominated sites.

The second CCA axis predicted the structure of amphibian communities in human-dominated landscapes at all four spatial scales. This was shown primarily by the green toad and the common toad, as they are placed along Axis 2. The number of nearby water bodies was a good indicator of the presence of the eastern spadefoot and the tree frog, but only at larger scales. Possibly as a result of its narrow range of occurrence, the southern crested newt was placed separately from other species on the diagrams. Finally, some species, such as the tree frog, the stream frog, the marsh frog and the smooth newt, are generalists and were placed in the centre of the diagrams.
Discussion

Breeding habitat selection

In this study, water body and disturbance–isolation characteristics significantly affected the pattern of the amphibians’ breeding distribution, but their impact differed from species to species. The results of the breeding habitat selection by the recorded species are in accordance with previous studies conducted in Mediterranean areas (Ildos and Ancona 1994; Sotiropoulos et al. 1995; Bousbouras and Ioannidis 1997; Ficetola De Bernardi 2004; Kati et al. 2007; Valakos et al. 2008).
Figure 3. Bi-plot of amphibian species with significant environmental and isolation variables at the 2000-m scale after a canonical correspondence analysis.

The effect of disturbance–isolation variables seems to be important for the fire salamander and the yellow-bellied toad, as both species inhabit forested areas and avoid anthropogenic landscapes (Table 2). According to Valakos et al. (2008), salamander populations found in anthropogenic landscapes and deforested habitats might be considered as relics of former forest occupants. The fire salamander and the yellow-bellied toad did not demonstrate anthropogenic preferences. Disturbance factors, such as human settlements and agricultural lands, might contribute to habitat isolation for these species resulting in a network of several patches. Any negative change in the landscape and an increase of patchiness may lead to an imbalance of local population dynamics.

The habitat of the southern crested newt’s habitat usually includes deciduous forest and agricultural sites up to 2000 m above sea level (Sotiropoulos et al. 1995). However, in our study, it was almost exclusively found in forested areas, but in only one case was
it detected in a human-dominated site. In particular, our work demonstrated a positive association with higher altitude for the southern crested newt.

As a rule, the agile frog occurs at sites far from cultivated areas (Kati et al. 2007) that have moderate solar exposure. For example, the agile frog occurs in sites covered with dense herbaceous vegetation (Valakos et al. 2008), which are in fact more abundant in forested landscapes. In contrast, the eastern spadefoot breeds in ponds with a clay bottom (where due to its fossorial habits it can easily dig a burrow in which it lives) and this species avoids rocky and compact soils with high solar exposure (Valakos et al. 2008). The stream frog avoids water bodies at high altitudes, with dense vegetation (Bousbouras and Ioannidis 1997) but selects sunny breeding sites in open landscapes. The marsh frog and the tree frog are highly opportunistic species that might occur in very heterogeneous habitats (Valakos et al. 2008); however, in our study they were detected mostly in cultivated sites and at low altitudes.

Lastly, the common toad and the green toad were found to avoid water bodies at high altitude (Kati et al. 2007) but were expected to occur close to human settlements (Valakos et al. 2008; Pafilis and Valakos 2012).

Species richness
Species richness seems to be influenced only by water body characteristics. We found that among our study water bodies, lush aquatic vegetation was an important habitat requirement for amphibian abundance (Ildos and Ancona 1994; Burne and Griffin 2005). More specifically, breeding-pond preference was positively influenced by vegetation complexity. The second habitat characteristic positively associated with species richness was solar exposure, as found previously by Ficetola and De Bernardi (2004). Sunny wetlands have a higher temperature with accelerated processes of decomposition and photosynthesis, and have consequently increasing food and dissolved oxygen availability (Dodd 2009). In general, shaded wetlands are associated with high tree canopy cover. Aquatic sites with low canopy closure provide better conservation value (greater species richness) than sites that are completely covered by forest canopy (Burne and Griffin 2005). In addition, forest succession can lead to an increase in canopy closure and thereby reduce the suitability of amphibian breeding habitats. Forest expansion is one of the main threats to overall biodiversity, especially for birds of prey in the Dadia-Lefkimi-Soufli Forest National Park (Catsadorakis et al. 2010). The indication from our study is that forest expansion may also have a high negative impact on amphibian species.

Care needs to be taken in implementing conservation measures aimed at increasing species richness because this might negatively influence some species. For example, the yellow-bellied toad was negatively associated with rich aquatic vegetation and the agile frog preferred less sun-exposed wetlands for breeding sites. These habitat characteristics were associated with reduced species richness.

Fish presence
Our analyses failed to reveal a relationship between amphibian occurrence and the presence of fish in a particular water body, even if such a link was discovered in other surveys (Ficetola and De Bernardi 2004). This relationship might have been
detected if the amount of eggs and larvae in each pond could have been estimated. Fish often prey on the larvae of newts and frogs and their occurrence regularly originates from human intervention, for instance by introduction of fish for recreational fishing. Further studies are needed to quantify the impact of fish on amphibian populations in a Mediterranean setting.

**Community structure at different spatial scales**

The most important and statistically significant environmental variables that influenced amphibian community distribution at all four spatial scales (Figures 2 and 3) were altitude, the presence of forest and cultivated areas, and the number of water bodies.

**Agricultural landscape**

The negative effect of cultivated landscapes is well recognized and reported by other researchers (Dodd and Smith 2003; Dodd 2009). Some amphibians might be adapted to human-altered habitat (Rubbo and Kiesecker 2005). These species tend to favour open areas and to be more abundant in agricultural landscapes than in forested ones (Wells 2007). Other species occurring in human-dominated habitats at lower altitudes, such as the common and green toads, benefit from small artificial ponds, which are more abundant in the cultivated zone, because they are used for irrigation and other agricultural purposes. Such pools are acknowledged as important amphibian breeding sites (Kati et al. 2007) only when the traditional agricultural practices are used and the surrounding habitat has not degraded.

Properly managed, the occurrence of ponds within agricultural land can effectively increase the total amount of breeding habitat and help to sustain amphibian populations (Knutson et al. 2004). In contrast, intensification and large-scale enlargement of agriculture (Helmer and Scholte 1985) does negatively affect the reproductive success of amphibians. In particular, the use of several fertilizers needs to be reduced, as it has been shown that nitrogen pollution elevates amphibian mortalities (Rouse et al. 1999; Ortiz et al. 2004). In this study we discovered a positive correlation of amphibian assemblages with cultivated sites. Therefore to minimize adverse impacts on amphibian communities, it is essential to promote traditional agriculture based on low chemical inputs and the cultivation of small fields comprising multiple cultivated crop rotation.

**Forested landscape**

Species like the fire salamander (Pafilis and Valakos 2012), the yellow-bellied toad, the southern crested newt and the agile frog are more common in forested landscapes with natural broad-leaved and mixed (coniferous with broad-leaved) stands at higher altitudes. The first two mentioned species are correlated with broad-leaved forested habitats, which provide suitable terrestrial microhabitats for foraging and hiding under fallen leaves (Valakos et al. 2008). The homogeneous coniferous forest seems to have no significant effect on amphibian representatives of forested habitats at all spatial
scales. The pattern of selection of forest type by the fire salamander and the yellow-bellied toad changed at increasing spatial scales from a preference for mixed and broad-leaved forest at small scales to only broad-leaved forest at larger scales. These species, as well as other taxa, and in fact the entire ecosystem may have been affected by the removal of the original oak forest and its replacement by pine plantations in the 1970s (Adamakopoulos et al. 1995). It has been shown that even broad-leaved stands of small size within conifer forests may effectively support the conservation of reptile diversity (Ioannidis et al. 2008). Forestry practices should lead to the least possible habitat alteration, especially of upland forests around vernal pools (like streams for the fire salamander) and other potential wetland breeding sites of high conservation concern.

Isolation characteristics
Isolation characteristics, such as the number of water bodies, were found to be a good indicator of the presence of both the eastern spadefoot at the three largest spatial scales (750 m, 1250 m and 2000 m) and the tree frog at the two largest spatial scales (1250 m and 2000 m). Amphibian metapopulation dynamics are influenced by the density and distribution of wetlands in the landscape (Semlitsch and Bodie 1998; Semlitsch 2000). Hence the positive relation between these species and the occurrence of nearby water bodies may suggest that both species live in metapopulations, with clustered ponds used as patches of suitable habitats. Hence, clustered wetlands can be collectively used by individuals of the same species, as they frequently move between nearby ponds, thereby characterizing the habitat network of the metapopulations (Ficetola and De Bernardi 2004).

Metapopulation capacity creates the threshold condition for persistence (Hanski and Ovaskainen 2000). As a result maximizing metapopulation capacity is equivalent to minimizing the risk of extinction. All things being equal, when the number of habitat patches is large, populations are more vulnerable, whereas when the number of habitat patches is small, populations are better adapted to survive (Ovaskainen 2002). Furthermore, environmental changes in habitat patches can turn suitable amphibian habitat into a population sink.

According to Vos and Stumpel (1995), tree frogs in richly structured areas are able to move across long distances of 1–2 km using the tree canopy. Due to the increasing presence of highways and roads, settlements and intensive farmland, the exchange of individuals of this species among neighbouring breeding sites may be hindered (Pellet et al. 2004).

Influence of spatial scales
In this study we discovered that at the smaller spatial scales the eastern spadefoot was positively affected by the presence of both homogeneous oak and mixed forests, whereas at larger scales the species was associated with cultivated landscapes, alternating between open habitats and mixed forest. Considering both the low resistance of this species to high air temperature and its fossorial life, the forest habitats seem to provide better refuges in times of drought. Besides, there was a positive correlation with the number of water bodies that might suggest the existence of a metapopulation where its
individuals might use two distinct landscape types, including forested and agricultural sites. Better reproduction conditions for the eastern spadefoot seem to occur in cultivated landscapes, as it breeds in ponds with a clay bottom (Table 2), which in this study area are more abundant in cultivated land. Consequently, the intensification of agriculture, especially arising from the use of nitrogenous fertilizers and the homogenization of landscapes, may negatively affect this species. Water pollution might therefore be contributing to the disappearance of the most sensitive species (Ortiz et al. 2004).

Conclusion
According to our results, amphibian populations need breeding sites that are characterized by a variety of aquatic conditions, which are surrounded with a mosaic landscape. Inclusions of provisions for habitat connectivity, particularly between aquatic and terrestrial habitats (Dodd 2009), and the management of all aspects of habitats at multiple spatial scales, instead of using individual components (Bosch et al. 2004; Lindenmayer et al. 2007), is required to ensure effective amphibian conservation efforts. Protection of sites where amphibians occur and especially of those habitat types that are characterized by an extremely high diversity of amphibians must be integrated in the management plans for the Dadia-Lefkimi-Soufli Forest National Park as well as in other protected areas.

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