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Primary Research Paper

Breeding habitat selection of *Salamandra salamandra* (Linnaeus, 1758) in the most arid zone of its European distribution range: application to conservation management

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Abstract

The influence of environmental variables on the selection of a water body as breeding habitat by *Salamandra salamandra* was studied in an arid zone located in the southwestern part of its distribution range. From November 2002 to October 2003, 50 water bodies were monitored in the south east of the Iberian Peninsula. Environmental data were submitted to a stepwise logistic regression analysis at macrohabitat, water body typology and microhabitat scales in order to establish the main factors influencing the use of a given water body as breeding habitat by this species. A significant degree of dependence between the reproduction of *Salamandra salamandra* and environmental variables was observed at all of these levels. These results should be taken into account when populations of this species are subjected to management and/or recovery programmes in arid areas.

Introduction

Demographic trends in amphibian populations may be caused by natural fluctuations (Pechmann et al., 1991), although a growing number of publications suggest that the decline of amphibian populations all over the world is due to anthropogenic factors (Scoccianti, 2001; Marco, 2002a, b; Semlitsch, 2003). These factors include aspects dealing with overexploitation, habitat loss, disease and climatic change (Stuart et al., 2004). It has also been suggested that amphibian population declines are the result of complex interactions among these threatening stressors, which act synergistically (Gardner, 2001; Blaustein & Kiesecker, 2002). The response of amphibian populations to the same combination of threatening factors may vary depending on numerous factors such as habitat type, life stage or history of experiencing particular stressors (Gardner, 2001; Blaustein & Kiesecker, 2002). To help stop amphibian decline and for the correct management and conservation of amphibian species, the ecology and biology of individual species needs to be known (Ancona & Capietti, 1995).

Arid regions are characterized by a negative water balance, which creates an unpredictable environmental stress (Vidal-Abarca et al., 1992). Aquatic systems in these regions are subject to natural disturbances, including droughts and floods, because of their irregular hydrological regime both on an annual and pluri-annual scale. In the south-east of the Iberian Peninsula such characteristics are drastic (Vidal-Abarca et al., 1992). This area represents the southeastern border of the worldwide distribution range of *Salamandra salamandra* (Linnaeus, 1758) (Alcobendas & Buckley, 2002).

Salamandra salamandra is included in Annexe III of the Bern Convention and is considered vulnerable in the Spanish red book of amphibians and reptiles (Pleguezuelos et al., 2002). This conservation status emphasizes the importance of studying the biological and ecological characteristics of this species in order to develop proper management strategies. Moreover, *S. salamandra* shows a great genetic variation across its distribution range (Steinfartz et al., 2000; Montori & Herrero, 2004) and it has been proposed that differences in evolutionary histories may be the cause of adaptive differences in this species (Weitere et al., 2004). This fact makes it interesting to compare the habitat requirements of *S. salamandra* populations differing for their evolutionary histories. Nevertheless, although some papers concerning the breeding habitat selection of *S. salamandra* in different regions of Europe have been published (Ancona & Capietti, 1995; Augert & Guyetant, 1995; Babik & Rafinski, 2001), no such investigation has been undertaken in the Iberian Peninsula.

The aim of this study is to establish the main environmental factors that influence the use of a particular water body as breeding habitat by *S. salamandra* in the southwestern and most arid zone of its European distribution range.

Materials and methods

The study area is located in an eco-geographical sector of the Segura River basin (UTM 30SWH; SE Iberian Peninsula) (Vidal-Abarca et al., 1990) which extends over an area of about 150 km^2. This river basin is in the most arid zone of the Iberian Peninsula (Vidal-Abarca et al., 1987) and, probably, of Europe (Geiger, 1973). This eco-geographical sector is characterized by 500 mm of annual precipitation, a 4 month negative water balance and hydrological cycles that are severely disturbed by flash floods. It represents the most arid zone in the distribution range of *S. salamandra* (Alcobendas & Buckley, 2002).

The study was carried out from November 2002 to October 2003. During this period of time a total of 50 water bodies (Fig. 1) were monitored monthly (every 2 weeks during the breeding season). The different types of methodology used in this study included: dip-net (Babik & Rafinski, 2001; Bradley et al., 1994), visual inspection (Babik & Rafinski, 2001) and minnow-traps (Harrison et al., 1986). The selection of each methodology was decided *in situ*, depending on the observed water body characteristics. However, dip-net and visual inspection were used in all cases. The reproduction of *S. salamandra* was established by the detection of larvae in the monitored water bodies and their presence/absence was recorded for each sampling site.

At each sampling site, environmental variables concerning the main water body features were collected. These variables were classified according to macro- (500 m around sampling site, except altitude) and micro- (within sampling site) habitat scales. At the macrohabitat scale, environmental variables related to land uses, lithology, topography and altitude were considered (Table 1), and at microhabitat scale, the water sheet area, aquatic and riparian vegetation, substrate and the physicochemical characteristics of the water (Table 2). At each sampling site, variables concerning physicochemical characterization were measured five times during each visit.

In addition to the above variables, the typology of the water bodies was considered as corresponding to an intermediate spatial scale. The water body typologies studied included: drinking troughs (lentic permanent artificial small water bodies where cattle drink; they have vertical walls but with medium and small sized stones nearby and inside to make them easily accessible to amphibians); cisterns and ponds (lentic permanent artificial but naturalized water bodies used for farming purposes; their intermediate slopes make them accessible to amphibians); artificial pools (lentic medium-sized or large artificial water bodies used for agricultural purposes; their walls are vertical and amphibians usually cannot get out of them); streams (intermittent and natural water courses, totally accessible to amphibians). The distribution of these water body typologies in the study area is presented in Figure 1.

The presence/absence of reproduction in *S. salamandra* (dependent variable) and environmental variables (independent variables) were submitted to a stepwise logistic regression analysis to establish breeding site selection. This statistical analysis is the most frequently used ecological

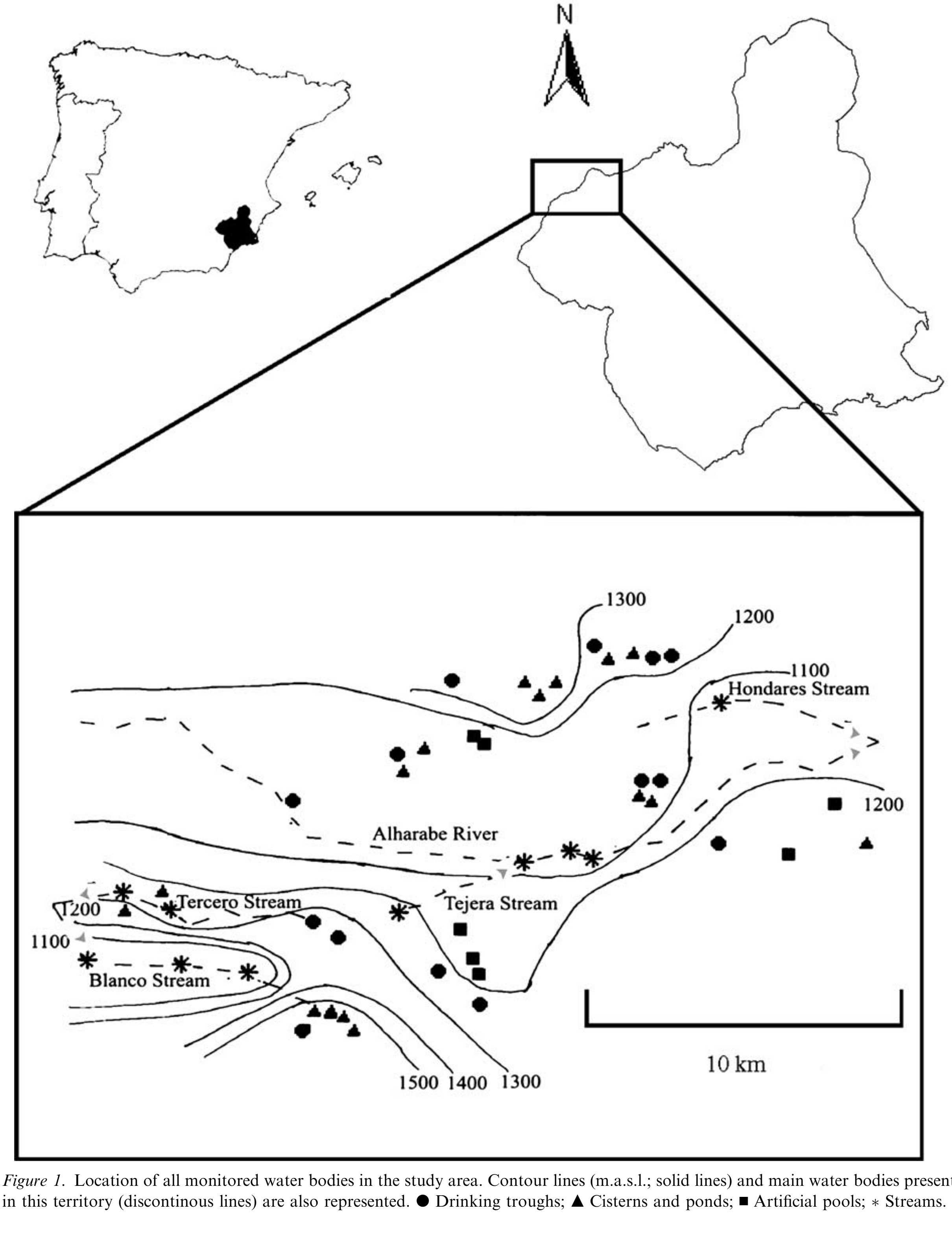

Figure 1. Location of all monitored water bodies in the study area. Contour lines (m.a.s.l.; solid lines) and main water bodies present in this territory (discontinous lines) are also represented. ● Drinking troughs; ▲ Cisterns and ponds; ■ Artificial pools; ∗ Streams.

Table 1. Variables considered at macrohabitat scale to establish breeding habitat preferences by *S. salamandra*

Variable	Units
Land use	Types: (1) Forest (Pine area, Holm-oak area, Savine area, Bush area); (2) Cattle; (3) Agricultural (Extensive arboreal crop area, Extensive herbaceous crop area); (4) Residential
Land use cover	Percentage of each land use type
Dominant land use	Dominant land use type measured *sensu* Bain (1999)
Land use mean	Mean of land use type measured *sensu* Bain (1999)
Land use heterogeneity	Typical deviation value of land use type measured *sensu* Bain (1999)
Lithology	Dominant lithology: (1) Limestone and compact dolomite; (2) Limestone and loam; (3) Limestone, loamy limestone and loam; (4) Lime conglomerate; (5) Loam, clay, limestone and sand; (6) Gypsum, loam and clay; (7) Loam; (8) Gravel and sand; (9) Blocks; (10) Pebble; (11) Rounded pebble. (García, 1999)
Topography	Types: (1) Steeply sloping; (2) Mountainous; (3) Intermediate
Altitude	Metres above sea level (m.a.s.l.)

modelling approach (Rushton et al., 2004) and has been successfully used in studies concerning different amphibian species (Vos & Stumpel, 1995; Hazell et al., 2001; Guerry & Hunter, 2002; Ensabella et al., 2003; Jakob et al., 2003; Ficetola & De Bernardi, 2004; Hazell et al., 2004). Stepwise logistic regression analysis were independently carried out on all environmental variables at each spatial scale.

To assess the influence of the environmental variables included in the logistic regression model on the reproduction of *S. salamandra*, deviance values were used. Differences between the null model and amplified model were tested through the Pearson chi-square (Silva & Barroso, 2004).

The statistical analyses were performed with the SPSS® statistical package and a significance level of 0.05 was accepted.

Table 2. Variables considered at microhabitat scale to establish breeding habitat preferences by *S. salamandra*

Variable	Units
Surface of water body	m^2 of water sheet
Aquatic vegetation	
Aquatic vegetation cover	Annual average percentage of aquatic vegetation cover
Aquatic vegetation heterogeneity	Annual typical deviation value of aquatic vegetation cover
Riparian vegetation	Types: (1) Absent; (2) Grass; (3) Bush; (4) Grass and bush
Riparian vegetation cover	Annual average percentage of riparian vegetation cover
Dominant riparian vegetation	Annual dominant riparian vegetation type measured *sensu* Bain (1999)
Riparian vegetation mean	Annual mean riparian vegetation type measured *sensu* Bain (1999)
Riparian vegetation heterogeneity	Annual typical deviation value of riparian vegetation type measured *sensu* Bain (1999)
Substrate	Types: (1) Living rock; (2) Sand and gravel; (3) Mud
Water body dominant substrate	Annual dominant water body substrate type measured *sensu* Bain (1999)
Water body substrate mean	Annual mean water body substrate type measured *sensu* Bain (1999)
Water body substrate heterogeneity	Annual typical deviation value of water body substrate type measured *sensu* Bain (1999)
Physicochemical characteristics of water	
Temperature	Annual average water temperature (°C)
Temperature heterogeneity	Annual typical deviation value of water temperature
PH	Annual average water pH-
pH heterogeneity	Annual typical deviation value of water pH
Conductivity	Annual average water conductivity (mS)
Conductivity heterogeneity	Annual typical deviation value of water conductivity
Spring and summer ion concentration	mg/L

Results

Salamandra salamandra larvae were detected in 44% of the sampling sites. 59% of these water bodies revealed the presence of *Alytes dickhilleni* Arntzen & García-París, 1995 larvae and 45.5% of them the presence of *Rana perezi* Seoane, 1885. No other amphibian species was detected in the same water bodies where larvae of the studied species were captured.

Table 3 shows the results of the stepwise logistic regression analysis carried out independently for macrohabitat, typology of water body and microhabitat scales.

The stepwise multiple logistic regression analysis revealed topography and altitude as the only significant variables ($p = 0.000$) on a macrohabitat scale influencing selection of breeding habitat by *S. salamandra* (Table 3). Figure 2 shows the significant positive selection this species presents in the study area for breeding in sampling sites located in mountainous zones. In addition to this, although the results obtained show that *S. salamandra* also selects as breeding habitat water bodies located at altitudes between 900 and 950 metres above sea level (m.a.s.l.), it mainly selects breeeding habitats located at altitudes higher than 1250 m.a.s.l. (Fig. 2).

In the intermediate spatial scale analysis, the environmental variable typology of water body was included in the logistic regression analysis. The results of this analysis showed the significance of this variable ($p = 0.011$), which is influencing the selection of breeding habitat by *S. salamandra* in the study area (Table 3). The reproduction of this species was confirmed in all the water body categories considered, except streams (Fig. 3). This result shows the significant positive selection this species presents for breeding in lentic water bodies in the study area.

As regards the microhabitat scale, sulphate concentration was shown to be the only significant variable ($p = 0.013$) determining selection of breeding habitat by *S. salamandra* (Table 3). This species selects with a significant degree of preference as breeding habitat water bodies exposed to low sulphate concentrations (Fig. 4).

Discussion

As in previous studies on breeding habitat selection of different amphibian species (Beebee, 1985; Ancona & Capietti, 1995; Augert & Guyétant, 1995; Ensabella et al., 2003), a large number of environmental variables was used to characterize the monitored water bodies as well as possible, due to the difficulty of foreseeing factors that may influence the selection of a certain water body as breeding habitat.

According to Krawchuk & Taylor (2003), a statistical hierarchical approach to data is essential for understanding the response of species to habitat structure. The statistical analysis presented in this paper avoid mistakes due to interactions between variables at each spatial scale, allowing for the influence of the environmental variables on the reproduction of the species to be ascertained at different scales separately. Consequently, the results obtained show the macrohabitat variables topography and altitude, water body typology and the microhabitat variable sulphate concentration as determining factors in the selection of a certain water body by *S. salamandra* in the study area.

At the macrohabitat scale, *S. salamandra* shows a preference for breeding in water bodies located in mountainous topography and at altitude higher than 1250 m.a.s.l. This could be related to the fact that areas located at high altitude and in mountainous areas permit the permanence of moister

Table 3. Result of multiple regression analysis for variables considered at macrohabitat, typology of water body and microhabitat scales

Spatial Scale	Deviance	Degrees of freedom	χ^2 Value	*p* Value	Cases correctly Classified (%)	Significant variables
Macrohabitat	20.961	13	44.381	0.000	88	Topography Altitude
Water body typology	54.286	3	11.056	0.011	66	Water body typology
Microhabitat	46.208	2	8.638	0.013	68.3	Sulphate concentration

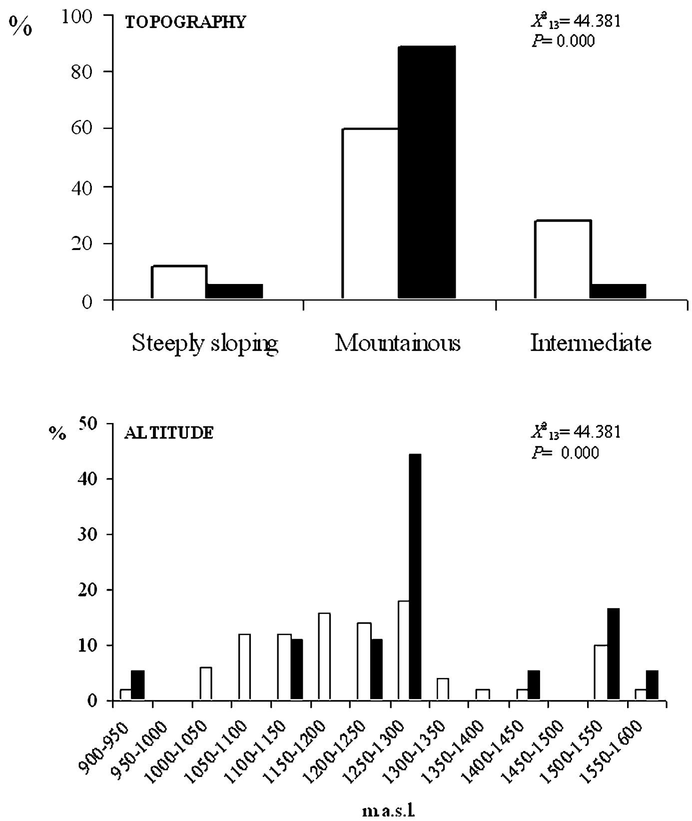

Figure 2. Bar chart showing the distribution of relative frequencies (%) of topography and altitude categories for the total number of sampling sites (white bars) and for the number of water bodies occupied by *S. salamandra* (black bars).

forests than in the rest of the study area. These forests are recognized as the typical environment to which adult *S. salamandra* individuals are associated (Bousbouras & Ioannidis, 1997; Salvador & García-París, 2001). So, like in the east of France (Augert & Guyetant. 1995), it is possible that *S. salamandra* breeds in water bodies located near forests where the terrestrial phases of this species

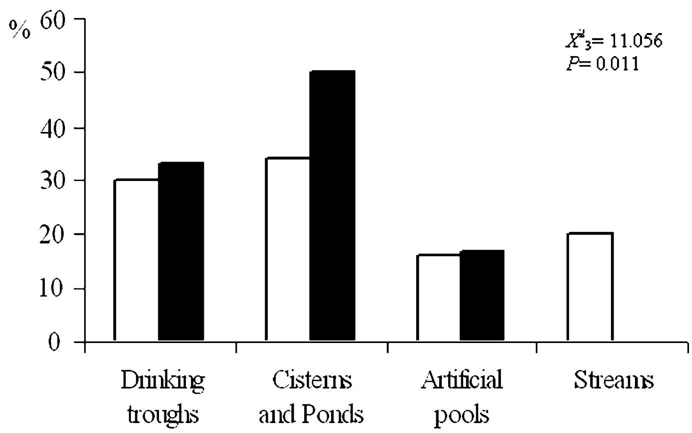

Figure 3. Bar chart showing the distribution of relative frequencies (%) of the categories of water body typology for the total number of sampling sites (white bars) and for the number of water bodies occupied by *S. salamandra* (black bars).

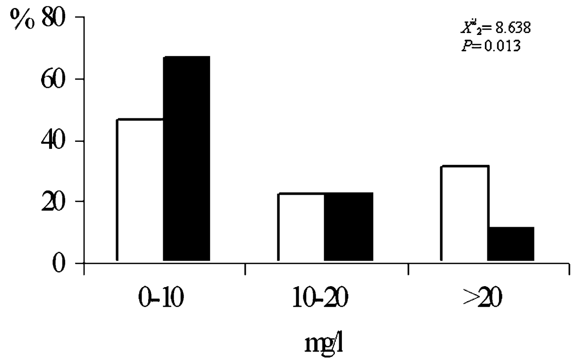

Figure 4. Bar chart showing the distribution of relative frequencies (%) of the categories of sulphate concentration for the total number of sampling sites (white bars) and for the number of water bodies occupied by *S. salamandra* (black bars).

inhabits, and no authentic breeding habitat selection exists at macrohabitat scale.

The results obtained in the present study differ from those presented by Ancona & Capietti (1995), who mentioned that *S. salamandra* preferred breeding sites located at low altitudes. This contradiction could be explained if it is taken into account that moist forests are restricted almost exclusively to high altitudes in the study area, while Ancona & Capietti's studied terrain can present these forests at low altitudes. The presence of moist forests would also explain the selection of water bodies located at altitudes comprised between 900 and 950 m.a.s.l.

As regards water body typology, the absence of reproduction of *S. salamandra* in any of the streams existing in the study area is remarkable. This fact contrasts with the information presented in previous studies performed in other areas (Ancona & Capietti, 1995; Bousbouras & Ioannidis, 1997; Babik & Rafinski, 2001; Weitere et al., 2004), where reproduction of this species was recorded in streams. Therefore, the results obtained show that *S. salamandra* selects lentic water bodies as breeding habitat in the study area. Although reproduction of this species has been detected in ponds (Degani & Kaplan, 1999; Reques, 2000; Spencer et al., 2002; Weitere et al., 2004), Babik & Rafinski (2001) and Salvador & García-París (2001) described streams as the main breeding habitat for this species in other regions of the Iberian Peninsula and Europe. However, according to Vargas & Real (1997), these differences could be explained by considering that *S. salamandra* would not be able to adapt to disturbances produced by floods, a phenomenon which often characterizes streams of semi-arid regions (Vidal-Abarca et al., 1992). Weitere et al. (2004) proposed that differences in ecological diversification within *Salamandra salamandra* are caused by local adaptations rather than environmental plasticity. So, the results obtained would suggest the possibility of genetic adaptations to an area with a very unpredictable water availability.

At the microhabitat scale, only sulphate concentration in the water body was seen to be determining breeding habitat selection by *S. salamandra*. In previous studies (Ancona & Capietti, 1995), the physicochemical variables of the water showed no effect on the selection of a given water body as breeding habitat. Moreover, Montori & Herrero (2004) point out that the reproduction of the species in clean and acid waters in northern areas of its distribution range is a consequence of available habitat rather than of a real selection. These considerations suggest that no real influence of sulphates on *S. salamandra* reproduction exists. As it has been previously mentioned, moist forests are important habitats for the survival of terrestrial phases of the species (Bousbouras & Ioannidis, 1997; Salvador & García-París, 2001), which selects lentic water bodies as breeding habitat in the study area. These water bodies present significantly ($U = 32.500$; $p = 0.003$) lower sulphate concentration (mean value: 1.66) than lotic water bodies (mean value: 2.83). So, the positive selection by the studied species of water bodies characterized by a low sulphate concentration may be a result of these water bodies corresponding mainly to lentic habitats located in areas where moist forests are well preserved. This reasoning would also explain the negative selection by *S. salamandra* of water bodies showing high sulphate concentrations, most of which correspond to streams, a habitat selected negatively as breeding habitat by the species.

In relation to water body substrate and to aquatic and riparian vegetation cover, *S. salamandra* showed its independence with regard to these variables. In the south of the Iberian Peninsula *S. salamandra* is an ovoviviparous species (García-Paris et al., 2003). So, it does not need a certain substrate or vegetation for spawning, in contrast to oviparous urodele species (Strijbosch, 1979; Miaud, 1995; Jakob et al., 1999; Babik & Rafinski, 2001).

In short, it has to be noticed that the results obtained point out the great importance of conserving the forests existing in arid areas where *S. salamandra* is present for the survival of its populations. Traditional farming is still practised close to these forests (Pérez & Lemeunier, 2003) and, as a consequence, the presence of numerous lentic water bodies is maintained. *S. salamandra* showed a breeding habitat preference for lentic water bodies (i.e. drinking troughs, cisterns and ponds, etc). This emphasizes the importance of recovering and conserving traditional farming to ensure the survival of this species, a measure already recognized as one of the most important actions for amphibian conservation (Scoccianti, 2001; Calhoun & Hunter, 2003). These conclusions should be taken into account when *S. salamandra* populations are subjected to management and/or recovery programmmes in arid zones.

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