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Conservation of Mediterranean temporary ponds under agricultural intensification: an evaluation using amphibians

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Abstract

This study examined responses of amphibians breeding in Mediterranean temporary ponds to a gradient of agricultural intensification, aiming to identify land uses and management prescriptions favouring the conservation of these habitats in farmed landscapes. Larval amphibian assemblages and habitat attributes were sampled at 57 ponds, 10 of which had been converted into permanent irrigation reservoirs. Species richness increased with area and hydroperiod in temporary ponds, with the addition of rare species to ponds with long hydroperiods resulting in a tendency for the less widespread species (e.g. *Triturus marmoratus* and *T. boscai*) to occur in the most species-rich ponds, while species-poor ponds consisted predominantly of widespread species only (nested pattern). However, one species (*Pelodytes punctatus*) was largely restricted to the most ephemeral ponds, whereas permanent irrigation reservoirs were species-poor and lacked most species occurring in temporary waters. The strongest negative correlates of amphibian abundances were the intensification of agricultural land uses, the transformation of ponds into permanent reservoirs and the introduction of exotic predators (fish and crayfish) from the irrigation channels. The results suggest that conservation of temporary pond amphibian assemblages in Mediterranean farmland requires networks of ponds with diverse hydroperiods, where the natural hydrologic regimes, less intensive land uses and isolation from irrigation waters should be preserved.

Keywords: Agroecosystems; Habitat modelling; Hydroperiod; Tadpoles; Wetlands

1. Introduction

Temporary ponds are relatively small and shallow waterbodies with an annual dry phase of variable timing and duration, which frequently hold unique communities of aquatic organisms (Collinson et al., 1995; Grillas and Roché, 1997). Although these ephemeral waters are highly vulnerable to human activities and threatened in many regions, their biodiversity value is frequently overlooked, contributing to their neglect and inadequate management (Semlitsch and Bodie, 1998; Snodgrass et al., 2000b). This is a case in point in the Mediterranean region, where the conservation of these habitats gained prominence only recently, after the European Union (EU) Habitats Directive (92/43/EEC) listed Mediterranean temporary waters as conservation priorities.

Mediterranean temporary ponds are poorly understood and highly endangered, suffering widespread degradation and loss due to increases in the area of land under intensive cultivation and urban use (Grillas and Roché, 1997). Many of the surviving temporary ponds remain in low-intensity farmland, where the traditional farming regimes may have contributed to their conservation for millennia (Grillas and Roché, 1997). However, low-intensity farmland is rapidly disappearing in southern Europe, with land abandonment and afforestation in the economically marginal farming areas, and agricultural intensification on the more productive soils (Stoate et al., 2001). The conservation of Mediterranean temporary ponds thus requires regulations promoting compatible agricultural land uses, such as the agro-environmental measures of the EU Common Agricultural Policy. These regulations are based on the

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compensation of farmers for maintaining farming practices supporting the conservation of threatened species and habitats. Shortage of ecological information on Mediterranean temporary ponds, however, makes it difficult to design management prescriptions specifically targeted at such habitats.

Amphibians are valuable indicators of habitat condition in small, temporary wetlands, where they are widespread and play a key role in structuring biological assemblages and moderating fluxes of energy and nutrients (Semlitsch and Bodie, 1998; Snodgrass et al., 2000a). Therefore, it is possible that changes to amphibian populations caused by agriculture intensification (e.g. Oldham, 1999), may reflect wider impacts on temporary pond biodiversity. In these circumstances, amphibians may provide clues to identify agricultural land uses and management prescriptions favouring the overall conservation value of temporary ponds, particularly where information on other aquatic organisms is lacking. Moreover, it is important to manage temporary ponds for breeding amphibians themselves, as there is concern over global declines in amphibian populations (Houlahan et al., 2000). This is particularly relevant in agricultural landscapes, where amphibians are strongly at risk from a wide range of impacts to their terrestrial and aquatic habitats (Berger, 1989; Bishop et al., 1999; Joly et al., 1999, Oldham, 1999).

This study examines the conservation of temporary ponds in Mediterranean farmland from the perspective of breeding amphibians, assessing the main factors affecting the assemblages across a gradient of agricultural intensification. The study was made in a typical Mediterranean landscape in southwest Portugal, where we (1) surveyed temporary ponds and characterized the dominant agricultural uses, (2) assessed the relationships between the attributes of amphibian assemblages (e.g. species richness and abundance, proportions of different species) and habitat variables reflecting gradients of natural and anthropogenic variation in habitat conditions, and (3) described the patterns of occurrence and abundance of individual amphibian species across the same gradients. Results of the study were then used to set recommendations for agro-environmental schemes improving the conservation of temporary ponds in Mediterranean farmland.

2. Methods

2.1. Study area

The study was carried out within a Natural Park on the coastal plain of southwest Portugal (37°30′ N, 8°57′ W) (Fig. 1). The 50–150 m high and 5–15 km wide coastal plateau runs North–South for about 100 km, bordered by a chain of small mountains. Climate is Mediterranean with oceanic influence. Aridity increases southwards, with annual mean temperature increasing from 15 to 16 °C, and annual precipitation decreasing from 650 to 400 mm, of which >80% falls in October–March. The landscape is predominantly flat, with tree cover restricted to a few small woods, windbreaks and stream valleys. The less intensive farming system is the extensive cultivation of winter cereals, on a cereal–fallow rotation basis. Beef cattle are also important, with large areas occupied by pastures, fodder crops, and silage corn or sorghum. Since about 1990 there has been a strong increase in irrigated crops, particularly vegetables for international markets, associated with frequent use of pesticides, chemical fertilizers and annual tillage.

Temporary ponds occur throughout the area on sandy soils, occupying small, shallow and discrete depressions. The functioning of the ponds is closely related to the hydrologic fluctuations of the water table, filling during the winter rains and drying out in summer. Some of the ponds are deepened by farmers and used as reservoirs, which are water-fed through irrigation channels. Exotic predators, such as the American crayfish (*Procambarus clarkii*) and the small-mouth bass (*Micropterus salmoides*), are common in the irrigation channels and frequently colonise the permanent reservoirs. Vegetation in the ponds depends on grazing and cultivation pressure, but it is usually a low humid prairie dominated by grasses, rushes and tussock-forming sedges. Under the lightest farming regimes there is a characteristic external belt of wet heath. Despite agricultural pressure, some ponds retain a considerable conservation value for vascular plants, branchiopod crustaceans, aquatic insects and amphibians (Beja, 2000).

2.2. Pond survey

A pond survey was carried out during autumn 1996, based on previous work conducted in 1993 (F. Faria and P. Cabrita, personal communication), aerial photography, and 1:25,000 topographic maps. Temporary ponds were defined as bodies of water occupying depressions, which are flooded during the rainy season for a sufficiently long period to allow the development of aquatic vegetation and hydromorphic soils, but which are not in contact with permanently flooded habitats such as rivers (Grillas and Roché, 1997). Former temporary ponds converted into irrigation reservoirs were recorded during the survey. Each pond was mapped and classified according to the dominant agricultural use: (1) semi-natural—lightly grazed, bordered by wet heath or by a well-developed community of tall rushes; (2) cultivated—tilled recently, with the presence of crops, ploughing furrows or stubbles; (3) grazed—regularly grazed by livestock, with shrubs and tall rushes absent, but no signs of recent cultivation; (4) reservoirs—converted into irrigation reservoirs.

2.3. Amphibian sampling

The coastal plateau was divided into seven sectors (Fig. 1), and three ponds within each dominant agricultural use were selected randomly in each sector. From the potential sample of 84 ponds only 57 were actually sampled, as there were fewer than three ponds of some agricultural types in some sectors. Amphibians were sampled in 1997 in three discrete periods (29 January–25 February, 13–26 March, 28 April–22 May), starting about 1 month after the ponds had flooded and ending up when all but the most long-lasting ponds had dried out; these encompass the main breeding season for the species occurring in the study area (Díaz-Paniagua, 1992; Malkmus and Schwarzer, 2000). This study was short-term, providing a snapshot of amphibian assemblages that are probably highly dynamic (e.g. Pechmann et al., 1989; Díaz-Paniagua, 1992; Hecnar and M'Closkey, 1996). Nevertheless, this kind of spatial survey is still useful to estimate changes in assemblage attributes across important environmental gradients (Snodgrass et al., 2000a).

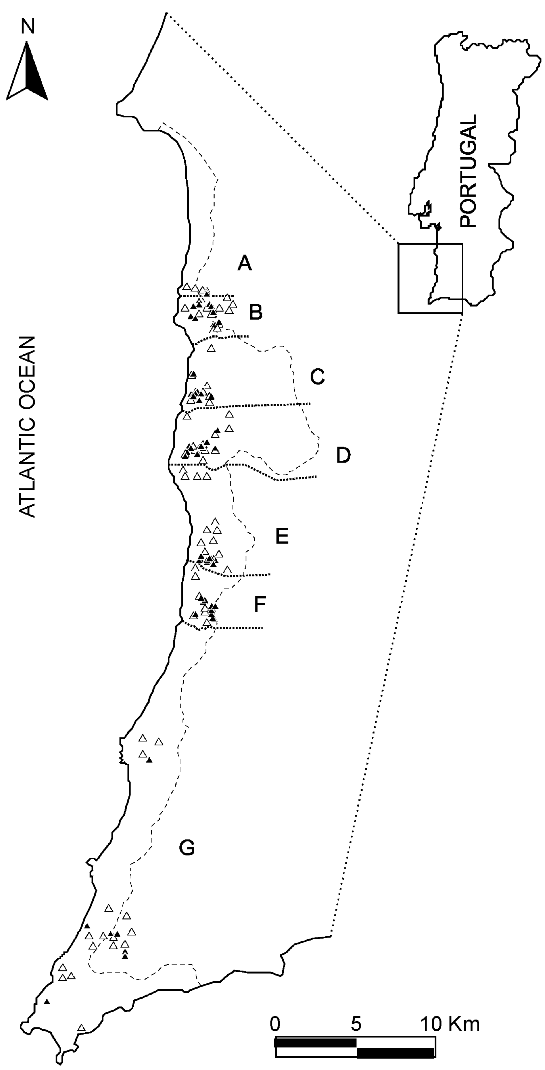

Fig. 1. Map of the study area in southwest Portugal, showing the limits of the Natural Park of southwest Alentejo and Vicentina Coast (dashed line) and the division of the coastal plateau in seven geographical sectors (A–G) used in stratified sampling. Triangles are the ponds recorded in the 1996 pond survey, with solid triangles indicating the subset sampled for amphibians in 1997.

Depending on pond size, each sample consisted of three to five 30-s blind sweeps (mean $=3.1\pm0.4$ SD, $n=139$) with a 30×20 cm aperture dip-net, conducted by one person wading across the pond and systematically covering all habitats available. Amphibians were identified to species and returned to water at the end of each sampling session; *Hyla* spp. tadpoles could not be reliably separated in the field and were thus identified to genus. Sweep sampling was used because the study required repeatedly surveying many ponds over a large region, and sampling larvae may be more efficient for inventorying amphibians than sampling adults (McDiarmid, 1994). However, the sampling protocol used may miss early-breeding species with short larval periods and species with low breeding success, and there may be biases due to differences in catchability among species (Snodgrass et al., 2000a). These problems, however, are unlikely to introduce major errors in comparative studies like this one, where a consistent sampling scheme was used across sites.

The abundance of a species on a sampling occasion was expressed in terms of catch-per-unit-effort (CPUE; mean number of individuals per sweep); for each pond and species, the sampling date yielding the largest CPUE was used in data analyses. This procedure was followed because the abundance of amphibian larvae vary widely across the season and the sample yielding the maximum catch may reflect more closely the size of the breeding population. The sampling dates selected varied among amphibian species to account for temporal succession in pond occupancy (Díaz-Paniagua, 1992). The overall abundance of amphibians at the ponds was estimated as the sum of maximum CPUE for all the species recorded at each pond.

2.4. Environmental variables

Ponds were characterised using 23 variables describing morphology, hydroperiod, potential agricultural impacts, and water chemistry (Table 1). Area and depth of ponds were measured in the field during the period of maximum flooding. Relative depth was computed as the maximum depth expressed as a percentage of area of the pond. A drying score was used to quantify the hydroperiod: 1—dry before the end of March (short); 2—dry before the end of April (intermediate); 3—dry before the end of summer (long); 4—permanent. The potential agricultural impacts considered were the ploughing regime, intensification of lands uses, conversion of ponds into irrigation reservoirs, and introduction of

Table 1
Summary statistics and details of environmental variables used to characterize 57 ponds in southwest Portugal (March 1997)[a]

Variables	Code	Units	Transformation	Median	(Range)
Morphology					
Area	AREA	ha	Log_{10}	0.24	(0.01–4.20)
Maximum depth	MDPT	m		0.40	(0.15–1.20)
Relative depth	RDPT	%		0.86	(0.19–7.74)
Hydroperiod					
Hydroperiod	HYD	Score (1–4)		3	(1–4)
Agriculture					
Ploughing	PLGH	Score (1–3)		2	(1–3)
Intensification	INT	Score (1–3)		2	(1–3)
Reservoir	RES	Presence (0/1)		0	(0–1)
Exotic predators	PRED	Presence (0/1)		0	(0–1)
Water chemistry					
pH	pH			6.6	(4.6–9.4)
Conductivity	COND	S/cm	Log_{10}	361	(86.0–2435)
Total hardness	HARD	mg/l	Log_{10}	77.0	(34.0–446)
Nitrate	NO_3	mg NO_3/l	Log_{10}	0.22	(<0.009[b]–19.8)
Nitrite	NO_2	mg NO_2/l	Log_{10}	0.004	(<0.003[b]–0.21)
Orthophosphate	P_2O_5	mg P_2O_5/l	Log_{10}	0.07	(<0.002[b]–1.4)
Silicates	SiO_2	mg SiO_2/l	Log_{10}	3.5	(0.8–16.7)
Chloride	Cl	mg Cl/l	Log_{10}	75.0	(17.0–804)
Sulphate	SO_4	mg SO_4/l	Log_{10}	14.0	(3.0–88)
Sodium	Na	mg Na/l	Log_{10}	35.2	(10–306)
Potassium	K	mg K/l	Log_{10}	3.2	(0.2–32.5)
Calcium	Ca	mg Ca/l	Log_{10}	12.0	(2.3–103)
Magnesium	Mg	mg Mg/l	Log_{10}	10.2	(2.0–47.0)
Iron	Fe	mg Fe/l	Log_{10}	1.2	(<0.005[b]–15.1)

[a] Variables were categorized according to whether they describe morphology, hydroperiod, potential agricultural impacts, and the water chemistry of ponds.
[b] Below the limits of detectability of the analytic methods.

exotic predators from the irrigation channels. Ploughing was quantified using three ordinal categories: 1—ponds not ploughed in recent years, as assessed by the presence of patches of wet heath or tall rushes; 2—ponds ploughed regularly but not in the last farming season, as assessed by the presence of a well-developed herb-layer covering the bottom and margins; 3—ponds ploughed in the last farming season, as assessed by the presence of ploughing furrows or stubbles. Intensity of land uses within 200 m from the edge of the pond was also scored: 1—grazed land (natural or sown pastures); 2—extensive cultivation of arable land, including periods of grazing fallow; 3—intensive cultivation of vegetables and other crops. The transformation into irrigation reservoirs and the presence of exotic fish or crayfish were coded as dummy variables. Water samples were collected between 4 and 17 March 1997, in 1-l plastic bottles, and deep-frozen before chemical analysis in a certified laboratory of the Ministry of the Environment using standard methods. Samples were obtained during the period of maximum flooding, from open surface water at about the middle of the pond, where aquatic vegetation was absent or scarce. During sampling, pH readings were taken using a pocket pH meter (Hanna Instruments—9025; ± 0.1 pH accuracy).

2.5. Data analysis

Amphibian abundance data and environmental variables showing skewed distributions were log-transformed to approach normality (Table 1). Because water chemistry data were strongly inter-correlated, Principal Component Analysis (PCA) was used to identify the main gradients of variation, and PCA scores were then taken as independent variables in subsequent analysis (ter Braak, 1995). Throughout the study, the significance level was set at $P<0.05$, except where indicated otherwise to permit the detection of subtle effects on amphibians despite the limited power of some statistical tests. Analyses were made using S-Plus 2000 (MathSoft, 1999).

Environmental correlates of amphibian species richness, distribution and abundance were examined using generalized linear modelling (GLM; McCullagh and Nelder, 1989). Species richness was modelled using Poisson regression corrected for over-dispersion, whereas the occurrence and abundance models were based on logistic and Gaussian regression, respectively. Model development involved a preliminary selection of significant variables using Wald tests and likelihood-ratio statistics. Combinations of significant variables and their interaction terms were then screened systematically, and the Aikaike Information Criteria (AIC) was used to select the best multivariate models (Burnham and Anderson, 1998). Percentage reduction in deviance between the null model (model fitted to the intercept only) and each model tested was taken to indicate the amount of variability explained by the models.

The incidence of nestedness in species distributions among ponds was examined using the system temperature (T) metric of Atmar and Patterson (1993). This metric gives a temperature of 0° for a perfectly nested system, where species found in depauperate assemblages are also found in progressively more species-rich assemblages, and 100° for a totally random system. The significance of T-values was estimated from Monte-Carlo derived probability (Atmar and Patterson, 1995). Species associations were examined by pairwise comparisons in 2×2 contingency tables using the phi correlation (ϕ), with the sequential Bonferroni technique (Rice, 1989) correcting significance levels for multiple comparisons.

Statistical associations between assemblage structure and environmental variables were quantified with canonical correspondence analysis (CCA; ter Braak, 1995), using CANOCO 4 (ter Braak and Šmilauer, 1998). Log-transformed CPUE data of amphibian species were used as dependent variables. Model building was based on the stepwise addition of environmental variables affecting significantly the assemblage structure, with significance assessed from Monte Carlo permutation tests. The overall significance of models was also based on permutation tests for the sum of all canonical eigenvalues.

3. Results

3.1. Habitat typing of ponds

The 237 temporary ponds recorded along the costal plateau were aggregated in small clusters (Fig. 1). Only 24.5% of ponds were classified as semi-natural, as most showed signs of recent cultivation (38.8%), regular grazing by livestock (23.2%) or transformation into irrigation reservoirs (4.2%). The status of 9.3% of ponds could not be determined. The 57 ponds selected for amphibian sampling were relatively small and shallow, with only five larger than 1.0 ha, and 11 deeper than 1.0 m (Table 1). Ten ponds had been transformed into irrigation reservoirs, including eight of the deepest ponds (>1.0 m). Reservoirs maintained water through the summer, whereas the dry phase in temporary ponds started between March and June. All fish and crayfish recorded in ponds were exotic species. Fish occurred in three reservoirs and one temporary pond, and crayfish were recorded at four reservoirs and seven temporary ponds. Every pond showed some kind of agricultural interference, with only four retaining wet heath.

Waters were circum-neutral (pH: 6.6–7.2) to slightly acidic (5.5–6.6), and sodium and chloride were the predominant cation and anion, respectively. Concentrations of calcium and magnesium were usually low, and waters could be considered soft to slightly hard. High nutrient concentrations were found at four ponds for NO_3 (>1.0 mg/l) and three for P_2O_5 (>0.4 mg/l). The four components (eigenvalues >1) extracted in the chemical PCA accounted for 76.6% of variance in the original data (Table 2). The strongest variations were in the salinity of water, with the concentration of major ions (Ca, Mg, Na, K, Cl, SO_4) increasing on the first PC axis, along with conductivity and hardness. The second axis defined a gradient from more acidic ponds, with higher concentrations of dissolved Fe and SiO_2, to circum-neutral ponds, with higher concentrations of SO_4 and NO_3. The third and fourth axes reflected essentially the enrichment of the waters with nitrogen and phosphorus, respectively.

3.2. Species richness and abundance

During the study we collected 2784 anuran tadpoles, and 311 larval and 17 adult caudates, representing nine species (Table 3). Pond occupancy ranged from 87.7% for *Pelobates cultripes* to 1.8% for *Bufo calamita*. Amphibians were absent from only four ponds (7.0%).

Mean species richness was 2.2±1.2 (SD), ranging from zero to five species per pond. The number of species increased with area and hydroperiod in temporary ponds, and declined with area in irrigation reservoirs

Table 2
Loadings of water chemistry variables on the first four axes extracted by PCA, and the proportion of variance accounted for by each axis, for 57 ponds in southwest Portugal (March 1997)[a]

Variable	PC axes			
	1	2	3	4
pH		−0.488	−0.438	
Conductivity	0.920			
Hardness	0.911			
NO_3		−0.545	0.729	
NO_2			0.886	
P_2O_5				−0.674
SiO_2		0.680		
Cl	0.929			
SO_4	0.472	−0.618		
Na	0.925			
K	0.537			
Ca	0.886			
Mg	0.948			
Fe		0.716		
Proportion of total variance (%)	41.1	15.3	12.3	7.9

[a] For clarity, only loadings >|0.40| were listed.

Table 3
Amphibian species collected in 57 ponds in southwest Portugal (January–May 1997)[a]

Species			Total captured	Number (%) of ponds	
Caudates					
Sharp-ribbed salamander	*Pleurodeles waltl*	Ad.	13	3	(5.3)
		Larv.	200	26	(45.6)
Marbled newt	*Triturus marmoratus*	Ad.	3	3	(5.3)
		Larv.	106	12	(21.1)
Bosca's newt	*Triturus boscai*	Ad.	1	1	(1.8)
		Larv.	5	3	(5.3)
Anurans					
Western spadefoot toad	*Pelobates cultripes*		2423	50	(87.7)
Parsley frog	*Pelodytes punctatus*		41	9	(15.8)
Natterjack toad	*Bufo calamita*		29	1	(1.8)
Common and Stripeless tree frogs	*Hyla arborea + H. meridionalis*		274	16	(28.1)
Marsh frog	*Rana perezi*		17	8	(14.0)

[a] Ad. = adult. Larv. = Larvae.

(Table 4, Fig. 2). The multivariate model for total catch of amphibians highlighted negative effects of land use intensification and the presence of exotic predators, and a unimodal response to the hydroperiod (Table 4). Univariate analysis also showed declining catches with water acidity (PC2; $P = 0.045$) and reservoirs ($P < 0.001$), and unimodal responses to maximum ($P = 0.012$) and relative depths ($P = 0.078$).

3.3. Models for individual species

Pond morphology and hydroperiod influenced strongly the occurrence of the six most widespread amphibians (Table 5), underlining a marked tendency for a turnover of species along the hydroperiod gradient (Fig. 3). The presence of two species peaked in shallow ponds with short hydroperiods, with *Pelodytes punctatus* largely confined to the most ephemeral ponds and *P. cultripes* occurring in about all but the most permanent ponds. The prevalence of *Pleurodeles waltl* was highest in intermediate hydroperiods, whereas both *Triturus marmoratus* and *Hyla* spp. occurred most frequently in temporary ponds with the longest hydroperiods, declining in permanent ponds. Only *Rana perezi* favoured deep, permanent ponds, though it was less prevalent in the deepest ones. Species occurrences were also influenced by agriculture (Table 5), with negative relationships found for the conversion of ponds into irrigation reservoirs (*P. cultripes*, *P. punctatus* and *P. waltl*), introduction of exotic predators (*P. punctatus*), intensification of land uses (*P. cultripes* and *P. waltl*), and ploughing of ponds (*T. marmoratus*). There were, however, a few positive relations with agriculture intensification, with increased prevalence of *R. perezi* in reservoirs and more intensive

Table 4
Generalized linear models relating amphibian species richness (Poisson regression) and catch per unit effort ($\log_{10}[CPUE + 1]$; Gaussian regression) to environmental variables, in 57 ponds in southwest Portugal (January–May 1997)[a]

Independent variables	Estimate	S.E.	*t*	*P*
Species richness (% DEV = 27.4)				
Constant	−0.650	0.424	−1.530	0.132
Log_{10}(Area)	0.288	0.123	2.348	0.007
Hydroperiod	0.238	0.096	2.474	0.036
Reservoir × $[\text{Log}_{10}(\text{Area})]^2$	−0.075	0.021	−3.618	<0.001
Catches (% DEV = 39.6)				
Constant	0.696	0.453	1.536	0.130
Intensification score	−0.347	0.099	−3.505	<0.001
Predators	−0.280	0.150	−1.870	0.067
Hydroperiod	0.738	0.338	2.179	0.034
Hydroperiod^2	−0.144	0.069	2.090	0.042

[a] %DEV represents the percentage of explained deviance. See Table 1 for definition of variables.

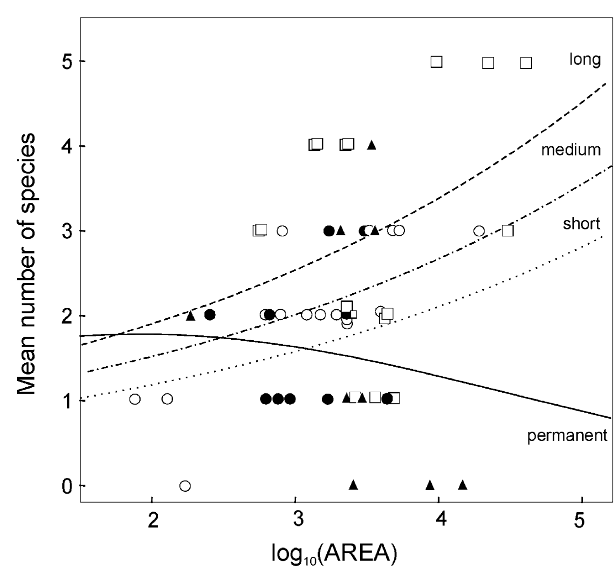

Fig. 2. Variation of mean amphibian richness with area ($\log_{10}$-transformed) in 57 ponds of different hydroperiod, sampled in southwest Portugal in January–May 1997. The lines represent the GLM fit to the data described in Table 4. Short hydroperiod—dotted line and solid circles; medium—dot-dashed line and open circles; long—dashed line and open squares; permanent—solid line and triangles.

Table 5
Summary of GLM analysis for the distribution and abundance of amphibian species in 57 ponds in southwest Portugal (January–May 1997)[a]

Species	%DEV	Morphology			Hydroperiod	Agriculture				Water chemistry PC2
		AREA	MDEPTH	RDEPTH	HYDROP	PLOUGH	INTENS	RESERV	PRED	
Presence/absence logistic models										
Pelobates cultripes	28.4		*0.008* (−)		*0.001* (−)		*0.003* (−)	< **0.001** (−)		
Hyla spp.	16.0	*0.015* (+)	0.067 (∩)		**0.004** (∩)					
Pelodytes punctatus	27.9		< **0.001** (−)	*0.005* (−)	*0.008* (−)	*0.015* (+)		*0.051* (−)	**0.031** (−)	
Rana perezi	32.0		< **0.001** (∩)	*0.007* (+)	*0.001* (+)		*0.063* (+)	*0.020* (+)		
Pleurodeles waltl	8.4				*0.041* (∩)		**0.010** (−)	*0.049* (−)		
Triturus marmoratus	23.0	**0.002** (+)		*0.045* (−)	*0.056* (∩)	**0.034** (−)				
Catch per unit effort (as $\log_{10}$[CPUE+1] and CPUE <0) models										
P. cultripes	33.6		*0.032* (∩)	*0.052* (−)	*0.049* (∩)		**0.001** (−)	*0.063* (−)	**0.001** (−)	*0.059* (−)
Hyla spp.	25.8						**0.045** (−)			
P. punctatus	88.5	*0.047* (−)		**0.001** (+)		*0.066* (−)	**0.016** (−)	–[b]	–[b]	0.039 (+)
R. perezi	47.6	**0.058** (+)								

[a] Significance levels assessed from likelihood ratio statistics and directions of association—positive (+), negative (−) or unimodal (∩)—are given for environmental variables showing significant relationships ($P<0.05$ except where shown) with the independent variable. Variables given in bold are those incorporating the best models (as judged by the AIC criteria) while in italics are given other variables showing significant univariate relationships. %DEV represents the explained deviance for each of the best AIC models. See Table 1 for definition of variables.

[b] The variable was not evaluated because the species was absent from ponds converted into irrigation reservoirs and where there were exotic predators.

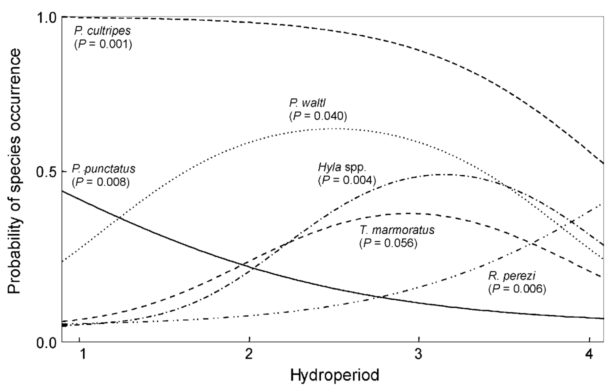

Fig. 3. Probabilities predicted from logistic regression models of ponds being occupied by each of the six most widespread amphibian species recorded in 57 ponds in southwest Portugal (January–March 1997), in relation to hydroperiod. Significance levels assessed from likelihood ratio tests are given in brackets.

land uses, and a positive association of *P. punctatus* with ponds ploughed recently.

The intensification of land uses showed the most consistent influences on the catches of amphibians, negatively affecting *P. cultripes*, *Hyla* spp. and *P. punctatus* (Table 5). The effects for the remaining variables were more varied. The catch of *P. cultripes* was lower in reservoirs and in the presence of exotic predators, declined with relative depth and acidity (PC2), and showed unimodal responses to maximum depth and hydroperiod. No *P. punctatus* were caught in reservoirs and in the presence of exotic predators, and its catch declined with area and ploughing, but increased with relative depth and water acidity. The catch of *R. perezi* increased with pond area.

3.4. Assemblage structure

Considering 53 ponds where at least one species was recorded, there was significant nestedness, with a clear tendency for the less widespread species to occur in the most species-rich ponds, while species-poor ponds consisted predominantly of widespread species only ($T=11.4°$, $P \ll 0.001$). The pattern remained identical when permanent ponds were excluded from the analysis ($T=9.4°$, $P \ll 0.001$). Contingency table analyses showed a strong negative association between *P. cultripes* and *R. perezi* ($\phi=-0.58$, $P<0.002$), and a positive association between *T. marmoratus* and *T. boscai* ($\phi=0.50$, $P<0.002$). There was also a weak association between *Hyla* spp. and *T. marmoratus* ($\phi=0.29$, $P<0.043$), but this was not significant when the sequential Bonferroni correction was considered.

The CCA model was significant ($P<0.001$), showing very clear effects (all $P<0.05$) of maximum depth and area of ponds, presence of exotic predators, and concentration of nitrates (PC3) on the amphibian assemblages (Fig. 4). The effects of the remaining agricultural variables were either very weak (ploughing; $P=0.092$) or lacking (reservoirs and the intensification of land uses; $P>0.1$), but they were forced into the model to illustrate their relations with other variables. The first CCA axis (16.9% of variability in the data) reflected a gradient of increasing depth and area, along which the relative abundance of *P. punctatus* decreased and that of *T. boscai*, *T. marmoratus*, *R. perezi*, and *Hyla* spp. increased (Fig. 3). Increasing size of ponds was associated with higher prevalence of exotic predators and

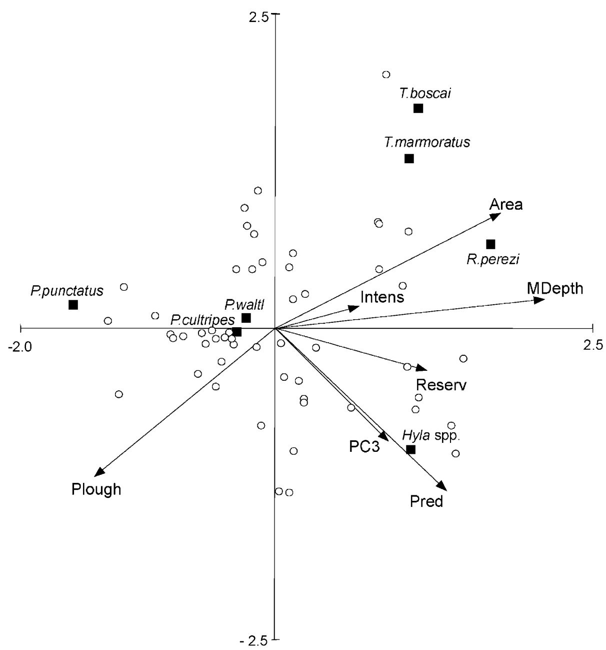

Fig. 4. Ordination diagram of the Canonical Correspondence Analysis (CCA) for the abundance of amphibians ($\log_{10}[CPUE+1]$) recorded in 57 ponds in southwest Portugal (January–May 1997), as a function of habitat variables. Open dots are the fitted scores for ponds, and black squares are the species. Arrows point in the direction of the environmental gradient defined by each habitat variable. See Table 1 for definition of variables.

reservoirs, and reduced incidence of ploughing. The second axis (6.7% of variability) represented a much weaker gradient, reflecting an increase in nitrate concentration (PC3) and incidence of exotic predators, which was positively linked to the relative abundance of *Hyla* spp. and negatively so with that of *Triturus* species. Distribution of ponds in the ordination space showed an apparent continuum in assemblage variation, with no evidence of discrete groups of ponds associating with particular clusters of species (Fig. 4).

4. Discussion

4.1. Gradients of natural variability

The dominant gradients of natural variability found in this study may largely be related to the hydroperiod, which is usually regarded as the primary factor affecting the assemblages of temporary pond amphibians (Pechmann et al., 1989; Snodgrass et al., 2000a,b). The hydroperiod influences the period during which a pond is available for colonisation, interacting with the timing and duration of the amphibians breeding season to limit the set of species that may colonise each pond. Pond colonisation occurs at different times across the breeding season, with some species occurring when ponds have just been flooded (e.g. *P. punctatus* and *P. cultripes*) and others breeding later in the season (e.g. *R. perezi*; Diáz-Paniagua, 1992). Therefore, the most ephemeral ponds main retain only early breeders, whereas long-lasting ponds may hold both early and late breeders. This may contribute to species richness increasing with hydroperiod via the addition of species that breed progressively later in the season, and thus to the nested assemblage structure reported in this and other studies (Hecnar and M'Closkey, 1997).

Despite the tendency for a nested system, there was some turnover of species along the hydroperiod gradient, with *P. punctatus* almost exclusive of very ephemeral ponds and only *R. perezi* widespread in permanent waters. This is consistent with the view that trade-offs among life-history traits result in the restriction of species occurrence along the gradient (Schneider and Frost, 1996; Wellborn et al., 1996). However, in our case there was a continuum in the replacement of species along the gradient, and no distinctive breaks in assemblage structure as reported elsewhere (Snodgrass et al., 2000a). This may because we did not sample very ephemeral ponds and natural permanent waters, where species not recorded in this study are known to occur (e.g. *Discoglossus galganoi*, *Bufo bufo* and *Salamandra salamandra*). Including these habitats might have contributed to define groups of species requiring habitats with similar duration and to reduce the incidence of nestedness.

The main influence of pond depth on the amphibian fauna was probably through the length of time the pond retained water, deeper ponds maintaining contact with the water table for longer. Pond area had less effect on the hydroperiod, though it did have an independent influence on the species present. Indeed, there was an interesting interaction between these two variables, with species richness increasing with area in temporary ponds, but showing an inverse relationship in permanent waters, probably due to the built-up of predator populations in large permanent ponds (Snodgrass et al., 2000a). A positive relationship between area and species richness is common among temporary pond organisms (Ebert and Balko, 1987; Brose, 2001), though several studies failed to document it for amphibians (Hecnar and M'Closkey, 1996; Snodgrass et al., 2000b). This discrepancy may be due to the strong relation between hydroperiod and amphibian species richness in systems where pond area and hydroperiod are not correlated (Snodgrass et al., 2000b). Also, the reverse responses of species richness to area in temporary and permanent waters, may mask any species–area relationship in studies that combine these two habitats and ignore hydroperiod effects.

4.2. Agricultural impacts

The change from temporary to permanent waters associated with the conversion of ponds into irrigation reservoirs, showed the strongest negative effects on amphibians, with marked reductions in the occurrence of all species but *R. perezi*. This may be due to habitat changes caused by the disruption of the natural hydrologic regime and/or to the colonisation of the reservoirs by exotic fish and crayfish via the irrigation channels. These predators are known to eat amphibian eggs and larvae, and may cause their decline or even local extirpation (Axelsson et al., 1997; Adams, 2000). There was certainly a strong negative relationship between the presence of predators and both the total amphibian abundance and the abundance of *P. cultripes*, though our methods were inefficient at recording fish and crayfish in the deepest ponds, making further investigations necessary. This is particularly important in the case of crayfish, which may be able to thrive in temporary waters due to their burrowing behaviour (Correia and Ferreira, 1995).

In the study area there has been a decrease in fallows and dry cereal cultivation, accompanied by increases in irrigated cereals, vegetables, sunflower and other oil seeds. Results from the current study suggest that these changes may have negative consequences on amphibian populations. Indeed, the ponds under the most intensive land uses showed lower prevalence of *P. cultripes* and *P. waltl*, and reduced abundance of *P. cultripes*, *P. punctatus*, and *Hyla*. This is consistent with the view that suitable terrestrial habitats around breeding ponds are critical for amphibians (Semlitsch, 1998; Joly et al., 1999; Hazell et al., 2001), and may be particularly affected by agricultural intensification (Oldham, 1999). Future studies should quantify in more detail the land uses surrounding each pond, to identify the quantity and quality of the habitats required by each species.

This study failed to detect marked effects of nutrient contamination on amphibians, though this factor has been implicated in the reduction of amphibian populations in other agricultural landscapes (Berger, 1989; Bishop et al., 1999). This was probably because high nitrate and phosphate loadings were found at a very few ponds, and thus nutrient contamination could at most have local effects. In a few cases, high nutrient loadings seemed to be reflected in the development of reed mace (*Typha* spp.) and other aquatic vegetation (R. Alcazar and P. Beja, unpublished data), which probably favoured the relative abundance of *Hyla* spp.

Ploughing showed inconsistent effects on the amphibian assemblages, apparently favouring the occurrence of *P. punctatus*, but affecting negatively the presence of *Triturus* species. The first effect may reflect the marked association of *P. punctatus* with very shallow and ephemeral ponds, which were the ones ploughed most regularly. In the case of *Triturus*, evidence from other studies suggest that there may be a genuine avoidance of ponds cultivated recently (Joly et al., 1999).

4.3. Conservation and management implications

Farmers usually regard temporary ponds as a nuisance, as they hinder farming operations and decrease yields. Therefore, conservation of these habitats requires the introduction of agro-environment subsidy schemes, whereby farmers complying with a set of management prescriptions would be compensated for losses in productivity (e.g. Stoate et al., 2001). Results of this study suggest that compensation should cover at least: (1) finding alternative locations and technical solutions for building irrigation reservoirs without affecting temporary ponds; (2) creating barriers to prevent water from irrigation channels reaching the ponds, thereby avoiding colonisation by exotic predators, particularly crayfish; (3) avoiding cultivation of at least the ponds inhabited by *Triturus* species; (4) maintaining less intensive land uses in a terrestrial buffer zone surrounding the pond. Definition of the adequate width and land uses for buffer zones will require additional information on the movements and habitat selection of amphibians around breeding ponds (e.g. Semlitsch, 1998).

Temporary ponds with long hydroperiods held the richest amphibian assemblages, and so they might be considered as top priorities for conservation. However, although they may also be the richest for a wide range of other aquatic organisms, they do not support the entire biodiversity associated with these systems (Schneider and Frost, 1996, Wellborn et al. 1996, Snodgrass et al., 2000a,b; Brose, 2001). Therefore, a conservation strategy for temporary pond biodiversity in Mediterranean farmland should be based on networks of ponds representative of the entire hydroperiod gradient. Future research should focus on the number and spatial configuration of ponds to be included in such networks, to maximise its effectiveness for biodiversity conservation.

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References

Adams, M.J., 2000. Pond permanence and the effects of exotic vertebrates on anurans. Ecology 10, 559–568.

Atmar, W., Patterson, B.D., 1993. The measure of order and disorder in the distribution of species in fragmented habitat. Oecologia 96, 373–382.

Atmar, W., Patterson, B.D., 1995. The Nestedness Temperature Calculator: A Visual Basic Program. AICS Research, Chicago,IL.

Axelsson, E., Nystrom, P., Sidenmark, J., Bronmark, C., 1997. Crayfish predation on amphibian eggs and larvae. Amphibia–Reptilia 18, 217–228.

Beja, P., 2000. Ecology and conservation of Portuguese temporary ponds. In: Pond Action (Ed.), Proceedings of the Ponds Conference 1998. The Ponds Conservation Trust, Oxford, pp. 91–95.

Berger, L., 1989. Disappearence of amphibian larvae in the agricultural landscape. Ecology International Bulletin 17, 65–73.

Bishop, C.A., Mahony, N.A., Struger, J., Ng, P., Pettit, K.E., 1999. Anuran development, density and diversity in relation to agricultural activity in the Holland River watershed, Ontario, Canada (1990–1992). Environmental Monitoring and Assessment 57, 21–43.

Brose, U., 2001. Relative importance of isolation, area and habitat heterogeneity for vascular plant species richness of temporary wetlands in east-German farmland. Ecography 24, 722–730.

Burnham, K.P., Anderson, D.R., 1998. Model Selection and Inference. A Practical Information–Theoretic Approach. Springer, New York NY.

Collinson, N.H., Biggs, J., Corfield, A., Hodson, M.J., Walker, D., Whitfield, M., Williams, P.J., 1995. Temporary and permanent ponds: an assessment of the effects of drying out on the conservation value of aquatic macroinvertebrate communities. Biological Conservation 74, 125–133.

Correia, A.M., Ferreira, Ó., 1995. Burrowing behavior of the introduced red swamp crayfish *Procambarus clarkii* (Decapoda: Cambaridae) in Portugal. Journal of Crustacean Biology 15, 248–257.

Díaz-Paniagua, C., 1992. Variability in timing of larval season in an amphibian community in SW Spain. Ecography 15, 267–272.

Ebert, T.A., Balko, M.L., 1987. Temporary pools as islands in space and time: the biota of vernal pools in San Diego, Southern California, USA. Archiv fur Hydrobiologie 110, 101–123.

Grillas, P., Roché, J., 1997. Vegetation of Temporary Marshes. Ecology and Management. Tour du Valat Arles, France.

Hazell, D., Cunningham, R., Lindenmayer, D., Mackey, B., Osborne, W., 2001. Use of farm dams as frog habitat in an Australian agricultural landscape: factors affecting species richness and distribution. Biological Conservation 102, 155–169.

Hecnar, S.J., M'Closkey, R.T., 1996. Regional dynamics and the status of amphibians. Ecology 77, 2091–2097.

Hecnar, S.J., M'Closkey, R.T., 1997. Patterns of nestedness and species association in a pond-dwelling amphibian fauna. Oikos 80, 371–381.

Houlahan, J.E., Findlay, C.S., Schmidt, B.R., Meyer, A.H., Kuzmin, S.L., 2000. Quantitative evidence for global amphibian population declines. Nature 404, 752–755.

Joly, P., Miaud, C., Lehmann, A., Grolet, O., 1999. Habitat matrix effects on pond occupancy in newts. Conservation Biology 15, 239–248.

Malkmus, R., Schwarzer, U., 2000. Die verbreitung der amphibien und reptilien in Südwest-Portugal. Zeitschrift für Feldherpetologie 7, 37–75.

MathSoft, 1999. S-Plus 2000: Modern Statistics and Advanced Graphics. MathSoft, Cambridge, MA.

McCullagh, P., Nelder, J.A., 1989. Generalized Linear Models, second ed. Chapman & Hall, London, UK.

McDiarmid, R.W., 1994. Amphibian diversity and natural history: an overview. In: Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C., Foster, M.S. (Eds.), Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institute, Washington, DC, pp. 5–15.

Oldham, R.S., 1999. Amphibians and agriculture: double jeopardy. In: Whitfield, M., Matthews, J., Reynolds, C. (Eds.), Aquatic Life Cycle Strategies. Survival in a Variable Environment. The Marine Biological Association of the United Kingdom, Plymouth, UK, pp. 105–124.

Pechmann, J.H.K., Scott, D.E., Gibbons, J.W., Semlitsch, R.D., 1989. Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. Wetlands Ecology and Management 16, 564–576.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.

Schneider, D.W., Frost, T.M., 1996. Habitat duration and community structure in temporary ponds. Journal of the North American Benthological Society 15, 64–86.

Semlitsch, R.D., 1998. Biological delineation of terrestrial buffer zones for pond breeding salamanders. Conservation Biology 12, 1113–1119.

Semlitsch, R.D., Bodie, J.R., 1998. Are small, isolated wetlands expendable? Conservation Biology 12, 1129–1133.

Snodgrass, J.W., Bryan Jr., A.L., Burger, J., 2000. Development of expectations of larval amphibian assemblage structure in southern depression wetlands. Ecological Applications 10, 1219–1229.

Snodgrass, J.W., Komoroski, M.J., Bryan Jr., A.L., Burger, J., 2000. Relationships among isolated wetland size, hydroperiod, and amphibian species richness: implications for wetland regulations. Conservation Biology 14, 414–419.

Stoate, C., Boatman, N.D., Borralho, R., Carvalho, C.R., de Snoo, G.R., Eden, P., 2001. Ecological impacts of arable intensification in Europe. Journal of Environmental Management 63, 337–365.

ter Braak, C.J.F., 1995. Ordination. In: Jongman, R.H.G., ter Braak, C.J.F., van Tongeren, O.F.R. (Eds.), Data Analysis in Community and Landscape Ecology. Cambridge University Press, Cambridge, UK, pp. 91–173.

ter Braak, C.J.F., Šmilauer, P., 1998. CANOCO Reference Manual and Users' Guide to CANOCO for Windows: Software for Canonical Community Ordination (Version 4). Microcomputer Power, Ithaca, NY.

Wellborn, G.A., Skelly, D.K., Werner, E.E., 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics 27, 337–363.