

## Breeding habitat selection of an endangered species in an arid zone: the case of *Alytes dickhilleni* Arntzen & García-París, 1995

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**Abstract.** The influence of environmental variables on the selection of a particular water body as breeding habitat by *Alytes dickhilleni* was studied in the southeastern and most arid zone 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. Statistical analysis showed that the reproduction of *Alytes dickhilleni* is associated with the macrohabitat variable topography, and the water body typology. This species breeds mainly in permanent water bodies located in mountainous topography in the study area. These results should be taken into account when populations of this species are subjected to management and/or recovery programmes in arid areas.

**Keywords.** *Alytes dickhilleni*, breeding habitat, Iberian Peninsula, reproduction, selection.

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### INTRODUCTION

Despite demographic trends in amphibian populations may be caused by natural fluctuations (Pechmann et al., 1991), a growing number of studies suggests that the worldwide decline of amphibian populations is due to anthropogenic factors (Wake, 1991; Pechmann and Wake, 1997; Semlitsch, 2003). The most important factors are overexploitation, habitat loss, disease and climatic change (Stuart et al., 2004), as well as complex interactions among these threatening factors, which may act synergistically (Gardner, 2001; Blaustein and Kiesecker, 2002). The response of amphibian populations to the same combination of threatening factors may vary depending on numerous factors such habitat type, life stage

or history of experiencing particular stressors (Gardner, 2001; Blaustein and Kiesecker, 2002). These aspects make it essential to know the ecology and biology of individual amphibian species to stop their decline and for their correct management and conservation (Ancona and 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, such as droughts and floods, because of their irregular hydrological regimes 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, considered as one of the most important areas in the Mediterranean region because of the high species diversity and/or endemic amphibians found there (Borkin, 1999), represents the southeastern border of the worldwide distribution range of *Alytes dickhilleni* Arntzen and García-París, 1995 (García-París and Arntzen, 2002).

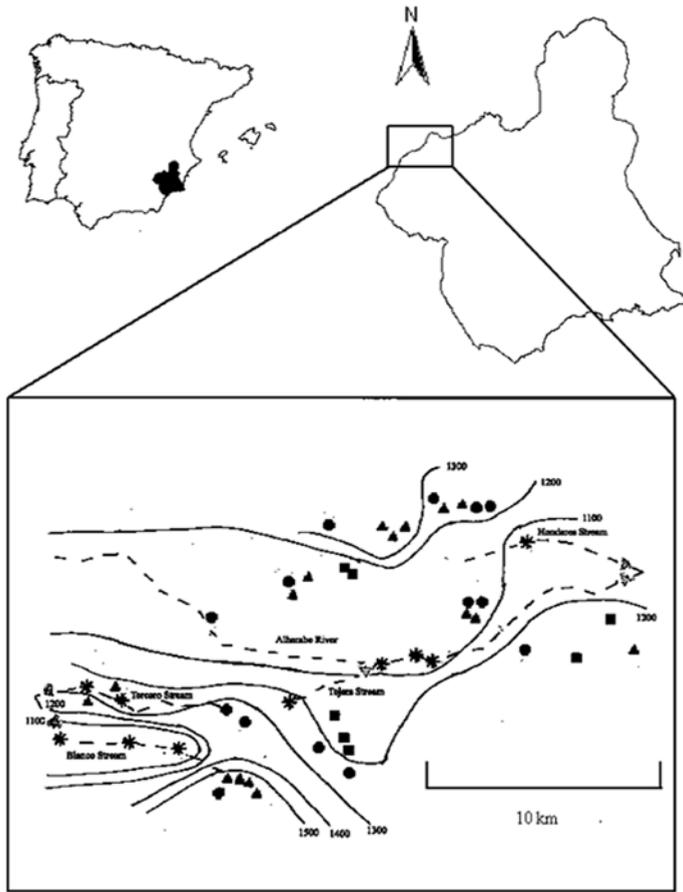
*Alytes dickhilleni* has been categorised as “vulnerable” by the IUCN (2004) and in the Spanish red book of amphibians and reptiles (Pleguezuelos et al., 2002). This conservation status emphasizes the importance of studying biological and ecological characteristics of this species in order to develop proper management strategies. Successful propagation of an individual’s genes depends on the selection of breeding habitat, among other factors (Duellman and Trueb, 1994). Nevertheless, although some publications describe the typologies of water bodies used for breeding by *Alytes dickhilleni* in the Iberian Peninsula, no detailed investigation about breeding habitat selection by this species has been undertaken. Therefore, 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 *Alytes dickhilleni* in the most southeastern and most arid zone of its worldwide distribution range.

## MATERIAL 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<sup>2</sup>. This river basin is included 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 *Alytes dickhilleni* (García-París and Arntzen, 2002).

The study was carried out from November 2002 to October 2003. During this period a total number of 50 water bodies (Fig. 1) were monitored monthly (every two weeks during the breeding season). The different types of methodology used in this study included: dip-net (Bradley et al., 1994; Babik and Rafinski, 2001), visual inspection (Babik and Rafinski, 2001) and minnow-traps (Harrison et al., 1986). The selection of each methodology was decided in situ depending on monitored water body characteristics. However, dip-net and visual inspection were used in all cases. The reproduction of *Alytes dickhilleni* was established by the detection of eggs and/or larvae in the water bodies monitored 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 macrohabitat (500 m around sampling site, except altitude) and micro- (within sampling site) habitat scales. Table 1 shows the variables considered



**Fig. 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 (discontinuous lines) are also represented.

● Drinking troughs; ▲ Cisterns and ponds; ■ Artificial pools; \* Streams

at the macrohabitat scale. Environmental variables land use cover and topography were determined by visual estimation during the field surveys. Altitude (error:  $\pm 5$  m) was established using a GPS receptor Garmin® eTrex Venture™ and lithology was obtained from the available cartography (García, 1999). At the microhabitat scale, environmental variables related to water sheet area, aquatic and riparian vegetation, substrate and the physicochemical characteristics of the water were recorded (Table 2). At each sampling site, variables for the physicochemical characterization (pH, temperature and conductivity) were measured five times during each visit using an universal pocket meter WTW® Multi340i. This measures were taken at surface level (< 15 cm depth) within a 5 h period (1100-1600 h). At the same time, in spring and summer, a water sample of each sampling site was taken and stored at -20 °C before being analysed by ionic chromatography to determine its ionic concentration. The water sheet area of each sampling site was in situ assigned to one of the classes shown in Table 2. In relation to the water body substrate, it was characterised using the methodology proposed by Bain (1999), which consists of categorizing the variable and of making at least 10 visual designations at each sampling site.

**Table 1.** Variables considered at macrohabitat scale to establish breeding habitat preferences by *Alytes dickhilleni*.

Variable	Units
Land use	Types: (1) Forest (dominant species: <i>Pinus nigra</i> Arnold, <i>Quercus rotundifolia</i> Lam., <i>Juniperus thurifera</i> L., <i>Brachypodium retusum</i> (Pers.) Beauv. and <i>Thymus</i> spp. L.); (2) Pasture; (3) Agricultural: Extensive arboreal crop area (species mainly harvested: <i>Prunus dulcis</i> (Mill) D.A. Webb) and Extensive herbaceous crop area (species mainly harvested: <i>Triticum</i> spp. L., <i>Hordeum</i> spp. L.); (4) Residential
Land use cover	Percentage of each land use type
Dominant land use	Dominant land use type measured <i>sensu</i> Bain (1999)
Mean land use	Mean of land use type measured <i>sensu</i> Bain (1999)
Land use heterogeneity	Typical deviation value of land use type measured <i>sensu</i> 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) Colluvial carbonated blocks; (10) Glacis carbonated pebbles; (11) Alluvial carbonated rounded pebbles. (García, 1999)
Topography	Types: (1) Steeply sloping; (2) Mountainous; (3) Intermediate
Altitude	Metres above sea level

This approach provides three new variables (mean water body substrate, dominant water body substrate and water body substrate heterogeneity). In order to obtain a more comprehensive environmental characterization of the sampling sites, Bain's methodology was also applied to the variables related to land use and riparian vegetation cover. In addition to the above variables, the typology of the water bodies was considered to be intermediate on a spatial scale.

The water body typologies studied included: drinking troughs (lentic permanent artificial small water bodies where cattle drink; although they have vertical walls, medium and small sized stones nearby and inside make them easily accesible to amphibians); cisterns and ponds (lentic permanent artificial but naturalized water bodies used for farming purposes; their intermediate slopes make them accesible to amphibians); artificial pools (lentic permanent medium-sized or large artificial water bodies used for agricultural purposes; their walls are vertical and amphibians usually cannot get out of them); streams (natural headwaters water courses, length < 2 km; totally accesible to amphibians).

The presence/absence of reproduction in *Alytes dickhilleni* (dependent variable) and environmental variables (independent variables) were submitted to a stepwise logistic regression analysis (backward method) to establish breeding site selection. This statistical analysis is the most frequently used ecological modelling approach (Rushton et al., 2004) and has been successfully used in studies on many amphibian species (Vos and Stumpel, 1995; Hazell et al., 2001; Guerry and Hunter, 2002; Ensabella et al., 2003; Jakob et al., 2003; Ficetola and De Bernardi, 2004; Hazell et al., 2004). Stepwise logistic regression analysis was carried out independently on all the environmental variables at each spatial scale.

Before performing the multiple logistic regression analysis, a multiple correspondence analysis was made at macro- and microhabitat scales in order to remove any interations between environmental variables which might result in false interactions between dependent and independent variables. Only the environmental variables which showed the highest value for one of the dimensions

**Table 2.** Variables considered at microhabitat scale to establish breeding habitat preferences by *Alytes dickhilleni*.

Variable	Units
Surface of water body	m <sup>2</sup> of water sheet. Classes: (1) 0-5; (2) 5-10; (3) 10-50; (4) > 50
Aquatic vegetation	
Aquatic vegetation cover	Annual average percentage of aquatic vegetation cover
Aquatic vegetation heterogeneity	Annual typical deviation value of aquatic vegetation cover Types: (1) Absent; (2) Herbaceous appearance vegetation (shorter than 10 cm); (3) Bushy appearance (taller than 10 cm); (4) Mixture of herbaceous and bushy vegetation
Riparian vegetation	
Riparian vegetation cover	Annual average percentage of riparian vegetation cover
Dominant riparian vegetation	Annual dominant riparian vegetation type measured <i>sensu</i> Bain (1999)
Mean riparian vegetation	Annual mean riparian vegetation type measured <i>sensu</i> Bain (1999)
Riparian vegetation heterogeneity	Annual typical deviation value of riparian vegetation type measured <i>sensu</i> Bain (1999)
Substrate	
Dominant water body substrate	Types: (1) Living rock; (2) Sand and gravel; (3) Mud Annual dominant water body substrate type measured <i>sensu</i> Bain (1999)
Mean water body substrate	Annual mean water body substrate type measured <i>sensu</i> Bain (1999)
Water body substrate heterogeneity	Annual typical deviation value of water body substrate type measured <i>sensu</i> 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

extracted by the analysis and the lowest for the rest were included in the multiple logistic regression analysis. Of all the variables initially included in the multiple correspondence analysis, these environmental variables can be considered as the main independent environmental variables.

To assess the influence of the environmental variables included in the logistic regression model on the reproduction of *Alytes dickhilleni*, deviance values were used. These values are a measure of the fitness of the logistic regression model, so that a low deviance value means high likelihood and, consequently, a good model (Silva and Barroso, 2004). Differences between the null model and amplified model were tested through the Pearson chi-square (Silva and Barroso, 2004).

Statistical analyses were performed with the SPSS<sup>®</sup> statistical package and a significance level of 0.05 was accepted (except for exceptions, where a significance level of 0.10 was accepted).

## RESULTS

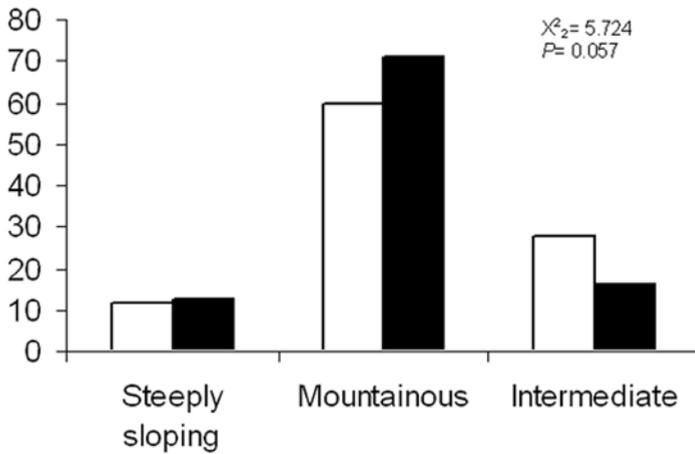
The reproduction of *Alytes dickhilleni* was confirmed in 62% of the sampling sites. Table 3 presents the proportion of these water bodies where companion amphibian species were detected.

**Table 3.** Proportion of sampling sites (inhabited by *Alytes dickhilleni*, n = 31) where companion species were found.

Species	% of localities
<i>Salamandra salamandra</i> (Linnaeus, 1758)	38.7
<i>Pelophylax perezi</i> Seoane, 1885	64.5
<i>Epidalea calamita</i> (Laurenti, 1768)	6.5
<i>Bufo bufo</i> (Linnaeus, 1758)	9.7
<i>Pelodytes punctatus</i> (Daudin, 1802)	6.5
<i>Discoglossus jeanneae</i> Busack, 1986	3.2

**Table 4.** Result of multiple correspondence analysis for variables considered at macrohabitat scale (in bold, variables included in multiple logistic regression analysis).

Variable	Dimension 1	Dimension 2
Land use		
Land use cover		
Forest use	0.919	0.916
<i>Pinus</i> area	0.245	0.151
<i>Quercus</i> area	0.0881	0.246
<i>Juniperus</i> area	0.111	0.157
<i>Brachypodium</i> and <i>Thymus</i> area	0.297	0.525
Cattle use	0.404	0.00281
Agricultural use	0.862	0.842
Extensive arboreal crop area	0.121	0.178
Extensive herbaceous crop area	0.838	0.474
Residential use	0.335	0.0402
Dominant land use	0.464	0.00841
Mean land use	0.780	0.854
Land use heterogeneity	0.244	0.610
Lithology	0.728	0.291
Topography	0.666	0.00780
Altitude	0.519	0.397



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

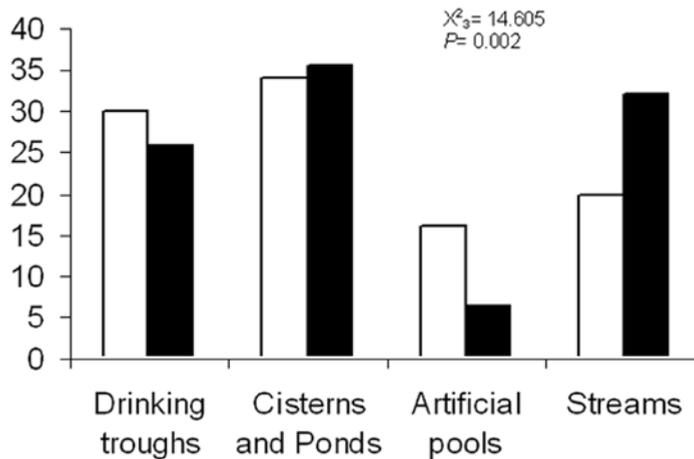
**Table 5.** Result of multiple regression analysis for variables considered at macrohabitat, typology of water body and microhabitat scales (\* significant  $P < 0.1$ ; \*\* significant  $P < 0.05$ ).

Spatial Scale	Deviance	Significative Variables	Degrees of Freedom	X <sup>2</sup> Value	P-Value	Cases Correctly Classified (%)
Macrohabitat	60.682	Topography	2	5.724	0.057*	70
Water body typology	51.802	Water body typology	3	14.605	0.002**	70
Microhabitat	48.114	-	4	-6.890	0.142	-

Table 4 shows the scores for each environmental variable in each dimension extracted by the multiple correspondence analysis at macrohabitat scale. Topography combined the highest value for dimension 1 (0.666) and the lowest for dimension 2 (0.00780). Land use heterogeneity presented the lowest value for dimension 1 (0.244) and the highest for dimension 2 (0.610). Only these two macrohabitat variables were included in the multiple logistic regression analysis.

The multiple logistic regression analysis revealed topography as the significant variable ( $\alpha = 10\%$ ;  $P = 0.057$ ) on a macrohabitat scale influencing the selection of breeding habitat by *Alytes dickhilleni* (Table 5). Fig. 2 shows the significant positive selection this species presents for breeding in sampling sites located in mountainous zones in the study area.

In the intermediate spatial scale analysis, the environmental variable typology of water body was included in the logistic regression analysis. The result of this analysis showed the significance of this variable ( $\alpha = 5\%$ ;  $P = 0.002$ ) in influencing the selection of breeding habitat by *Alytes dickhilleni* in the study area (Table 5). The reproduction of this species was confirmed in all the water body categories considered (Fig. 3). The results point to a significant negative selection for artificial pools and a positive selection of streams as breeding habitat in the study area. Although a high proportion of the sites where the reproduction of the spe-



**Fig. 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 the number of water bodies occupied by *Alytes dickhilleni* (black bars).

cies has been detected correspond to drinking troughs and cisterns and ponds, these typologies are not obviously selected by *Alytes dickhilleni* as breeding habitat.

As regards microhabitat scale, Table 6 shows the scores for each environmental variable in each dimension extracted by the multiple correspondence analysis at this spatial scale. Mean riparian vegetation showed the highest value for dimension 1 (0.718) and the lowest for dimension 2 (0.217). Temperature heterogeneity combined the lowest value for dimension 1 (0.169) and the highest value for dimension 2 (0.571). Only these two microhabitat variables were included in the multiple logistic regression analysis. None of these variables provided a significant multiple logistic regression model (Table 5).

## DISCUSSION

As in previous studies on breeding habitat selection by different amphibian species (Beebee, 1985; Ancona and Capietti, 1995; Augert and Guyétant, 1995; Ensabella et al., 2003), a large number of environmental variables was used to characterize the monitored water bodies as fully 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 and Taylor (2003), a statistical hierarchical approach to data is essential for understanding the responses of species to habitat structure. The statistical analysis presented in this paper avoids 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 variable topography and the variable water body typology as determining factors in the selection of a given water body by *Alytes dickhilleni* in the study area.

**Table 6.** Result of multiple correspondence analysis for variables considered at microhabitat scale (in bold, variables included in multiple logistic regression analysis).

Variable	Dimension 1	Dimension 2
Surface of water body	0.611	0.224
Aquatic vegetation		
Aquatic vegetation cover	0.263	0.393
Aquatic vegetation heterogeneity	0.194	0.253
Riparian vegetation		
Riparian vegetation cover	0.764	0.280
Dominant riparian vegetation	0.476	0.0698
<b>Mean riparian vegetation</b>	0.718	0.217
Riparian vegetation heterogeneity	0.437	0.286
Substrate		
Dominant water body substrate	0.00539	0.0339
Mean water body substrate	0.0366	0.0244
Water body substrate heterogeneity	0.0161	0.00583
Physicochemical characteristics of water		
Temperature	0.0823	0.241
<b>Temperature heterogeneity</b>	0.169	0.571
pH	0.129	0.0353
pH heterogeneity	0.0574	0.281
Conductivity	0.475	0.193
Conductivity heterogeneity	0.0719	0.302
Spring and summer ion concentration		
Fluorides	0.0440	0.125
Chlorides	0.121	0.132
Nitrates	0.130	0.186
Phosphates	0.0479	0.261
Sulphates	0.308	0.141
Sodium	0.369	0.288
Potassium	0.255	0.455
Magnesium	0.284	0.422
Calcium	0.498	0.0706
Lithium	0.346	0.139

At the macrohabitat scale, *Alytes dickhilleni* shows a preference for breeding in water bodies located in mountainous topography. This preference was seen to be significant at a level of 0.10. This positive selection could be explained if it is considered that traditional land uses are almost restricted to mountainous topography, where they allow the existence

of pine, holm-oak, savine and bush areas. These areas are recognized as the environment to which the presence of *Alytes dickhilleni* adult individuals is associated (Salvador and García-París, 2001). Hence, *Alytes dickhilleni* would breed in available water bodies located near the habitats where the terrestrial phases of this species are lived out, there being no an authentic breeding habitat selection at macrohabitat scale.

As regards water body typology, the reproduction of *Alytes dickhilleni* in the study area has been confirmed in water bodies from all the different typologies considered in the present study, which confirms the results presented in previous studies (París et al., 2002; Martínez-Solano et al., 2003). This environmental variable influences the reproduction of this species, which shows a positive preference for breeding in streams and a negative preference for artificial pools as breeding habitat. The influence that streams have on the reproduction of *Alytes dickhilleni* could be due to the fact that this typology consists of permanent small headwater watercourses. This would allow the species to finish its long larval development (García-París, 2004), and, additionally, would provide a way of dispersing larvae. The negative selection shown by the study species for breeding in artificial pools could be due to the fact that most of these water bodies in the study area are exposed to agricultural-related activities. Such activities include drastic changes in water level and, eventually, total dessication of the water body, cleaning and the addition of chemical products to kill aquatic flora and fauna. As a consequence of these actions, most *Alytes dickhilleni* larvae would die. Moreover, these water bodies have vertical walls, which represent an important obstacle for both breeding adults and metamorphic individuals of the species, which simply drown.

The results obtained show that *Alytes dickhilleni* shows no obvious preference for drinking troughs or cisterns and ponds as breeding habitat in the study area. Nevertheless, a high proportion of the sampling sites where the reproduction of this species has been detected corresponds to these typologies, which represent permanent water bodies where the species can complete its development (García-París, 2004). This suggests the importance of conserving drinking troughs, cisterns and ponds for the conservation of *Alytes dickhilleni* in arid zones, because these water bodies represent a shelter in areas where streams are characterized by disturbances such as droughts and floods (Vidal-Abarca et al., 1992), which could prevent larval development of this species.

At the microhabitat scale, no influence of environmental variables studied on *Alytes dickhilleni* reproduction was detected. This result would suggest the absence of any selection of a particular breeding habitat by *Alytes dickhilleni* at this spatial scale, probably as a result of the reproductive strategy of the species. The genus *Alytes* reproduces on land (Márquez, 1992) and male individuals carry the strings of eggs on their hindlimbs (Duellman and Trueb, 1994). When the larvae begin to hatch, the male toads sit in water and the larvae are released. Therefore, *Alytes dickhilleni* does not need a particular type of substrate or vegetation for spawning in contrast to other species of anuran amphibian such as *Pelodytes punctatus* (Guyétant et al., 1999) or *Hyla meridionalis* (Salvador and García-París, 2001). Hence, the detected independence of this species with respect to the microhabitat environmental variables considered suggests that the presence of water in a given water body for a long period of time would be the only condition for reproduction of *Alytes dickhilleni*, so that it could finish its larval phase, as was shown by Salvador and García-París (2001). Nevertheless, variables such as aquatic vegetation may have impor-

tant effects on larval survival, which would affect population recruitment rates and, lastly, breeding habitat selection. So, further studies concerning the growth, development, survival and condition of larvae or metamorphic individuals need to be performed to determine whether there is a real independence of the studied species in relation to microhabitat variables.

In short, it has to be noticed that the results obtained point to the great importance of conserving autochthonous vegetation in arid areas where *Alytes dickhilleni* is present for the survival of its populations. Traditional farming is still practised in regions where this vegetation is present (Pérez and Lemeunier, 2003), and so natural water quality and natural fluctuations of the water level in streams are preserved, as well as the presence and maintenance of numerous lentic water bodies (i.e. drinking troughs, cisterns and ponds, ...). *Alytes dickhilleni* showed a breeding habitat preference for permanent water bodies. 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 in amphibian conservation (Scoccianti, 2001; Calhoun and Hunter, 2003). So, although further studies, such as those concerning larval survival rates and body condition of metamorphic individuals, must be performed to assess fitness differentials for the studied species in different breeding habitats, these conclusions should be taken into consideration when *Alytes dickhilleni* populations are subjected to management and/or recovery programmes in arid zones.

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