



Amphibians in a human-altered wetland landscape: water matters, even when there is plenty

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Amphibians play an important role in ecosystems and are one of the most threatened taxa worldwide. In order to protect their populations, it is essential to understand the factors affecting their distribution, abundance and diversity. Baixo Vouga Lagunar is a heterogeneous, human-modified, coastal wetland in north-west Portugal, with seasonal or permanently flooded habitats. We characterised the patterns of distribution, abundance and diversity of amphibians and aimed to identify its environmental drivers. Species richness was best explained by distance to the nearest well, followed by presence of temporary water bodies. Distance to the nearest well was also the most important driver of the composition of local amphibian assemblages, followed by the distance to forested areas. Our results reflect the findings of other studies in the Mediterranean region that have revealed the importance of seasonally available habitats, and stress the need for conservation of natural as well as artificial water bodies and traditional agricultural practices which promote a mosaic of amphibian habitats.

Key words: Amphibians, anthropogenic habitats, conservation, Mediterranean wetlands, natural habitats, temporary water bodies

INTRODUCTION

Around 40% of amphibian species are threatened with extinction, and even a larger number present negative population trends (Hoffmann et al., 2010; IUCN, 2014). In Europe, about 25% of amphibian species are endangered (Temple & Cox, 2009). The high vulnerability of amphibians is in part due to features such as relatively low vagility (Weyrauch & Grubb Jr., 2004), dependence on highly specific microhabitat conditions (Cushman, 2006), and high philopatry (Wind, 1999). Because of their complex life cycle, amphibians are also susceptible to changes in aquatic and terrestrial environments such as habitat loss and fragmentation (Wilbur, 1980; Lehtinen et al., 1999).

Landscapes across Europe and, in particular, in the Mediterranean region have experienced significant changes in recent decades (Atauri & de Lucio, 2001; Moreira & Russo, 2007). Frequently, agricultural intensification can be harmful to amphibians (Beja & Alcazar, 2003; Pellet et al., 2004). However, there is evidence that some amphibian populations can also benefit from agricultural activity, and decline as a consequence of the abandonment of agricultural practices (Gray et al., 2004; Moreira & Russo, 2007).

In order to efficiently preserve amphibian populations, it is essential to understand the factors influencing their distribution. In the Mediterranean region, landscape

heterogeneity generates areas of high environmental value (Atauri & de Lucio, 2001; Martins et al., 2014). Due to a lifecycle that is tightly bound to water, amphibian distributions are influenced by the specific characteristics of aquatic sites (Beebee, 1983). For example, permanent water bodies characterised by a diversity of habitats can harbour rich amphibian communities conditionally that they are free of predatory fish (Laan & Verboom, 1990; Ficetola & Bernardi, 2004). Terrestrial habitats are further important for pond-breeding amphibians, providing feeding, overwintering or nesting sites (Marnell, 1998; Guerry & Hunter, 2002; Semlitsch & Bodie, 2003; Denoël & Ficetola, 2008). The occurrence of amphibians can be negatively affected by the presence of roads (Hels & Buchwald, 2001; Ficetola et al., 2009; Beebee, 2013) and positively influenced by the presence of habitat corridors, the extent of open areas, or riparian woodlands (Hartel et al., 2008, 2010; Ficetola et al., 2009; Hamer & Parris, 2011). In agricultural areas, small patches of forest combined with ponds located at the interface between cultivated areas and more natural landscape components have been shown to be favourable (Beebee, 1983; Beja & Alcazar, 2003; Weyrauch & Grubb Jr, 2004). Amphibian species richness generally decreases with increasing human population density (Pellet et al., 2004; Hamer & Parris, 2011), although for example constructions built for water retention such as wells and tanks can have a positive effect (Laan & Verboom, 1990).

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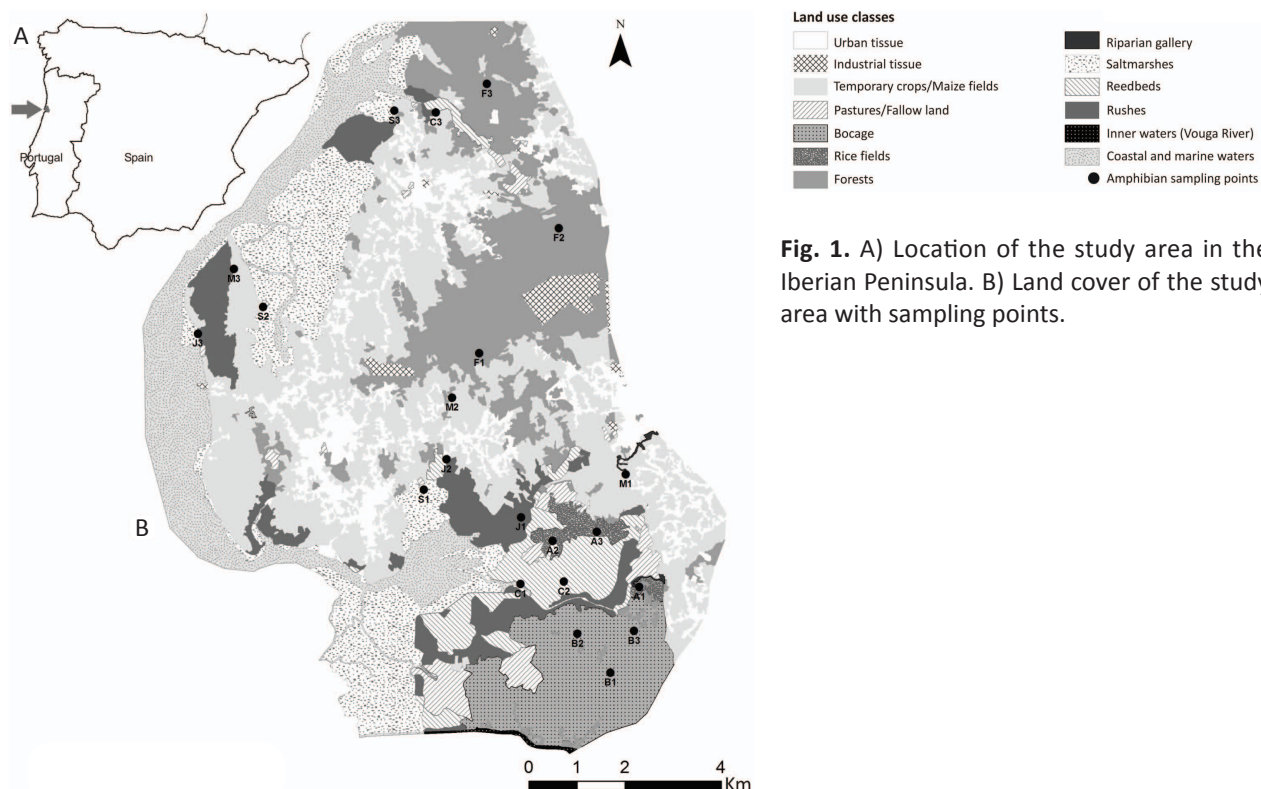


Fig. 1. A) Location of the study area in the Iberian Peninsula. B) Land cover of the study area with sampling points.

In the present study, we aim to identify the drivers of the distribution and diversity of amphibians in the human-altered Mediterranean wetland of Baixo Vouga Lagunar, Portugal. We predict that amphibian abundance and diversity will be negatively influenced by (i) the presence of the exotic crayfish *Procambarus clarkii*, a known predator of amphibians, and (ii) human activities such as urbanization, agriculture or forestry. We further predict that natural habitats will harbour a high diversity of amphibians. To test both predictions, we relate amphibian diversity measures with the distance and area of landscape features. We also predict that amphibian abundance and diversity will be enhanced by the abundance of insects, which represent part of their prey spectrum. Our study area comprises a mosaic of salt, brackish and freshwater, and we also predict that amphibian diversity will be higher where freshwater is more readily available.

MATERIALS AND METHODS

Study Area

The study area, Baixo Vouga Lagunar, is located approximately between 40°41'–40°50'N and 8°–8°41'W, and covers around 12,000 ha. This area corresponds to the north-east region of the Ria de Aveiro, a coastal lagoon wetland in north-west Portugal integrated in the Natura 2000 Network (PTZPE0004, PTCON0061).

The landscape of Baixo Vouga Lagunar consists of a mosaic of natural and human-modified habitats (Fig. 1). Natural habitats consist mainly of reedbeds (*Phragmites australis*, 4.5% of the study area), saltmarshes (mostly *Spartina maritima*, 12.8%) and rushes (*Juncus maritimus*, 6.7%). Anthropogenic habitats comprise forests (15%) mainly dominated by the exotic *Eucalyptus globulus* and the native maritime pine, *Pinus pinaster*. Farmlands

(29%) include maize fields (*Zea mays*, 28%) and rice paddies (*Oryza sativa*, 1%), and bocage (7.1%) consisting of cropland, pastures or fallow land divided by water ditches and hedgerows dominated by native trees and shrubs such as *Salix alba* and *Rubus ulmifolius* (Brito et al., 2010), harbouring high faunal richness (terrestrial mammals: Bandeira et al., 2013; bats: Mendes et al., 2014; birds: Special Protection Area for Birds PTZPE0004). The climate is Mediterranean, with a strong Atlantic influence (Bonmatí et al., 2006), with an annual average temperature of approximately 15–16°C and an annual average rainfall of 950 mm (IPMA, 2013). Air humidity ranges between 79% and 88% throughout the year, varying little throughout the day (Bonmatí et al., 2006). Strong winds are common during most of the year.

Data Collection

Fieldwork took place between October 2011 and September 2012. Sampling was performed at 21 sampling points, divided into groups of three replicates for each of the seven dominant habitats in the landscape mosaic (saltmarshes, rushes, reedbeds, rice paddies, maize fields, forests and bocage). Sampling points were at least 1 km apart in order to maximise sample independence. Several methodologies were applied. At each sampling point we installed a line of four pitfalls (with 3 m between buckets connected by a 50 cm high drift fence; Heyer et al., 1994; Machado, 2008), six arboreal pipe refuges (Johnson, 2005; Ferreira et al., 2012), and two aquatic funnel traps (Heyer et al., 1994). All traps were active for five nights every two months, and checked every morning. Additionally, we employed 500 m line transects, and sampled water bodies with a fish net at dusk and early in the night once a month (30 minutes/person each, Heyer et al., 1994; Machado, 2008). During the reproductive season of most species (March–May),

Table 1. Number of adults (and larvae) of each species, species richness (S), total number of adults (and larvae) (N), for each sampling point.

Species/Sampling Point		A1	A2	A3	B1	B2	B3	C1	C2	C3	F1	F2	F3	J1	J2	J3	M1	M2	M3	S1	S2	S3	Total
Alytes obstetricans																	(1)						(1)
Bufo spinosus				1					(1)								(2)						1(3)
Discoglossus galganoi									11(1)								1	5	2	6(5)			25(6)
Hyla molleri		1(11)	26	46		2		22	19		6(1)	(11)		17			2			7(4)			148(27)
Pelobates cultripes											1											1	
Pelodytes punctatus		1									6			3			2			17			29
Pelophylax perezi		145(89)	82(3)	232	1		3	28	30	72(15)	69(21)	12(12)		2(9)			52(28)	88(202)		2(8)		8	826(387)
Pleurodeles waltl																		1				1	
Rana iberica																	(7)					(7)	
Salamandra atra										(5)	4	1	5					9(1)					19(6)
Lissotriton boscai										(7)								1					1(7)
Triturus marmoratus										1	4	(1)			14(3)		1	1				13(1)	34(5)
N		147(100)	108(3)	279	1	2	3	50	49	84(29)	90(22)	13(24)	5	22(9)	14(3)	0(0)	58(38)	105(203)	2	32(17)	0(0)	21(1)	1085(449)
S		3	2	3	1	1	1	2	2	6	6	4	1	3	1	0	8	6	1	4	0	2	

an additional daytime sampling was performed on water bodies. Tadpoles were identified using Almeida et al. (2001).

In order to evaluate the effect of predation by the invasive crayfish species *P. clarkii* on amphibians, crayfishes captured in funnel traps and during water body sampling with fish nets were recorded and divided into adults and juveniles. The influence of the abundance and diversity of insects on the amphibian community was also assessed. Flying insects were recorded during nocturnal samplings with light traps placed on the ground, sufficiently far from amphibian traps and transects to reduce bias in amphibian sampling and active for a minimum of 2 hours per sampling occasion (Nabli et al., 1999). Insects were collected, stored in vials of 70% alcohol and subsequently identified to the level of Order.

Mapping of the study area and measurement of environmental descriptors

A detailed GIS database for the study area was developed in ESRI® software ArcGIS v.10.1. Mapping was primarily based on land-cover maps produced by Instituto Geográfico Português (COS2007) and complemented with information available from military maps (Instituto Geográfico do Exército, Portugal), satellite images (®ESRI Imagery Data) and field surveys. From the resulting GIS database, a set of environmental variables likely to affect the distribution of amphibians was measured within a buffer area of 250 m around each sampling point following Trenham (2001) and Trenham & Shaffer (2005). Initially, 59 environmental descriptors (Online Appendix 1) were quantified and grouped into six categories:

anthropogenic influence (all variables directly related to human presence in the area); distance to habitats (distance of each sampling point to the nearest point of each considered habitat); habitats (area of each considered habitat within the buffer and the Shannon Habitat Diversity Index of each buffer); water availability (all variables related to fresh and salt/brackish water); predation by crayfish (mean number of juveniles, adults, and the total number of individuals of *P. clarkii*); and insect availability (insect prey: average number of individuals (N), number of insect orders (S), diversity (Shannon-Wiener H index), and evenness (Pielou J index)).

Data Analysis

Data collected with all methods and from all sampling periods were pooled by sampling point. Analyses were based on tables of (1) abundance per species and sampling point (only adult captures), and (2) presence-absence (incidence) of each species (adults and larvae). We assessed the level of correlation between the different environmental descriptors for each category using the Spearman correlation coefficient estimated in R v. 2.15.3 (R Core Team, 2011). For each pair of descriptors that were highly correlated (Spearman correlation coefficient above 0.6), only the descriptor with the higher level of correlation with three different response variables (S, Shannon-Wiener H index, Pielou J index) was retained (taking the biological meaning of the correlation into account, Mladenoff et al., 1999). This approach resulted in a set of 21 variables used for GLMM analysis. The descriptors pertaining to *P. clarkii* numbers were tested separately. Environmental descriptors were not divided into classes for the ordination analysis. Therefore, we

Table 2. Selected environmental descriptors and best models ($\Delta AIC_c < 2$) explaining the variation in amphibian species richness, from different categories, with reference to the weight (ω_i) of the models in each category and to the percentage of explained deviance relative to the null model. The way each descriptor affects the species richness is given by the -/+ signal. Environmental descriptors with a significant effect on each of the best models: $p < 0.1$ (*); $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***)).

Environmental descriptors by category	Best Models ($\Delta AIC_c < 2$)	ω_i in category	% explained deviance
Anthropogenic influence	- <i>D_well</i> **	49%	11.5%
<i>L_asph</i> ; <i>Urban</i> ; <i>D_well</i>	- <i>D_well</i> ** - <i>L_asph</i>	31%	14.5%
Distance to habitats	- <i>D_forest</i> °	48%	4.9%
<i>D_forest</i> ; <i>D_fallow</i> ; <i>D_bocage</i>	+ <i>D_bocage</i>	18%	2.3%
Area of habitats	+ <i>Maize</i> ° - <i>Bocage</i> °	19%	12.0%
<i>Maize</i> ; <i>Fallow</i> ; <i>Bocage</i> ; <i>Salt</i> ; <i>Reedbed</i>	+ <i>Maize</i> **	16%	7.4%
	- <i>Bocage</i> *	16%	7.3%
	- <i>Bocage</i> * - <i>Reedbed</i>	7%	9.6%
Water availability	+ <i>Temp_water</i> **	32%	9.0%
<i>N_well</i> ; <i>D_brack</i> ; <i>L_fresh</i> ; <i>Temp_water</i>	- <i>L_fresh</i> + <i>Temp_water</i> *	14%	11.1%
Predation	- <i>PC_Adult</i>	35%	1.3%
<i>PC_Juv</i> ; <i>PC_Adul</i> ; <i>PC_Tot</i>	- <i>PC_Tot</i>	33%	1.1%
	- <i>PC_Juv</i>	32%	1.1%
Food availability (Insects)	+ <i>N_insect</i> *	51%	6.8%
<i>N_insect</i> ; <i>Hyme</i> ; <i>Dipt</i>			

also tested for correlation between environmental descriptors from different categories. We were able to further exclude five more variables (Maize, D_Bocage and D_fallow, PC_Tot and PC_Juv) that were highly correlated with at least two other descriptors.

Constrained analysis of principal components (CAPC) was performed with *R* software using the commands *step*, *anova.cca* and *capscale* from the package *vegan* (Oksanen et al., 2011), based on the set of 16 environmental descriptors mentioned above. The following approach was done separately for the incidence and the abundance data matrices. For selecting the most relevant set of descriptors, we performed a forward model selection (commands *step* and *capscale*) based on an increasing number of descriptors and weighted the models using Akaike's information criterion. This approach generated a best model with a subset of the environmental descriptors. We then tested the significance of the effect of each descriptor on the constrained ordination (command *anova.cca*, permutation test on each term). We retained the descriptors with a significant effect and repeated the previous analysis without these descriptors. We stopped the procedure when no more descriptors with significant effects were found. Finally, we performed a CAPC including only the environmental descriptors with significant or marginally significant effects.

In order to understand which environmental descriptors affect amphibian species richness, generalized

linear mixed models (GLMM) were performed using *R* and the *glmer* function of the *Lme4* package (Crawley, 2012). Models were built using a Poisson distribution of the error and a Log link function. Information on the *a priori* assignment of each point to one of the seven most representative habitats was included in the analysis as a random effect in order to discard its possible influence from the analysis (Crawley, 2012). First, models within each category (Online Appendix, Table 2) were sorted using the corrected Akaike Information Criterion (AIC_c , Burnham & Anderson, 2004), calculated from AIC estimated by the package *Lme4*. All models within each category that exhibited a ΔAIC_c lower than 2.0 were selected as plausible best models (Burnham & Anderson, 2004). In a second stage, we assessed the level of significance of the contribution of each environmental descriptor in each of the selected models in each category. We retained as plausible best models only those models where all the descriptors had a significant or marginally significant contribution to the model. Best models from all categories were sorted together by ΔAIC_c , after their relative weight and ΔAIC_c was re-estimated based on this set of best models. These best models were then compared based on their ΔAIC_c , weight and percentage of explained deviance relative to the deviance of the null model.

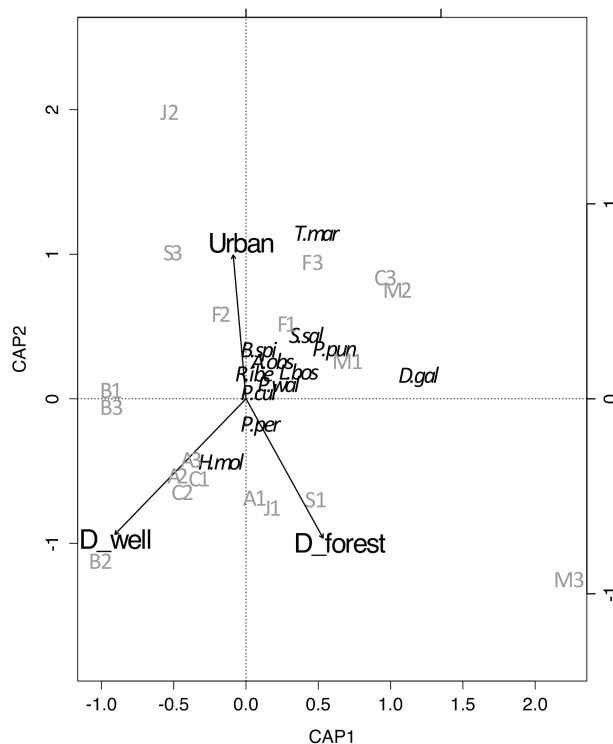


Fig. 2. Bidimensional plot resulting from the constrained analysis of principal components, based on species incidence data and a set of environmental descriptors with significant – D_well ($p=0.001$); D_forest ($p=0.003$) – or marginally significant effects – Urban ($p=0.065$) – on the constrained ordination. Sites are represented in bold grey font, species in black italic font and environmental descriptors by black vectors with regular black font.

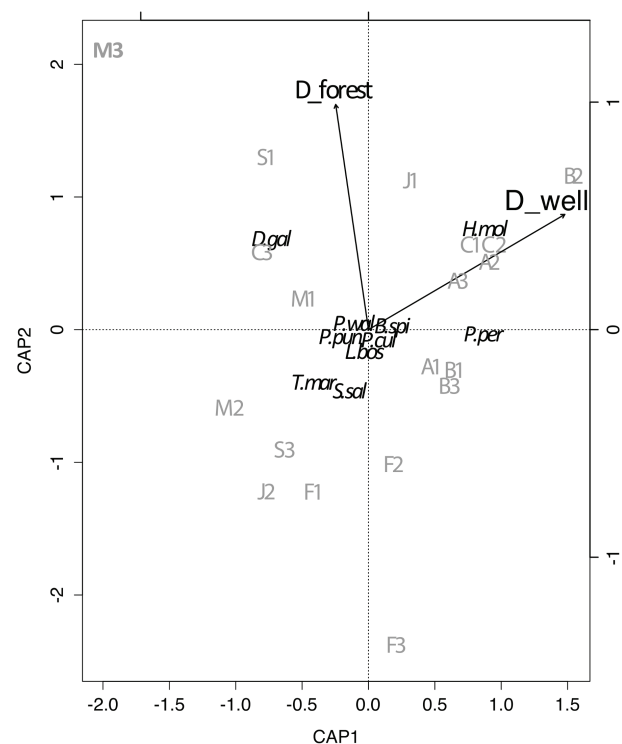


Fig. 3. Bidimensional plot resulting from the constrained analysis of principal components, based on abundance species data and a set of environmental descriptors with a significant effect – D_well ($p=0.002$); D_forest ($p=0.01$) – on the constrained ordination. Sites are represented in bold grey font, species in black italic font and environmental descriptors by black vectors with regular black font.

Table 3. Best models explaining the variation in amphibian species richness, from all categories of environmental descriptors, which include only significant or marginally significant descriptors. K - number of parameters in the model; AIC_c - corrected Akaike information criterion; ΔAIC_c - difference to the smallest AIC_c value; w_i - weight of model i in the set of partial models. The way each descriptor affects species richness is given by the +/- signal. Environmental descriptors with a significant effect on each of the best models: $p < 0.1$ (°); $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***).

Best Models (environmental descriptors)	K	AIC_c	ΔAIC_c	w_i	deviance	% explained deviance
- <i>D_well</i> **	3	76.6	0.00	43.9%	69.01	11.5%
+ <i>Temp_water</i> **	3	78.6	1.99	16.2%	71.00	9.0%
+ <i>Maize</i> ° - <i>Bocage</i> °	4	79.5	2.91	10.3%	68.66	12.0%
+ <i>Maize</i> **	3	79.8	3.22	8.8%	72.23	7.4%
- <i>Bocage</i> *	3	79.9	3.27	8.6%	72.28	7.3%
+ <i>N_insect</i> *	3	80.3	3.69	6.9%	72.70	6.8%
- <i>D_forest</i> °	3	81.8	5.19	3.3%	74.20	4.9%
Null model (Intercept***)	2	82.8	6.14	2.0%	78.00	-

RESULTS

Relationship between environmental descriptors and the local amphibian assemblages

Over the 12 sampling months, we captured 1534 amphibians from 12 species (Table 1; 449 larvae and 1085 metamorphosed individuals), largely corresponding to the species found by Loureiro et al. (2010). *Pelophylax perezi* and *Hyla molleri* were recorded in 15 and 11 of the 21 sampling points, respectively. *Pleurodeles waltl*, *Pelobates cultripes* and *Alytes obstetricans* were detected only once. Local species richness (Table 1) at the sampling points varied between zero (points J3 and S2) and eight (point M1).

Three environmental descriptors had a significant or marginally significant effect on the constrained ordination (CAPC) of the local species assemblages, represented by the matrix of the incidence of twelve species over the sampling sites: distance to closest well ($p=0.001$), distance to forest ($p=0.003$) and extent of urban area in the buffer ($p=0.065$, Fig. 2). The CAPC with this set of environmental descriptors explained a highly significant amount (47%, $p < 0.001$) of the total variation in the species incidence data. Only two environmental descriptors had a significant effect on the constrained ordination (CAPC) of the local species assemblages, represented by the matrix of the abundance of adults of ten species over the sampling sites (Fig. 3): distance to closest well ($p=0.002$) and distance to forest ($p=0.010$). The CAPC with this set of environmental descriptors explained a highly significant amount (31%, $p < 0.001$) of the total variation in the species abundance data.

Relationship between environmental variables and amphibian species richness

The number of selected environmental descriptors in each category ranged from three (anthropogenic influence, distance to habitats, insect availability) to five (habitats). Among the different categories, 14 models showed ΔAIC_c values below 2.0 (Table 2). The weight

of each model relative to all the models in the same category ranged from 7.0 to 49.0%. Explained deviance by each model relative to the deviance of the null model ranged from 1.1% to 12.0%. Not all models with $\Delta AIC_c < 2$ included environmental descriptors with a significant effect on the model.

Of the 14 best models, seven included only environmental descriptors with significant or marginally significant effects (Table 3). The percentage of explained deviance varied between 4.9% and 12.0%. The ΔAIC_c of the models ranged from 0 to 5.19 (6.14 including the null model). Only two models (*D_well* and *Temp_water*) had a ΔAIC_c below 2. Taking these seven models into account, the weight of each model ranged from 3.3% (*D_forest*) to 43.9% (*D_well*), with the first two models ($\Delta AIC_c < 2$) accounting for 60.1% of the weight of the different models. Four additional models, related to the extent of maize fields and bocage within the buffer and to the abundance of insects, had ΔAIC_c values below 4.

DISCUSSION

Despite the high level of human influence, Baixo Vouga Lagunar is characterised by high levels of vertebrate diversity boosted by a mosaic of habitats (Alves et al., 2014; Mendes et al., 2014; Marques et al., 2015). Our results support a high amphibian diversity for the area overall, although only four out of 21 sampling points harboured more than six species, and only two species (*P. perezi* and *H. molleri*) were recorded in more than half of the sampling points. We found that the main drivers of amphibian diversity were related to freshwater availability, in addition to other factors such as insect abundance.

The influence of predators and prey

Amphibians are predators and prey of both vertebrate and invertebrate species. Therefore, we expected that amphibian presence, abundance and species richness is negatively affected by the presence of an invasive predator, *P. clarkii*, and positively influenced by the abundance of prey such as insects. *Procambarus clarkii* is

widely recognised as a predator of amphibian larvae (Cruz et al., 2008). Food intake, on the other hand, positively influences amphibian development and reproductive performance (Wells, 2010) and may reduce also inter-specific competition (Toft, 1985; DuRant & Hopkins, 2008).

We found no effect of the abundance of *P. clarkii* on amphibian species diversity or composition, suggesting that the negative effects of predation are masked by other drivers of diversity. The abundance of insects as potential prey, on the other hand, was significant and explained almost 7% of species richness. None of the individual insect orders had a significant effect on its own, suggesting that the availability of nocturnal flying insects generally parallels favourable condition for amphibians (Davic & Welsh, 2004; Kovács et al., 2007).

The influence of human-altered and natural habitats

We expected amphibian assemblages to be more diverse in pristine habitats, and that, due to the combination of abundant freshwater, shaded areas provided by hedgerows and open areas provided by pastures, bocage landscapes would accommodate a wide variety of amphibians (Denoël & Ficetola, 2008; Hartel et al., 2008; Hamer & Parris, 2011). However, the extent of bocage area within buffer zones had a negative effect on the species richness. We observed that water in the bocage ditches was frequently stagnant and, perhaps, not suitable for amphibians. We also detected the presence of other aquatic predators such as fish (Hartel et al., 2007) that might limit the presence of amphibians.

Despite negative correlations between agricultural areas and amphibian diversity (Findlay & Houlihan, 1997; Vallan, 2002; Beja & Alcazar, 2003; Pellet et al., 2004), the negative effects of agricultural practices can be minimised if amphibians have access to less impacted areas (Knutson et al., 1999; Piha et al., 2007). Contrary to expectations, the extent of maize fields had a positive effect on species richness in our study. Maize fields in our study area are frequently associated with an abundance of fresh water bodies such as wells, since maize is a very water-demanding crop. We suspect that the positive influence of maize is actually determined by its close relationship with water availability.

The distance to the nearest forest had a negative effect particularly on urodeles such as *Salamandra salamandra* and *Triturus marmoratus*. This was expected given the importance of forests for this group (Gustafson et al., 2011, see also Welsh, 1990; Werner & Glennemeier, 1999; Herrmann et al., 2005; Hartel et al., 2010). Forests provide shelter for amphibians outside of the breeding season, and the close relationship between amphibian presence and forests has been reported previously (Hecnar & M'Closkey, 1996; Demaynadier & Hunter, 1998; Knutson et al., 1999; Guerry & Hunter, 2002; Rubbo & Kiesecker, 2005; Eigenbrod et al., 2008; Hamer & McDonnell, 2008). However, distance to forested areas had only a rather minor influence on species richness, which is likely due to some species (such as *H. molleri*) preferring open areas. The lack of a detectable effect by other habitats (fresh or brackish water, open or forested,

agricultural or natural) might be related to the mosaic of small habitat patches characterising Baixo Vouga Lagunar, which makes it difficult to isolate the influence of specific habitat types on amphibian richness.

The influence of habitat heterogeneity and key landscape features

Habitat heterogeneity is well known as a major driver promoting diversity (Pianka, 1966; Martins et al., 2014; see also Marques, 2013; Alves et al., 2014; Mendes et al., 2014; Marques et al., 2015 for examples from the study area). However, the Shannon index applied to habitat areas (SHDI) was excluded from the analysis at an early stage due to low performance, which might be due to heterogeneity measured only based on the proportion of major habitat patches. Species occurrence and diversity might also be influenced by the presence of artificial landscape features such roads (Fahrig et al., 1995; Hels & Buchwald, 2001; with a negative influence) or wells and tanks (Galán, 1999; Almeida et al., 2001; with a positive influence). We found a positive correlation between the presence of several amphibian species and the distance to the nearest well, reinforcing the importance of these structures despite the presence of streams and ponds. Their importance has also been highlighted elsewhere (Hachtel et al., 2003; Knutson et al., 2004; Brand & Snodgrass, 2010; Ferreira & Beja, 2013). In Baixo Vouga Lagunar, five species (*P. perezi*, *T. marmoratus*, *Discoglossus galganoi*, *P. waltl* and *Lissotriton boscai*) were frequently found in wells and fountains. The extent of asphalt roads was included in the second best model of anthropogenic influence, and had a non-significant negative influence on amphibian species richness. None of the remaining landscape features related to human activities influenced patterns of assemblage composition and species richness.

The influence of freshwater availability

Wells are landscape features that provide a source of freshwater, and their positive effect is thus not surprising. Freshwater is generally abundant in the landscape, but does not appear to be equally suited for amphibians. The presence of temporary water bodies was the second best descriptor explaining patterns of species richness, confirming previous studies on their importance for amphibians (Pierce, 1993; Beja & Alcazar, 2003; Calhoun et al., 2005; Fonseca et al., 2008). However, temporary water bodies are disappearing in the Mediterranean region (Fonseca et al., 2008; Ferreira & Beja, 2013), although they are likely of particular importance for regions which are highly modified by humans, such as the Baixo Vouga Lagunar.

Relevance for conservation

Considering the current conservation status of amphibians and the high diversity of species present in Baixo Vouga Lagunar, it is essential to adopt management measures for this area, particularly considering key habitats such as freshwater bodies in the form of temporary ponds. Agricultural activity in Baixo Vouga Lagunar is characteristically low intensity, and our results

suggest that the abandonment of traditional agricultural practices could be harmful to amphibians (see also Hartel et al., 2010). Particularly in areas with a high abundance of brackish water bodies, one of the best management measures for the Baixo Vouga Lagunar may involve the promotion of traditional agricultural practices to increase the number of lentic waterbodies (Galán, 1999).

This study also reinforces the importance of temporary water bodies for amphibians. This however requires that area of ponds must be maintained intact even when ponds are dry. Human activities which compress the soil change the local hydrology and have an impact upon the flooding regime (Calhoun et al., 2005). It is essential to adopt conservation measures to terrestrial areas surrounding temporary ponds, for example to promote microclimates suitable for juvenile amphibians (Dodd & Cade, 1998; Calhoun et al., 2005). The improvement of irrigation ditches, such as those existing in bocage could be achieved by, for example, preventing the disposal of branches from the pruning of riparian vegetation into the ditches. The control of wildfires in forested areas and the maintenance of riparian vegetation may generally be beneficial to the amphibians in the Baixo Vouga Lagunar. We suggest that conservation guidelines for the area should be compiled into an integrated management plan to be implemented with the collaboration and training of local communities and stakeholders, following the strategy proposed by the European 2020 Biodiversity Strategy for local action.

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