

APPLIED ISSUES

Ecological assessment of an intermittent Mediterranean river using community structure and function: evaluating the role of different organism groups

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SUMMARY

1. Reliable lotic ecological monitoring requires knowledge of river typology, environmental factors, the effect of stressors known here as ‘pressures’ and appropriate indicators of anthropogenically induced change. We sampled benthic macroinvertebrate, fish, bird and macrophyte communities along an intermittent Mediterranean river and analysed community structure (relative abundance) and function (metrics) relative to environmental and pressure gradients in order to identify suitable indicator group(s) for future monitoring and mitigation programmes.

2. Principal components analysis revealed that scale-dependent longitudinal differences in valley form separated narrower higher lying sites and tributaries with good quality habitats from more open degraded sites lower down the river continuum on a small floodplain and large scale pressures describing changes in land use related to agriculture with associated physical bankside and channel impacts.

3. Forward selection of variables in redundancy analysis (RDA) showed that reach scale environmental variables were selected more frequently than pressure variables for each organism group. Altitude and pH were highly redundant within and between groups, indicating essentially longitudinal structural and functional distribution patterns. Redundancy was far lower between selected pressure variables, but single or no pressure variables were retained for some organism groups indicating poor association of functional data, in particular, with the identified pressures. All RDA results indicated a longitudinal pH gradient, highlighting the combined effect of multiple environmental and pressure based mechanisms on organism groups.

4. Large, mobile organisms such as fish and birds provided a reliable link between organism structure and function, environmental factors and physical disturbance of the channel, bankside and wider river corridor. Benthic macroinvertebrate and macrophyte structural data revealed distribution patterns in relation to water velocity, a key parameter for developing appropriate compensation measures.

5. Results clearly show the importance of assessing patterns of both functional and structural change across multiple organism groups in order to identify typologically

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appropriate links with complex environmental and pressure gradients and develop and implement appropriate monitoring systems.

Keywords: bioassessment, birds, fish, function, macroinvertebrates, macrophytes, Mediterranean rivers, structure

Introduction

Ecological assessment methods of lotic systems have tended to focus on single groups, in particular benthic macroinvertebrates, fish, diatoms and macrophytes (Barbour *et al.*, 1999; Furse *et al.*, 2006). More recently, the wider stream environment has been successfully assessed using birds (Bryce, Hughes & Kaufmann, 2002; Vaughn, Noble & Ormerod, 2007b). Given the hierarchical, multivariate complexity of river systems, it could be argued that single group monitoring methods have limited applicability and that multiple organism group approaches provide a more comprehensive ecological image of river health. The multiple organism group approach has gained considerable momentum in recent years, both in the U.S.A. (Barbour *et al.*, 1999) and Europe, in particular since the implementation of the EU Water Framework Directive (O'Connor, Walls & Hughes, 2000; Bryce *et al.*, 2002; Hering, Johnson & Buffagni, 2006b; Hering *et al.*, 2006c; Johnson *et al.*, 2006a; Johnson & Hering, 2009). This approach assumes that different life history strategies of different communities will respond (in a measurable or quantifiable way through structural or functional changes in the community assemblage) to different types of stressors, providing complementary and comprehensive information on ecological status and pressures affecting the system (Hering *et al.*, 2006c). Arguments against the multiple organism group approach include experimental design flaws, high costs, complicated logistics concerning implementation, potentially high levels of redundancy (i.e. fewer groups or even a single group can potentially provide relevant information on ecological status for management needs) and often inconclusive results (Resh, 2008).

Lotic biomonitoring programmes must be appropriate to river typology and based on biological indicators that respond to pressures in a way that is distinct from natural variation (Hering *et al.*, 2006c). This means that suites of ecological indicators can vary between river types within a given ecoregion.

For example, Johnson *et al.* (2006a) found that fish, macroinvertebrates, macrophytes and benthic diatoms exhibited different degrees of response to stress gradients in European mountain and lowland streams. Mediterranean river typology is characterised by a predictable annual cycle of flood and drought that varies in intensity according to levels and duration of annual and interannual rainfall (Gasith & Resh, 1999; Pires *et al.*, 2004; Bêche & Resh, 2007a,b; Bonada, Rieradevall & Prat, 2007). This regime acts as an extremely strong environmental filter, shaping biological community traits (Poff, 1997; Statzner, Dolédec & Hugueny, 2004; Bonada *et al.*, 2005), a potential source of environmental covariance concerning biotic reactions to anthropogenic pressures. Iberia's Mediterranean rivers have a long history of human intervention including intensive agriculture and forestry, damming, abstraction and urbanisation, resulting in habitat fragmentation, soil erosion, reduced connectivity and disruption of natural flow regimes. These impacts compromise lotic structure and function in systems already subject to a harsh natural seasonal cycle of drought and flood (Aguar & Ferreira, 2005; Hooke, 2006; Hughes, Ferreira & Cortes, 2008).

The incumbent Portuguese Government has authorised completion of a partially constructed dam on the Odelouca River by 2010 in order to augment and improve water supply in the Algarve region, but has demanded environmental mitigation and compensation measures to offset impacts caused during and after construction. The Odelouca, an intermittent Mediterranean river in the Algarve region of southern Portugal has been subject to human intervention along much of its catchment, but has high conservation value due to relatively intact and floristically unique riparian galleries found along stretches of the river corridor and the presence of two critically endangered endemic fish species (Santos & Ferreira, 2008), the Iberian Chub *Squalius aradensis* (Coelho *et al.*, 1998) and the Iberian nase *Iberochondrostoma almaiai* (Coelho, Mesquita & Collares-Pereira, 2005).

This study aims to assess the structural and functional response of four organism groups (benthic macroinvertebrates, fish, birds and macrophytes) to environmental factors and pressures in an intermittent Mediterranean system and assess their suitability for future monitoring programmes and mitigation measures. In this study we have divided variables into (i) environmental variables, which essentially describe natural features such as valley form, altitude, the presence of depositional bars and pH and (ii) pressure variables describing types of impact such as changes in land use (agriculture, urbanisation) bankside disturbance and discharge into the watercourse. We also adopt the definition of structural (taxonomic composition and abundance) and functional composition (groups of metrics covering several properties of the communities under study) described by Feld & Hering, 2007. Although metrics (i.e. functional data) are more commonly used for expressing ecological condition, changes in relative abundance can also provide important information on ecosystem patterns and may even contribute to the development of new metrics. A study of the effect of environmental stress on benthic macroinvertebrate assemblages in Central European lowland rivers found that levels of explained variance were higher for taxa (structure) than for metrics (function) (Feld & Hering, 2007). We have investigated the relation of these organism groups to natural and stressor gradients in an intermittent Mediterranean river in order to compare the results of structural and functional data and assess candidate organism groups that best describe human induced change within the study area.

Methods

Study area

The Odelouca river (511 km²), a sub-catchment of the Arade basin (987 km²) is a medium-sized, low-gradient, lowland stream running through predominantly schistose areas characteristic of southern Portugal (Fig. 1). The climate is typically Mediterranean; mean monthly precipitation levels (Bravur a Reservoir, 15 km west of the study area) exemplify the seasonal rainfall pattern with a range of 59–102 mm from October 2004 to April 2005 and 2–38 mm from May to September 2005. Precipitation patterns result in a relatively slow running river subject to 'flashy' spates



Fig. 1 Map of the Odelouca basin with sampling points.

in winter that dries to form unconnected, temporary pools (intermittent reaches *sensu* Gasith & Resh, 1999) in the river bed during the summer.

Catchment topography varies from narrow steep sided valley walls to restricted meander valleys and small floodplains in the lower reaches. Temporary side channels, backwaters and dense riparian galleries occur in less disturbed mid sections and tributaries, which will be submerged following dam completion. Dominant woody riparian plants comprise *Alnus glutinosa* (L.) Gaertner, *Salix salviifolia* Brot. spp. *australis* Franco, *Nerium oleander* L, and *Fraxinus angustifolia* Vahl.. Stands of *Tamarix africana* Poir et and *Nerium oleander* L. occur along the drier lower reaches.

Agriculture (primarily extensive citrus groves) and grazing of goats and sheep have replaced the natural

Mediterranean cork-oak (*Quercus suber* L.) woodland on the floodplain below the partially built dam (a cofferdam and an excavated subterranean flow diversion gallery). Observed impacts in the study area include diffuse organic pollution, nutrient enrichment, physical disturbance (riparian clearance, bank reinforcement) and reduced longitudinal connectivity (low-step damming and abstraction for irrigation by pumping from the riverbed). Urbanisation is relatively scant and *Eucalyptus globulus* Labill. and *Pinus pinaster* Aiton plantations are present in the higher areas of the basin. Tributaries suffer little physical disturbance, however the Monchique River receives organic input from piggeries and the small village of Monchique, whilst the lower reaches of the Monchicão tributary are affected by abstraction for agriculture. The Ribeira de Carvalho tributary is far less impacted.

Field sampling and data collection

Habitat assessment. Mediterranean systems are subject to flooding and drying (Gasith & Resh, 1999), where a cycle of abiotic (scouring and drying) and biotic/physicochemical factors (increased water temperature, and increased levels of predation in dry summer pools) exert a notable influence on river communities. Thus, all fieldwork was carried out between spring and early summer (February–May 2006), when full flow connectivity existed and levels of habitat diversity and available resources were highest. Data were collected from a total of 30 sites (Fig. 1): 25 sites along the main channel and five sites along the tributaries of Ribeira de Carvalho (1), Ribeira de Monchique (2) and the Ribeira de Monchicão (2).

Habitat structure, diversity and quality were assessed over a 500 m reach using an adapted version of the U.K. River Habitat Survey (RHS; addition of land use categories and plant species found on the Iberian Peninsula). RHS records substrata and flow type, natural features and modifications of the margins and river bed, land use, presence and complexity of riparian vegetation, together with measurements of stream and bank dimension (Raven *et al.*, 1997). Features were recorded at 10 spot checks situated at 50 m intervals and then assessed over the whole 500 m stretch ('sweep up'). Recorded features were used to assess the extent of natural or artificial features and characterise river habitat condition.

Other important data on geology, climate, temperature, altitude, relief, land use, land cover, organic and industrial discharge and the presence of roads were obtained using GIS sources (the Portuguese National Water Institute – INAG, CORINE), aerial photography and downloadable catchment data from the INAG website (see Fernandes *et al.*, 2007).

Lotic communities. All organism groups considered in this study were sampled within each 500 m RHS reach, using European STAR project methodologies (except for the avifauna), modified for application in Portuguese lotic systems (manuals published by INAG downloadable at http://dqa.inag.pt/dqa2002/port/docs_apoio/nacionais.html).

Benthic macroinvertebrate samples were taken along a 50–100 m long stretch at the beginning of each 500 m reach using an adapted AQEM multihabitat sampling protocol [AQEM, 1999; Hering *et al.*, 2004; Instituto Nacional da Água (INAG), 2008a]. The sampling area was selected in order to cover the greatest possible diversity of habitats representative of the reach, including (if present) a riffle and areas of deposition. Types and extent of habitats were visually estimated and six 1-m long sampling units of the most representative habitats were taken (0.25 m × 0.25 m handnet; habitats with <5% cover were excluded). The composite sample was placed in a labelled plastic flask and fixed *in situ* using 4% formaldehyde. In the laboratory, samples were washed, sieved, sorted and identified using a low-power stereo microscope. All individuals were picked from the samples; subsampling was used when more than 200 individuals of a given taxon were present in the sample. Macroinvertebrates were identified to the lowest possible taxonomic level, which was mostly genus or species. Where higher levels of taxonomic resolution were not possible (e.g. for many Diptera) the AQEM protocol for taxonomic adjustment was applied (Hering *et al.*, 2002).

Fish were sampled using an adapted STAR methodology (INAG, 2008b) with an Electracatch International, SAREL model WFC7-HV electrofisher (applying 300 V and maintaining a 3-A output to a 40 cm stainless steel anode). Sampling was carried out (area sampled was 20 times the mean width of the survey reach, minimum length 100 m starting at RHS spot check 1) by walking upstream in a zig-zag pattern (Lyons, 1996) or sampling from a boat at sites

over 1 m deep (Godinho, Ferreira & Santos, 2000). Captured fish, held in large plastic containers, were identified to species, counted, and immediately returned to the river.

Bird surveys were carried out during the nesting period, ensuring the maximum number of nesting species, at three equidistant points (250 m distance between points) including the riparian gallery and surrounding area (Bibby *et al.*, 2000). All birds observed or heard were recorded over a 10-min period at each point. The distance of the bird from the observer was estimated when the distance was inferior to riparian gallery width. Birds were also surveyed on the flood plain c. 100 m perpendicular to each point in the riparian gallery. All recorded birds were identified to species.

Macrophyte inventories were carried out in spring and early summer when the greatest number of species was present and water transparency and depth were most favourable for survey work [Comité Européen de Normalisation (CEN), 2003] along 100 m reaches (at the start of the 500 m RHS reach), taking into account the percentage cover of each species in the sample area, evaluated as if observed macrophyte populations occurred as a single agglomeration (INAG, 2008c). Epiphytic bryophytes above the splash/humid zone were not included in the surveys. Species level determinations were made on site; specimens that could not be identified were taken to the Superior Agronomy Institute herbarium (Technical University of Lisbon) and bryophytes to the herbarium of the Botanical Garden (Science Faculty of Lisbon) for identification. A list of recorded taxa and metrics calculated for each group is given in Appendix S1.

At each macroinvertebrate and fish sampling site, physicochemical measurements of temperature, conductivity, pH and dissolved oxygen were taken with hand held electronic field probes. Depth was measured with a metre rule to the nearest centimetre and water velocity estimated with an ultrasonic flow meter (FP101 Global Flow Probe). Mean water velocity was measured at 0.6 total depth when total depth was <0.8 m; otherwise velocity was measured at 0.2 and 0.8 of total depth (Bovee & Milhous, 1978).

Based on the literature and available data, a list of biological metrics was drawn up (Appendix S1a) covering, enumeration, diversity measures, tolerance/intolerance measures, habitat preference traits (e.g. zonation, habitat or flow preferences), feeding

traits, mode of existence (e.g. locomotion) and taxonomic groups (see Feld & Hering, 2007). Macroinvertebrate metrics were calculated using the ASTERISC software (version 3, downloaded from the AQEM website <http://www.aqem.de>).

Data analyses. Redundant environmental and pressure parameters covering three spatial hierarchical levels (basin, reach and habitat) were removed using the Spearman Rank Correlation analysis method (Hering *et al.*, 2006a,c; Feld & Hering, 2007). If two environmental or pressure variables were highly correlated (threshold value of $r \geq 0.7$) the variable with a higher overall mean correlation coefficient was excluded from further analysis. A similar approach was used for the metrics calculated for each biological group (threshold of $r > 0.8$ or $r < -0.8$). Considerable numbers of highly correlated variables and metrics still remained after this process, so it was repeated with a threshold of $r \geq 0.5$ or $r \leq -0.5$ for environmental and pressure variables and $r \geq 0.6$ or $r \leq -0.6$ for metrics.

Principal components analysis (PCA) was carried out on environmental and pressure data sets at each spatial level to reduce data dimensionality and identify the principal environmental and pressure gradients (May & Brown, 2002). From an initial list of 38 environmental variables and 36 pressure variables, a total of 9 environmental and 10 pressure variables were retained (Table 1). The same procedure was carried out for functional data (metrics) for each organism group. A total of 22 metrics were retained from an initial list of 125 metrics (Appendix S1a) and a list of taxa for each organism group is given in Appendix S1b.

Structural and functional data for each organism group [$\log(x + 1)$ or arcsin square root transformed according to data type; singly occurring specimens and those occurring at less than three sampling sites excluded] were analysed using detrended correspondence analysis (DCA) to assess the biological turnover or gradient within each data set and determine *a posteriori* use of unimodal or linear constrained response analyses (Gauch, 1982a). Gradient lengths obtained for all organism groups (<3.0 SD units) indicated the use of a linear model. Manual forward selection using a cut off point of $P > 0.1$ (Magnan & Rodriguez, 1994; Griffith *et al.*, 2003; Aguiar & Ferreira, 2005) was used to retain non-redundant subsets of environmental and pressure variables explaining functional and structural data distribution patterns for each organism

Table 1 Environmental (E) and pressure (P) variables divided over three spatial scales (basin, reach and habitat) retained for PCA and RDA after analysis for redundancy following comparison of the Spearman Correlation Coefficient

Variable & spatial scale	Unit/expression	Abbreviation	Source of data
Basin			
E Valley form	Class 0–4	VAL_FRM	GIS
P Urban area	% Catchment area	URB_A	GIS
P Monocultures	% Catchment area	MONO_A	GIS
P Agriculture	% Catchment area	AGRI_A	GIS
Reach			
E Altitude	m.a.s.l	ALT	GIS
E Number of bars	Count	BARS	RHS
E Bankfull width (m)	Metres	BKFULL	RHS
E Average riparian width	Metres	WDTRIP	RHS
E Land use natural/semi-natural	Class 0–4 (% area)	LU250_NAT	RHS/GIS 250 m bankside buffer
P Bank reinforced	Count derived score 0–4	BK_RI	RHS
P Bank embanked	Count derived score 0–4	BK_EM	RHS
P Land use agriculture	Class 0–4 (% area)	LU250_AG	RHS/GIS 250 m bankside buffer
Habitat			
E pH	Sorensen scale	PH	Sample site
E Dissolved oxygen	mg L ⁻¹	DO	Sample site
E Water velocity	m s ⁻¹	WVEL	Sample site
P Bank modification		BNK_MOD	RHS/sample site
P Channel modification		CH_MOD	RHS/sample site
P Banktop landuse agriculture	Class	BT_AG10	RHS/sample site 10 m bnkside buffer
P Banktop landuse pasture	Class	BT_RP10	RHS/sample site 10 m bnkside buffer

group. RDA was carried out on selected environmental and pressure variables for the functional and structural data of each organism group. A Monte-Carlo permutation test (999 permutations) on the first axis eigenvalue and the sum of all canonical eigenvalues or 'trace' evaluated the significance of the environmental and pressure effects for each analysis. To further aid interpretation of results, Spearman rank correlations ($P < 0.05$) were calculated between retained organism group metrics/taxa (explained variance values $\geq 15\%$) and the samples scores of RDA ordination axes 1 and 2 (where most of the variance tends to reside). CANOCO 4.5 (ter Braak & Smilauer, 1998) was used for DCA and RDA analyses and Statistica (version 6.0, Statsoft, Tulsa, OK, U.S.A.) for the calculation of correlation coefficients. For clarity, only taxa and metrics with explained variance values $\geq 15\%$ are shown in the ordination plots (Feld & Hering, 2007).

Results

Environmental and pressure gradients

PCA indicated higher levels of explained variance at the highest and lowest spatial scales, followed by

Table 2 PCA eigenvalues and loadings (axes 1 and 2) for retained environmental and pressure variables divided over three spatial scales of habitat, reach and basin. Only variables with loadings ≥ 0.5 on at least one of the first two PC axes are listed

Environment	Pressures		Eigenvalue	Eigenvalue	
	Axis 1	Axis 2		Axis 1	Axis 2
Basin					
Eigenvalue	0.726	0.274	Eigenvalue	0.539	0.332
VAL_FRM	1	0.004	URB_A	-0.626	0.049
			MONO_A	-0.963	0.189
			AGRI_A	-0.281	-0.959
Reach					
Eigenvalue	0.393	0.252	Eigenvalue	0.329	0.244
ALT	-0.712	0.650	BK_RI	0.511	-0.376
BARS	0.655	0.613	BK_EM	0.845	0.503
WDTRIP	-0.772	0.013	LU250_AG	0.427	-0.7675
LU250_NAT	-0.526	0.241			
Habitat					
Eigenvalue	0.518	0.348	Eigenvalue	0.444	0.315
WVEL	-0.749	-0.660	BNK_MOD	0.659	0.713
pH	-0.800	0.599	CH_MOD	0.062	0.574
DO	-0.539	0.016	BT_AG10	-0.068	0.578
			BT_RP10	0.888	-0.427

reach scale for both sets of variables (see Table 1 for details of retained variables and abbreviations and Table 2 for PCA results). For clarity, only variables with loadings ≥ 0.5 are described. The first two axes

of the basin level environmental PCA explained 100% of the variance (axis 1 = 72.6%, axis 2 = 27.4%), separating tributary sites and headwater sites situated in deep vee valleys from more heterogeneous mid section sites and shallow vee valley sites downstream of the cofferdam. The first two axes of reach level environmental variables ($n = 4$; loadings ≥ 0.5) explained 64.5% of the variance (axis 1 = 39.3%, axis 2 = 25.2%), separating sites with lower levels of human intervention situated higher up the river corridor from those below the cofferdam. Higher lying sites were characterised by riparian galleries, natural adjacent land use and instream habitat heterogeneity (the presence of bars). Habitat level PC axes explained 86.6% of the variance (axis 1 = 52.8%, axis 2 = 34.8%); the highest loadings along axes 1 and 2 were water velocity (WVEL, -0.749 and -0.660 respectively) and pH (PH, -0.800 and 0.599 respectively). Habitats in the middle and upper reaches were characterised by higher water velocity levels (WVEL), while lower lying sites had higher pH, temperature and dissolved oxygen levels (DO). Higher dissolved oxygen and to some extent even pH levels can be attributed to the considerable

daytime photosynthetic activity of dense stands of macrophytes and filamentous algae recorded at lower sites.

Basin level PC analysis of pressures (total explained variance 87.1%; axis 1 53.9%, axis 2 = 33.2%) illustrated the effect of large scale changes in land use, attributed primarily to agriculture (MONO_A and AGRI_A). The first two axes of the reach level pressure PCA explained 57.3% of the variance. Loadings (≥ 0.5) along axis 1 clearly described physical bankside disturbance (BK_RI and BK_EM) and changes in adjacent land use (LU250_AG), mostly at sites on the floodplain below the cofferdam. Habitat level PCA (axes 1 and 2 explained 75.9% of the variance) indicated variables (loadings ≥ 0.5) describing physical disturbance including bankface/top and the river channel modification and agricultural and pastoral activities (grazing at the banktop).

Forward selection of variables

Forward selection created non-redundant subsets of variables describing higher levels of variance in relation organism group structure and function

Table 3 Results of the manual forward selection procedure of environmental and pressure variables relative to the functional (F) and structural (S) data for each organism group (spatial scale: B = basin, R = reach, H = habitat)

Feature and corresponding spatial scale	Invertebrates		Fish		Birds		Macrophytes		Σ
	F	S	F	S	F	S	F	S	
Environmental variables									
PH H		•	•	•	•	•		•	6
ALT R		•	•	•	•	•			5
WVEL H	•	•						•	3
BARS R			•	•					2
WDTRIP R				•					1
LU250_NAT R					•				1
VAL_FRM B					•				1
Σ ES	1	3	3	4	4	2	0	2	
Σ organism group	4		7		6		2		
Pressure variables									
LU250_AG R		•				•		•	3
CH_MOD H			•	•					2
BK_RI R					•	•			2
BT_AG10 H					•				1
BNK_MOD H							•		1
MONO_A B								•	1
Σ ES	0	1	1	1	2	2	1	2	
Σ organism group	1		2		4		3		
Overall total per organism group	1	4	4	5	6	4	1	4	

(Table 3). The number of variables shared between organism groups ranged from two to six; five selected variables were unique to the structural or functional data of a particular organism group.

The variables pH (PH) and altitude (ALT) were highly redundant between all four organism groups. Forward selection retained more environmental variables than pressure variables for all organism groups, except macrophytes (environmental variables $n = 2$, pressure variables $n = 3$). A maximum of seven environmental variables were retained for fish, followed by six for birds, four for macroinvertebrates and only two (structural data) for macrophytes. More environmental variables were retained for organism group structural data, with the exception of avian data. Intra group redundancy was highest within the fish for pH (PH), altitude (ALT) and the presence of bars (BARS) and inter group redundancy was highest between fish and bird structural and functional data (pH and altitude). Fewer pressure variables were retained across the organism groups and intra group and inter group redundancy levels were lower. However, LU250_AG, indicating agricultural land use was selected for macroinvertebrate, avian and macrophyte structural data. Intra group redundancy was evident for channel and bankside disturbance (CH_MOD and BK_RI) for fish and bird groups. The highest number of retained pressure variables ($n = 4$) was for birds followed by macrophytes ($n = 3$). A single variable was retained for benthic macro-

invertebrate structural data and none for functional data indicating that the selected metrics do not appear to reflect the principal detected pressures.

Most retained variables derived from reach scale (46.15%), followed by habitat (38.46%) and basin level (15.37%). Similar to Feld & Hering (2007), the larger number of selected reach level variables may highlight the importance of processes at this spatial scale in determining group structure and function or the pervasive effect of predominantly reach level RHS recorded variables in the dataset.

RDA results

The extracted first axis and trace for RDA ordinations were mostly highly significant (Monte Carlo test; Table 4), with the exception of benthic macroinvertebrate and macrophyte functional data. Fish and bird structural and functional data provided the highest axis 1 eigenvalues. RDA ordination plots revealed the relation between organism groups and retained variables (Figs 2 & 3).

Water velocity (WVEL) was the only variable retained for the macroinvertebrate functional RDA (Fig. 2a). Metrics (>15% explained variance) were negatively correlated with axis 1, indicating the presence of organisms with rheophilic current preferences (%_RHEOPHIL, $r = -0.812$), lithal microhabitat preferences (% LITHAL, $r = -0.661$), higher numbers of Trichoptera taxa (N_TRICH, $r = -0.866$) and EPT

Table 4 Results of RDA and Monte Carlo permutations (F statistic, 999 permutations) for testing the significance of environmental and pressure variables retained by forward selection in relation to structural and functional data of benthic macroinvertebrates, fish, birds and macrophytes

	1st canonical axis			Sum of all canonical axes (trace)				
	Eigenvalue	axes 1–3	F ratio 1st axis	P 1st axis	Eigenvalue	F ratio all axes	P all axes	
Invertebrates								
F	0.078	0.741	0.098	–	–	0.078	2.382	0.054 ns
S	0.215	0.067	0.038	6.867	0.001***	0.353	3.406	0.001***
Fish								
F	0.304	0.138	0.028	10.493	0.001***	0.472	5.356	0.001***
S	0.199	0.179	0.064	5.731	0.009**	0.466	4.018	0.001***
Birds								
F	0.312	0.154	0.043	10.447	0.004**	0.515	4.072	0.001***
S	0.166	0.064	0.039	4.971	0.001***	0.284	2.476	0.001***
Macrophytes								
F	0.083	0.443	0.313	–	–	0.083	2.537	0.061 ns
S	0.157	0.077	0.049	4.665	0.001***	0.303	2.712	0.001***

F, function; S, structure; ns, not significant.

** $P < 0.01$; *** $P < 0.001$.

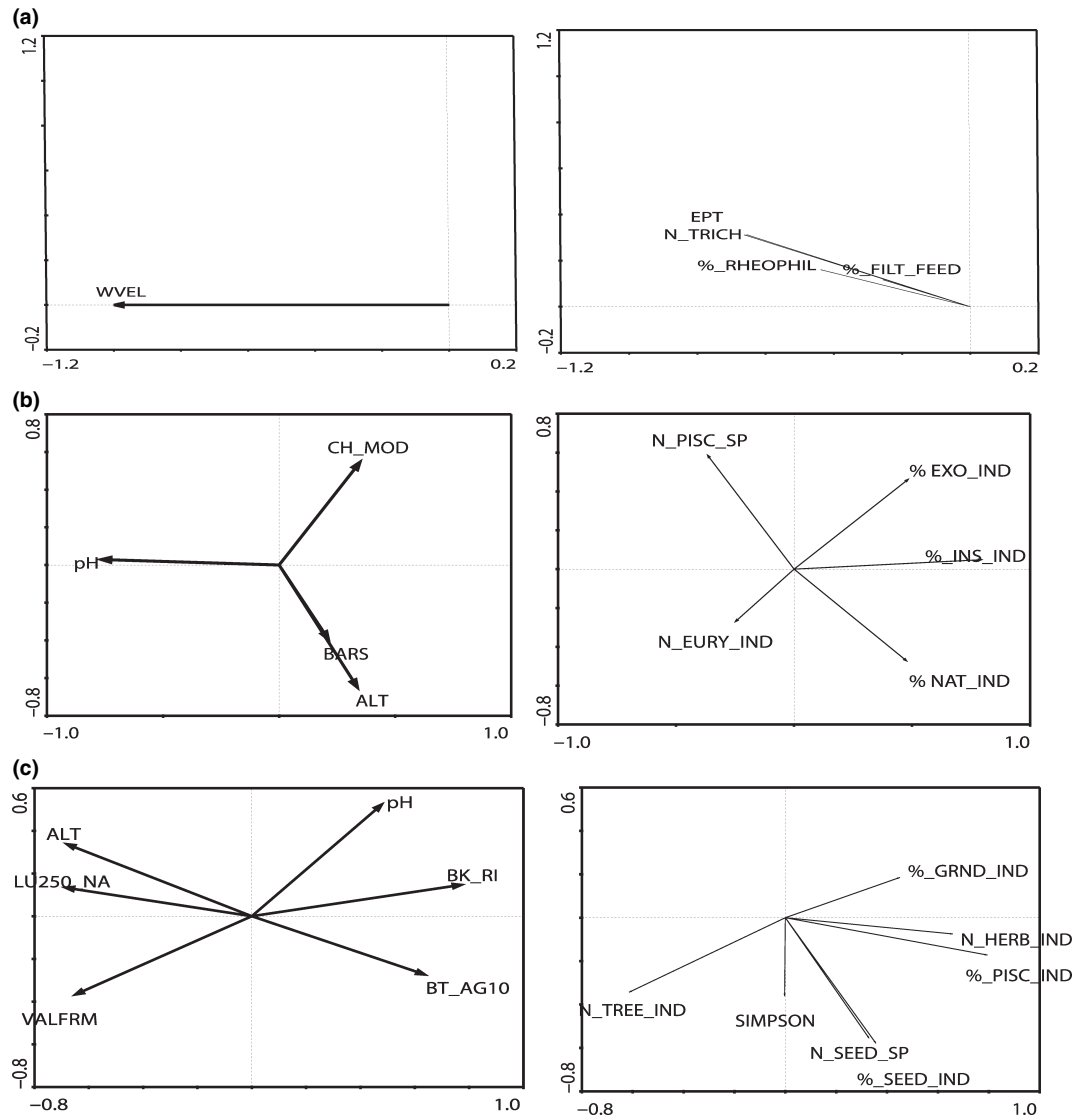


Fig. 2 RDA ordination plots of environmental and pressure variables and (a) macroinvertebrate functional data, (b) fish functional data and (c) bird functional data. Only functional organism group descriptors with >15% explained variance are shown (no macrophyte functional descriptors explained >15% variance). Biplots in the left hand column describe environmental and pressure variable distribution in the ordination space with those in the right hand column describe the distribution of metrics in the ordination space.

(Ephemeroptera, Plecoptera and Trichoptera) taxa (EPT, $r = -0.821$). Despite initial sorting using the Spearman Rank Correlation (see Hering *et al.*, 2006a,c; Feld & Hering, 2007), the proximity of the metrics in the ordination space suggests collinearity.

The taxa based RDA (Fig. 3a) indicated strong environmental/pressure gradients along axes 1 and 2, illustrated by the length of the arrows attributed to the ordinated variables. Axis 1 revealed a longitudinal pH (intra-set correlation $r = -0.889$) and altitude gradient

(intra-set correlation $r = 0.586$), essentially representing the river continuum. Axis two separated faster flowing sites (WVEL, intra-set correlation $r = -0.454$) from slow flowing sites in areas affected by agricultural land use (LU250_AGR intra-set correlation -0.869). A longitudinal taxonomic distribution pattern showed that taxa occurring more abundantly in the lower reaches of the Odelouca (higher pH axis 1) included *Baëtis* sp. ($r = -0.831$), Chironomidae ($r = -0.831$), *Dugesia* sp. ($r = -0.764$), *Gyraulus* sp.

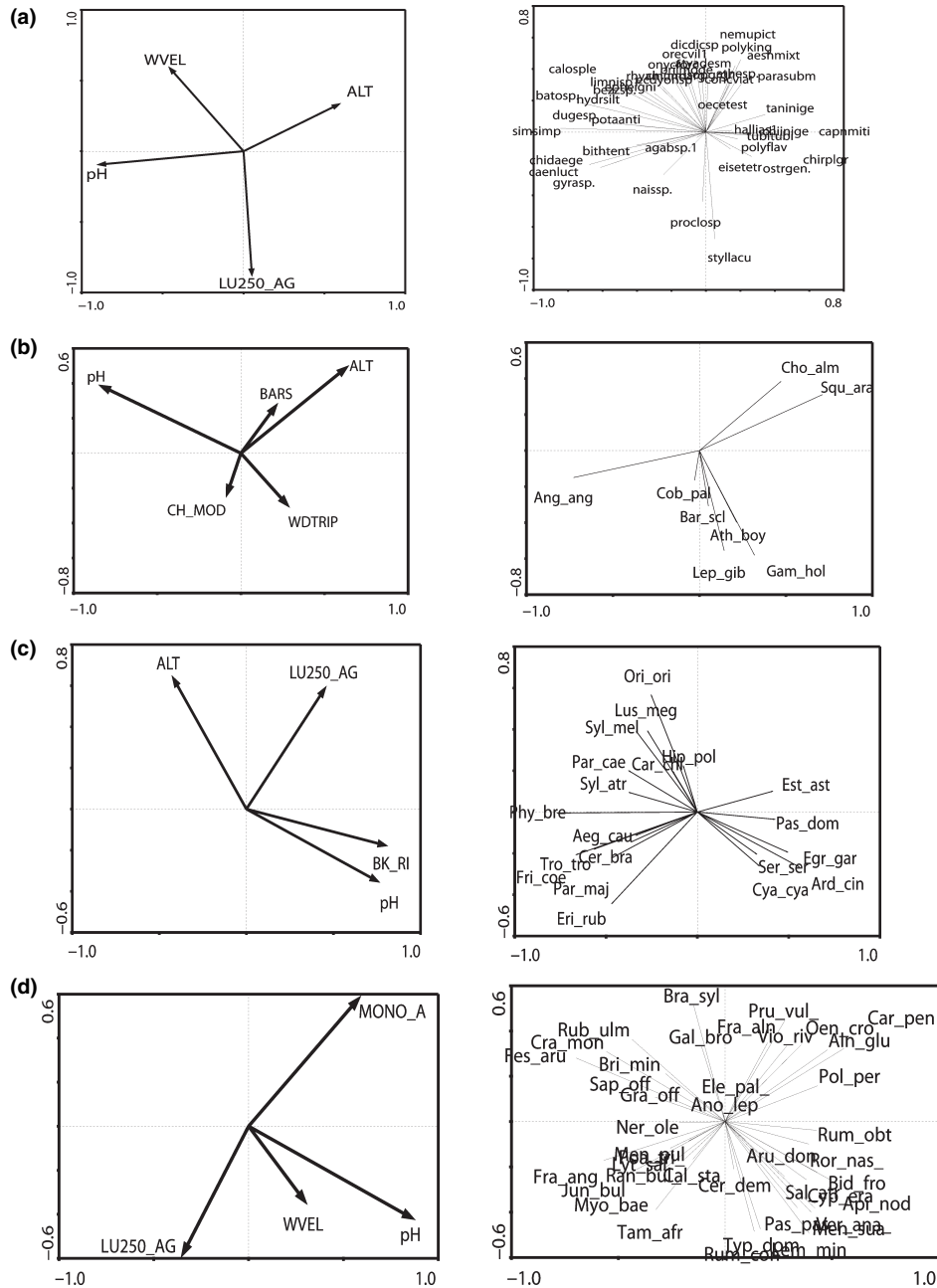


Fig. 3 RDA ordination plots of environmental and pressure variables and (a) macroinvertebrate structural data, (b) fish structural data (c) bird structural data (d) macrophyte structural data. Only structural organism group descriptors with an explained variance >15% are shown. Biplots in the left hand column describe environmental and pressure variable distribution in the ordination space with those in the right hand column describe the distribution of taxa in the ordination space.

($r = -0.0.688$) and *Simulium* sp. ($r = -0.849$). Taxa at the other end of this gradient were *Capnioneura mitis* Despax ($r = 0.469$), Tanytarsini, and *Chironomus plumosus-gr* (both not significant). *Stylaria lacustris* L. ($r = -0.822$) and *Procloeon* sp. ($r = -0.577$) were strongly associated with the agricultural land use

(axis 2), while *Dicronata* sp. ($r = 0.680$), *Atherix* sp. ($r = 0.670$) and *Onychogomphus forcipatus* L. ($r = 0.653$) were associated with higher water velocity.

Axis 1, revealed as a longitudinal pH gradient (intra-set correlation $r = -0.765$), was also evident for fish functional data (Fig. 2b), while axis 2 represented

changes in instream properties from less disturbed higher reaches (ALT intra-set correlation -0.650 , BARS intra-set correlation -0.394) to lower lying reaches affected by channel modification (CH_MOD intraset correlation 0.546). Fish metrics clearly separated sites along this gradient, describing shifts in exotic or native species status and feeding or habitat preferences. No diversity indices were selected, a facet of the harsh Mediterranean intermittent habitat template resulting in depauperate fish assemblages (Magalhães *et al.*, 2002). The percentage of insectivore fish species ($r = 0.969$) clearly separated sites along axis 1, while metrics describing percent exotic and native individuals reflected physical habitat change ($r = 0.666$ and $r = -0.868$ respectively). Piscivorous species were negatively associated with less disturbed sites.

Patterns in fish structural data closely mirrored metric distribution patterns, since metrics were derived from a small number of ecologically distinct species (Fig. 3b). Axis 1 also described a longitudinal pH gradient, while axis 2 described a shorter gradient related to channel modification and riparian gallery width (intraset correlation CH_MOD = -0.228 , WDTRIP = -0.292). Threatened native species were clearly associated with instream habitat quality and negatively associated with downstream habitat degradation (axis 1: *S. aradensis*, $r = 0.763$, *I. almaçai*, $r = 0.51$; axis 2: *S. aradensis*, $r = 0.533$, *I. almaçai*, $r = 0.413$). Although widespread, the eel *Anguilla anguilla* L. was more abundant at lower lying sites (axis 1, $r = -0.770$). A mixture of exotic (*Gambusia holbrooki* Girard and *Lepomis gibbosus* L.) and native (*Atherina boyeri* Risso, *Barbus sclateri* Günther and *Cobitis paludica* de Buen) species were associated with channel modification and riparian width. The presence of the native Iberian barbel, *B. sclateri* reflected its preference for pool-like conditions (Magalhães *et al.*, 2002) within the vicinity of the cofferdam, while the exotic *G. holbrooki* comprised just over 50% of the total catch at sites close to the cofferdam, where flow conditions were much reduced but riparian galleries were still present.

Bird functional data (Fig. 2c) strongly separated sites along distinct environmental and pressure gradients (axis 1) related to land use and bankside physical impacts. Most retained metrics described either feeding or habitat preferences; only a single diversity index (Simpson) was retained. Parameters on the left side of the biplot (Fig. 2c) separated higher

lying sites with natural land use situated in asymmetrical or narrower valleys (intraset correlations ALT = -0.681 , LU250NAT = -0.685 , VALFRM = -0.649) with good riparian stands, reflected in the metric describing the number of tree dwelling individuals (N_TREE_IND). Parameters on right side of the biplot separated lower lying sites subject to physical impacts such as bank reinforcement (intraset correlation BK_RI = 0.771 and BT_AG10 = 0.636). Associated metrics included the number of herbivore individuals (N_HERB_IND, $r = 0.564$) and the percentage of piscivorous individuals (%_PISC_IND, $r = 0.672$). Metrics describing seed eating species and individuals along axis 2 tended to be higher in areas affected by agriculture (N_SEED_SP, $r = -0.789$; %_SEED_IND, $r = -0.659$), reflecting a change in vegetation resulting from riparian clearance and the natural presence of more open ground. The number of tree dwelling individuals was highly correlated with both axes 1 and 2 (N_TREE_IND, axis 1, $r = -0.692$, axis 2, $r = -0.585$).

Avian structural data revealed distinct groups of species (Fig. 3c). The longitudinal gradient from higher lying sites to lower lying degraded sites was discernible (axis 1 intraset correlation: pH, $r = 0.750$, BK_RI, $r = 0.794$; axis 2 intraset correlation: ALT, $r = 0.633$, LU250_AG, $r = 0.582$). Species clearly associated with bankside disturbance (reinforcement) at lower lying sites were the Grey Heron *Ardea cinerea* L. (axis 1, $r = 0.452$), the little Egret *Egretta garzetta* L. (axis 1, $r = 0.557$) and the house sparrow *Passer domesticus* L. (axis 1, $r = 0.557$). Woodland species negatively associated with agricultural areas were the Iberian Chiffchaff *Phylloscopus brehmii* von Homeyer, the chaffinch *Fringilla coelops* L. (axis 1, $r = -0.706$), the Great Tit *Parus major* L. (axis 1, $r = -0.516$) and the Northern Wren *Troglodytes troglodytes* L. (axis 1, $r = -0.825$). A third group of predominantly woodland/scrub species was also associated with less impacted sites situated further upstream. Species significantly correlated with axis 2 were the Nightingale *Luscinia megarhynchos* Brehm (axis 3, $r = 0.641$), the golden Oriole *Oriolus oriolus* L. (axis 2, $r = 0.781$) and the Sardinian Warbler *Sylvia melanocephala* Gmelin (1axis, $r = 0.518$).

No macrophyte metric explained more than 15% variance relative to the single retained pressure variable describing bank modification, hence no figure is given. Macrophyte structural data revealed distinct

distribution patterns related to longitudinal changes in the hydric regime and habitat degradation associated with land use (Fig. 3d). The longitudinal pH gradient (pH, axis 1 intraset correlation, $r = 0.859$; axis 2, intraset correlation, $r = -0.416$) and land use gradient was evident (LU250_AG, axis 1 intraset correlation, $r = 0.344$; axis 2, intraset correlation, $r = -0.576$; MONO_A, axis 1 intraset correlation, $r = 0.576$; axis 2 intraset correlation, $r = 0.576$). Woody riparian species tolerant of wetter conditions, such as the common alder *Alnus glutinosa* (L.) Gaertner (axis 1, $r = 0.577$; axis 2, $r = 0.484$), the glossy Buckthorn *Fraxinus alnus* P. Mill (axis 2, $r = 0.495$), the dog violet *Viola riviniana* (Rchb.) and Common Selfheal *Prunella vulgaris* L. subspecies *vulgaris* (axis 2, $r = 0.450$), were associated with tributary sites where narrow strips of monocultural cereal crops (MONO_A) were present beyond the riparian zone. Woody shrubs tolerant of arid and disturbed conditions such as *Tamarix africana* Poiret (axis 1, $r = 0.368$; axis 2, $r = 0.663$) the Desert or Narrow Leaved Ash *Fraxinus angustifolia* Vahl. (axis 1, $r = -0.804$) and *Juncus bulbosus* L. (axis 1, $r = -0.514$) were present at sites affected by agriculture. Degraded lower lying sites were characterised by the instream presence of false water cress *Apium nodiflorum* (L.) (axis 1, $r = 0.671$) and Common Duckweed *Lemna minor* L. (axis 2, $r = -0.505$) and common tickseed *Bidens frondosa* L. (axis 1, $r = 0.766$), *Cyperus eragrostis* Lam (axis 2, $r = -0.405$) on the bankside. Species at main channel sites with lower levels of human intervention included woody shrub species such as the bramble *Rubus ulmifolius* Scott (axis 1, $r = -0.618$), the common Hawthorn *Crataegus monogyna* Jacq (axis 1, $r = -0.509$) and *Festuca arundinacea* Schreber (axis 1, $r = -0.749$).

Discussion

The Habitat Templet Theory (Southwood, 1977, 1988) describes organism structural and functional traits as a reflection of contemporary habitat conditions. Integrated multiple organism approaches use responses from a range of different life histories under certain habitat conditions to provide an early warning system to particular pressures with hopefully little or no redundancy (Furse *et al.*, 2006; Hering *et al.*, 2006b). Ideally, organism group response to stressors should be type specific and easily distinguishable from responses to environmental gradients (Johnson *et al.*,

2006a). The results of this study highlight important considerations in selecting suitable indicator organism groups for effective biomonitoring programmes, such as river typology, the identification of environmental gradients, the types of impact affecting the system under study and links between organism groups and stressors (Hering *et al.*, 2006a,c; Resh, 2008).

PCA revealed scale dependent environmental and pressure factors and potentially high levels of covariance (Frissell *et al.*, 1986; Allan, Erickson & Fay, 1997; Allan & Johnson, 1997; Poff, 1997; Hawkins *et al.*, 2000; Allan, 2004). European Mediterranean river basins have a long history of human disturbance from changes in land use on the floodplain to management of the channel and riverbanks for flood protection and to increase water supply. The history of human activity in Iberian Mediterranean systems, combined with the natural temporal and spatial fluvial patterns in these systems (Gasith & Resh, 1999) obscures the ready distinction of these two sources of variability (Hooke, 2006; Díaz, Suárez Alonso & Vida-Abarca Gutiérrez, 2008).

A purported advantage of the multi organism approach is that the range of responses distinguishes different types of stressors that are ideally distinct from responses to natural phenomena. Redundancy analysis clearly showed environmental factors to be major drivers of change in group structure and function, directly and indirectly describing essentially longitudinal distribution patterns, resulting in high levels of intra and intergroup redundancy for parameters such as pH and altitude. Ostensibly classified as an environmental factor in this study, the chemical pH gradient illustrates the combined effect of multiple mechanisms operating over several spatial scales as a result of underlying natural biotic and abiotic gradients, changes in land use and enrichment. Background pH levels are normally determined by catchment geology and biotic processes such as decomposition, photosynthesis and respiration (Townsend, Hildrew & Francis, 1989). Less shaded lower reaches of the Odelouca (increased bankfull width, riparian clearance and changes in land use) were characterised by dense aquatic macrophyte stands of false water cress *Apium nodiflorum* (L.), *Potamogeton* and filamentous algae (recorded using RHS). Higher pH levels were almost certainly partly attributable to increased runoff and photosynthetic activity which influenced instream physicochemical

processes. A prior study on pH gradients and land use in Southern English streams has described the reason for the strong pH gradient and its obvious influence upon macroinvertebrate community structure as 'undoubtedly complex' (Townsend *et al.*, 1989).

Retained pressure variables described changes in land use or physical disturbance of the bankside or channel. The number of pressure variables retained and levels of redundancy were lower and it became clear that benthic macroinvertebrate and macrophyte based metrics were not reliable indicators of the physical sources of disturbance recorded in the study area. By contrast, Feio *et al.* (2007) found macroinvertebrate assemblages in the Mondego River, a transitional river system in central Portugal, to be sensitive to morphological bankside and channel disturbance. These different responses are almost certainly related to typology. The Mondego is a predominantly perennial river system compared to the highly intermittent Mediterranean character of the Odelouca, where benthic macroinvertebrate communities are naturally tolerant of periods of physical disturbance such as scouring caused by winter rainfall runoff followed by drought. Hering *et al.* (2006b) consider benthic macroinvertebrate communities to be unsuitable for monitoring hydromorphological degradation in southern European rivers due to the lack of taxonomical knowledge. However, macroinvertebrate structural distribution patterns were clearly related to large scale changes in land use and water velocity, a vital environmental variable that will be drastically altered upon completion of the Odelouca dam.

Mediterranean flow patterns strongly affect macroinvertebrate communities; water velocity was shown to be an important environmental variable for benthic macroinvertebrate structure and function in this study. Marked shifts in benthic macroinvertebrate taxonomic and biological traits, community structure and abundance have been found in long term studies of wet and dry years in intermittent Mediterranean systems (Bêche, McElravy & Resh, 2006; Bêche & Resh, 2007b; Bonada *et al.*, 2007) and in Mediterranean systems with permanent, intermittent and ephemeral flow regimes (Bonada *et al.*, 2007). Bonada *et al.* (2007) also found a predominance of invertebrate taxa with pool like strategies in intermittent streams (such as the Odelouca), highlighting the functional constraints imposed by the Mediterranean habitat template. Fur-

ther, findings from the structural data could be used to develop new, possibly trait based, metrics specific to the study area.

Although macroinvertebrate metrics did not successfully detect the physical impacts acting upon the Odelouca, macroinvertebrate metrics in rapid bioassessment protocols (RBP's) have successfully detected water quality impairment in Iberian and South African Mediterranean streams (Bonada *et al.*, 2006). Macrophyte functional data also did not successfully reflect community changes along the PCA pressure gradients. Forward selection retained a single pressure variable (bank modification) and metrics described only very small amounts of variance. Similar to findings from other Mediterranean systems such as the Tagus River (Ferreira & Moreira, 1999; Aguiar & Ferreira, 2005), macrophyte structural data clearly indicated longitudinal species distribution patterns related to changes in land use, water velocity and water availability. Flow and substratum heterogeneity resulting from flood events are fundamental to riparian seed dispersal, recruitment (Dixon, 2003) and habitat heterogeneity which ultimately favour more trophically complex lotic communities (Pearson, Li & Lamberti, 1992). Aquatic macrophyte and woody riparian communities play a vital role in increasing habitat diversity and creating aquatic refugia for invertebrates and juvenile fish (Pinto *et al.*, 2006) and riparian birds (Jansen & Robertson, 2001b). Important stands of riparian woody plants species were identified along the tributaries and main channel sites (which will be inundated once the dam is completed) where levels of human intervention were low. Woody riparian species in the lower reaches comprised species typically resistant both to drought and human disturbance, highlighting again the innate covariance that exists between natural factors and stressors in the river landscape. The permanent change in flow patterns caused by the construction of the Odelouca dam will profoundly affect macroinvertebrate and macrophyte community structure and function. This must be redressed via appropriate compensatory measures, such as the establishment of a typologically suitable annual environmental flow regime.

The results of this study agree with the findings of Hering *et al.* (2006b) in linking larger, more mobile organisms that occupy a range of habitats with environmental and pressure factors operating at higher spatial scales. Results clearly emphasise the

importance of birds as indicators of wider river corridor health together with fish as more direct indicators of river health. Marked structural and functional distribution patterns were linked to the major PCA gradients, namely changes in land use, physical impacts to the bankside and channel and habitat quality. Fish community structural and functional changes have been marked following the construction of the cofferdam and flow diversion tunnel between 2001 and 2003. Surveys prior to construction recorded *S. aradensis* and *I. almaçai* as the two most abundant species, occurring along the entire length of the Odelouca (Pires *et al.*, 2004). RDA ordination biplots from this study clearly illustrate how these species are now confined to the upper reaches where habitat heterogeneity is greater, that the eel *A. anguilla* is more abundant in the lower reaches and that exotic species, previously described as having “no relevance for the fish assemblage”, are far more prevalent (Pires *et al.*, 2004) in areas suffering channel modification. Increased incidence of exotic species due to habitat degradation has also been observed in the Guadiana catchment, another Portuguese Mediterranean river system where eight native high conservation status species are known to occur (Collares-Pereira *et al.*, 2000; Bernardo *et al.*, 2003). Mediterranean fish communities tend to be depauperate but highly endemic because of the harsh Mediterranean regime. Native species are well adapted and respond via migration and/or tolerance of residual surface water refugia during the dry season to avoid changes in habitat conditions (Magalhães *et al.*, 2002; Bernardo *et al.*, 2003; Magoulick & Kobza, 2003; Davey & Kelly, 2007). Flexibility in habitat use as a response to the strong changes that characterise Mediterranean rivers is another tactic to increase overall survival by avoiding unfavourable habitats, e.g. those prone to scouring during flood or drying during the summer (Godinho, Ferreira & Cortes, 1997; Godinho *et al.*, 2000; Morán-López *et al.*, 2006). In a study on the effect of multi-year droughts on fish assemblages in the Torgal stream, situated only 40 km from the Odelouca study area, Magalhães *et al.* (2007) found little change in species richness and composition metrics but significant variation in individual species abundances in relation to interannual cycles of summer drought severity and the occurrence of rainy springs. Our results from a single snap-shot study confirm the limited application of species richness or

diversity metrics and highlight the importance of measures related to the abundance of native or exotic species and feeding or habitat preference. However the latter two traits may vary seasonally and require further study.

Avian structural and functional distribution patterns were strongly associated with habitat quality, changes in landuse and physical disturbance to the bank profile, highlighting the important role the avian community plays in direct riparian quality assessment along the Odelouca. Bird assemblages have been used as indicators of woodland and riparian condition (Bryce *et al.*, 2002; Hinsley *et al.*, 2008), large scale changes in land use (Ormerod *et al.*, 2000) and hydromorphological gradients (Vaughn *et al.*, 2007b). Further, studies on dippers in relation to acidification (Ormerod *et al.*, 1986) and water quality (Feck & Hall, 2004) have clearly illustrated the link between riparian birds and instream degradation resulting from global and local processes. Although adopting a different analytical approach, Vaughn *et al.* (2007b) used RHS data and bird census data to identify river bird distribution patterns and longitudinal environmental gradients linked to flow type, bankside and channel vegetation, depositional features and manmade structures. Bryce *et al.* (2002) used bird based metrics to develop a riparian bird integrity index for an integrated assessment approach including fish and invertebrate indicators and watershed data. The strong response of bird functional data to bankside/riparian habitat fragmentation has also been observed in another Mediterranean river system, the River Sado (Moreira, Saraiva & Pinto, 1997) and in Australian river systems (Jansen & Robertson, 2001b). Our results further emphasise the importance of bird communities as effective indicators of the ecological integrity of the wider river landscape (Vaughn *et al.*, 2007b).

This study across several communities was carried out over a single season (spring) in a single year during conditions of full connectivity. Annual and interannual cycle of flooding and subsequent drought in Mediterranean systems influence spatial and temporal cycles in fluvial, biotic and abiotic processes (Gasith & Resh, 1999; Hooke, 2006), with long term implications for biological communities. These predictable natural processes strongly influence organism group traits, a fundamental tenet of the River Habitat Templet (Townsend & Hildrew, 1994;

Magalhães *et al.*, 2002; Bernardo *et al.*, 2003; Bêche & Resh, 2007a,b; Bonada *et al.*, 2007; Díaz *et al.*, 2008) and have implications concerning the accurate interpretation of community responses to perturbation. These factors must be taken into account for developing and implementing effective mitigation and compensation measures during and following dam construction.

This study has shown how selected organism group functional and structural data can be used to detect redundancy and establish typologically appropriate links with stressors and environmental factors in an intermittent Mediterranean river system. It has also shown that detected gradients can comprise a complex interaction between natural and manmade factors. In the case of the Odelouca, fish and bird metrics and species appear to be reliable indicators of the large scale physical impacts, such as riparian habitat fragmentation, physical impacts to the riverbank and channel and flow disruption. Macroinvertebrate and macrophyte species distribution patterns in relation to flow and large scale change in land use can be applied in the testing and development of a multi-organism, multimetric system (Hering *et al.*, 2006a) to assess the ecological condition of the Odelouca and the effect of future mitigation and compensation measures. The permanently compromised flow regime in a system previously defined by dramatic, seasonal patterns of flood and drought must be taken into consideration once the dam comes into operation. This permanent impact will undoubtedly affect all the organism groups considered in this study, emphasising the importance of temporal studies for the development of appropriate mitigation and compensation measures.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. (a) Final list of metrics calculated for each organism group and retained following Spearman correlation analysis and PCA. (b) List of taxa recorded for each organism group with individual taxon codes and distribution data (% of total abundance) in the upper reaches, lower reaches and tributaries of the Odelouca River (●1–35%; ●36–60% ●>61%).

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