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3b2 v7 AQC : 866 PROD.TYPE: COM ED: VIJAYA PAGN: BV.R SCAN: XXX
pp.1–19 (col.fig.: NIL)

AQUATIC CONSERVATION: MARINE AND FRESHWATER ECOSYSTEMS
Aquatic Conserv: Mar. Freshw. Ecosyst. (in press)
Published online in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/aqc.866

Hierarchical spatial patterns and drivers of change in benthic macroinvertebrate communities in an intermittent Mediterranean river

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ABSTRACT

1. The imminent damming of the Odelouca River, an intermittent Mediterranean river situated in the south-west Algarve region of Portugal with valuable stands of riparian vegetation, has called for the compulsory implementation of compensatory measures.

2. In order to assess the primary environmental and human factors that drive change in the benthic macroinvertebrate assemblages of the Odelouca, and the spatial scale at which they occur, 30 sites were sampled for benthic macroinvertebrates and extensively surveyed using River Habitat Survey (RHS) in spring 2005.

3. A hierarchical cluster analysis of selected physical and RHS variables clearly indicated gradients of habitat quality (instream and riparian corridor) along both main channel and tributaries. Analysis of macroinvertebrate metrics by parametric and non-parametric ANOVA showed the derived clusters of groups to be biologically distinct.

4. From a total of 64 variables, divided into two explanatory variable groups (environmental or pressure) over three spatial scales (habitat, reach and basin), just 20, predominantly environmental, variables were retained for subsequent analyses.

5. Partial canonical correspondence analyses of the selected environmental and pressure variables over the defined spatial scales showed that environmental variables contributed most significantly over all of the spatial scales and that pressure variables related to land-use only contributed significantly at the level of the river basin.

6. Variables recorded by RHS contribute successfully to the detection of habitat quality gradients in a Mediterranean river system and the strongest drivers of macroinvertebrate change are primarily, but not exclusively, environmental factors occurring at middle and higher spatial scales.

7. Compensatory measures must therefore be implemented across a range of spatial scales, taking into account abiotic and biotic processes characteristic of disturbance-driven Mediterranean systems that contribute to habitat heterogeneity and quality and confer functional and trophic diversity to the macroinvertebrate assemblages.

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Received 16 October 2006; Revised 22 February 2007; Accepted 19 March 2007

KEY WORDS: benthic macroinvertebrates; Mediterranean rivers; River Habitat Survey; spatial scale; habitat; reach; basin; partial canonical correspondence analyses

INTRODUCTION

Lotic systems comprise a heterogeneous array of habitats formed by a myriad of physical, chemical and biological processes distributed over a widely recognized hierarchy of spatial scales (Frissell *et al*., 1986; Allan *et al*., 1997). Lotic biological assemblages are shaped by both natural and man-made variables that act as environmental filters along this spatial gradient (Karr and Schlosser, 1978; Davies *et al*., 2000; Malmqvist, 2002; Boyero, 2003; Bonada *et al*., 2005; Hughes, 2006). Thus, the biological community occurring at a given site is a subset of the potential regional pool of colonizers that has passed through a system of filters related to environmental variables and human activity. The resulting freshwater community itself has an important role in the lotic biotic system via the processing, consumption, transport and subsequent availability of nutrients to other members of the biota situated downstream (Vannote *et al*., 1980; Newbold *et al*., 1981; Malmqvist, 2002).

It is widely accepted that variables at higher spatial scales (ecoregion, catchment) are major determinants of factors at lower scales such as reach and habitat (Frissell *et al*., 1986; Davies *et al*., 2000; Allan, 2004a) which are said to have a more direct influence on species traits and taxonomic, trophic and structural complexity of the freshwater biota (Davies *et al*., 2000; Malmqvist, 2002; Boyero, 2003).

Recognizable changes in the biological community, factors and spatial scales described above, can be used as sentinels of change and as a tool by ecologists and natural resource managers to understand, predict and associate alterations or impacts resulting from these phenomena (Karr and Schlosser, 1978; Turak *et al*., 1999; Hawkins and Norris, 2000; Sandin and Johnson, 2004). Thus, it is essential to be able to recognize the appropriate factor(s) that relate landscape structure and land-use to the stream ecosystem and its level of biotic integrity as well as the spatial scale(s) at which they occur (Allan *et al*., 1997; Allan, 2004a). Freshwater biological assessment methods such as the Indices of Biotic Integrity (IBIs) use this approach: community components of the aquatic biota and indicators of basin health are used to assess the state of the lotic ecosystem (Karr and Chu, 2000; Allan, 2004a). Benthic IBIs are widely employed to monitor impacts across spatial scales, since, amongst the range of benthic macroinvertebrate adaptive strategies to the environmental gradients measured by IBI metrics, some will provide response data on ecosystem health (Karr and Chu, 2000; Lee *et al*., 2001).

Historically, the river basins and the watercourses of the southern European Iberian Peninsula have been subject to high levels of human intervention including intensive agriculture and forestry, damming, abstraction and urban development (Aguiar and Ferreira, 2005). These activities degrade habitat integrity and reduce longitudinal connectivity, resulting in isolated patches of disconnected lotic habitats and riparian galleries on the floodplain, clearly compromising lotic function. At the same time, Mediterranean lotic systems are highly characteristic and naturally 'stressed' as a result of the naturally harsh but highly predictable seasonal cycles of drying and flooding they are subject to (Gasith and Resh, 1999; Magalhães *et al*., 2002).

Pristine conditions rarely occur in Mediterranean systems, given the historically high level of intervention they have suffered. However, some rivers, such as the Odelouca River in the Algarve Region of southern Portugal still contain considerable stands of mature riparian vegetation and faunal elements which are of high conservation value. As part of an ongoing government programme to augment and improve water supply in the Algarve Region, the partially constructed dam situated in the Odelouca basin will be completed before 2010. However, the conservation value of the Odelouca catchment is high, owing to the presence of relatively intact and floristically unique riparian galleries along the river corridor and the presence of two threatened endemic fish species, the Arade nase *Chondrostoma almacai* (Coelho *et al*., 2005)

DOI: 10.1002/aqc

and the Arade loach *Squalius aradensis* (Coelho *et al*., 1998). As a result, environmental mitigation and compensation measures must be undertaken in order to offset the impacts caused by the construction of the dam. This study aims to identify the variables that drive patterns of change in the composition of functional guilds in the benthic macroinvertebrate assemblages of the Odelouca River as well as the scale at which they occur (habitat, reach or basin). They will be used as sentinels of change which will allow the development and implementation of spatially appropriate compensation measures to restore system functioning and complexity at suitable sites.

STUDY AREA

The study area, the Odelouca River (511.4 km^2) is a sub-catchment of the Arade basin (987.37 km^2), situated in the Algarve region of south-west Portugal (Figure 1). The Odelouca is a medium-sized, low-gradient, incised lowland stream running through the predominantly schistose areas of southern Portugal, although catchment geology also includes sienites, quartzites, granites and meta-volcanic deposits on the Serra of Monchique, with alluvial deposits in the lower reaches of the river. Catchment topography varies from narrow, steep-sided valley walls to restricted meander valleys and small floodplains. Connected temporary side channels and backwaters occur in less disturbed areas of the river corridor. The climate is typically Mediterranean, with annual rainfall following a predictable seasonal pattern with a wet season from October to March, and a dry season from June to August. This results in a relatively slow-running river subject to 'flashy' high discharge peaks during the winter, but running dry in the summer, leaving temporarily unconnected pools in the river bed.

Stands of riparian woody plants comprise *Alnus glutinosa* (L.) Gaertner, *Salix salviifolia* Brot. ssp. *australis* Franco, *Nerium oleander* L, and *Fraxinus angustifolia* Vahl. *Tamarix africana* Poiret and *N. oleander* in particular are associated with the lower reaches of the Odelouca basin. Intact riparian corridors occur in the mid-section of the Odelouca, the area to be submerged by the reservoir once the dam becomes operational.

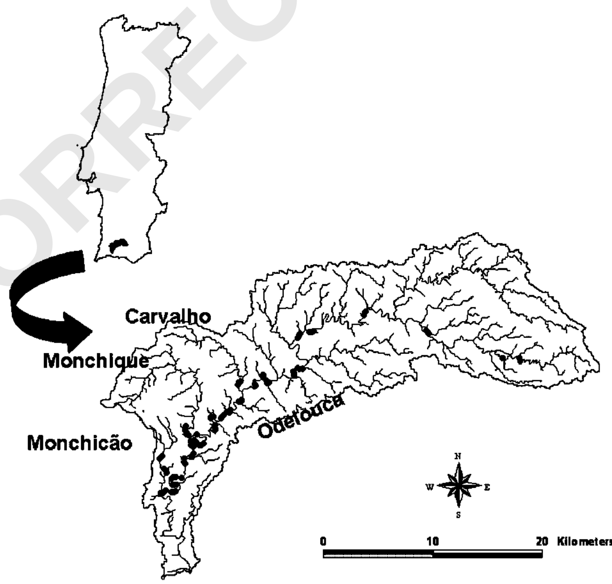

Figure 1. The Odelouca river basin, situated in south-west Portugal. Samples were taken along the main channel and the tributaries of Ribeira de Carvalho, Ribeira de Monchique and the Ribeira Monchicão.

Land-use on the floodplain is predominantly agricultural. Extensive citrus groves occupy the lower catchment below the dam, replacing the natural Mediterranean scrubland and cork-oak woodland vegetation (*Quercus suber* L.). Impacts from these activities include diffuse organic pollution and nutrient enrichment, destruction of the riparian corridor, bank reinforcement and reduced longitudinal connectivity via low-step damming and abstraction for irrigation by pumping from artificially deepened pools dug into the river bed. Urban development is relatively scant, represented by the two small urban conurbations of São Barnabé and São Marcos da Serra and isolated agricultural hamlets. Small-sized monocultures of *Eucalyptus globulus* Labill. and *Pinus pinaster* Aiton are present. Physically, the Odelouca's tributaries are relatively undisturbed. However, the Ribeira de Monchique is affected by organic input from several piggeries and the small town of Monchique, while the Monchicão tributary is affected by abstraction for agriculture in its lower reaches. The Ribeira de Carvalho, situated in the upper Odelouca catchment, is far less disturbed.

METHODS

Habitat assessment

Field data were collected in spring 2005 from 30 sites in the Odelouca basin; 25 sites were situated along the main channel and five in the tributaries of Ribeira de Carvalho, Ribeira de Monchique and the Ribeira Monchicão. Habitat structure, diversity and quality were assessed over a 500-m reach using an adapted version of the UK River Habitat Survey (RHS) method, with the addition of land-use categories and plant species typical of the Iberian Peninsula. RHS records more than 120 variables that describe instream characteristics such as substrate and flow type, character and modification of the margins, land-use, presence and complexity of riparian vegetation and other modifications to the river habitat. RHS comprises 10 'spot-checks' carried out at 50-m intervals of all the features present (assessed over 1 m and 10 m width from bank to bank) followed by a 'sweep-up' assessment of predominant habitat features and modifications together with measurements of stream and bank dimension over the 500-m survey reach (Raven *et al*., 1997). GPS readings of the start, mid-point and end-point of the 500-m reach were registered and at least two photographs of each site were taken. RHS software (version 3.3) was used to calculate the Habitat Quality Assessment index (HQA) and the Habitat Modification Score (HMS). The former metric expresses the structural diversity of natural features of known wildlife interest along the river corridor, while the latter is a measure of the extent to which the river has been modified (Table 1).

Extensive field surveys of riparian vegetation and land-use were taken over a 250 m wide buffer zone on each bank at the 30 survey sites and the entire river corridor. A geographical information system of land-use and the quality, conservation and continuity of the riparian corridor was created from the survey data and aerial photography of the study area. Catchment data on geology, climate, temperature, altitude, relief, land-use, land-cover, organic and industrial discharge and the road network were also added from other geographical information systems (Corine Land Cover, 2000; Instituto da Água, INAG; Instituto Superior de Agronomia, ISA).

Benthic macroinvertebrates

Benthic macroinvertebrate samples were taken in the area of the first RHS spot-check of the surveyed reaches situated, wherever possible at a site that included a riffle. Benthic macroinvertebrates were collected following a protocol adapted from the methodology used in the EU STAR project (http://www.eu-star.at). Six 0.25 m by 1 m (total area 1.5 m^2) samples were taken using a handnet (0.5 mm mesh, 25 cm width) and a standardized kick sampling method. The six hand-net samples were subdivided in proportion to the number and extent of substrate types in the following substrate categories: boulders and cobbles (> 256 mm), stones

Table 1. List of the recorded RHS features used to derive the Habitat Quality Assessment (HQA) and Habitat Modification Score (HMS) indices

Recorded feature	Basis for attribution of HQA score
Flow type	Diversity of flow types
Substrate (river bed)	Predominant natural substrate types
Channel features	Presence and extent of recorded 'natural' features, e.g. exposed bedrock and boulders, vegetated rock
Bank features	Presence and extent of recorded 'natural' features, e.g. eroding cliff, point and side bars
Bank vegetation	Presence and complexity of vegetation
Point bars	Count of total number of point bars along reach
Instream vegetation	Number of types of vegetation present in the stream (filamentous algae do not score)
Land use within 50 m	Broadleaf woodland, native pinewood, moorland/heath and wetlands
Trees and associated features	Tree density and continuity; presence of associated features (hanging boughs, exposed bank-side roots, coarse woody debris, fallen trees)
Special features	Waterfall > 5 m high, braided or side channel, debris dams, natural open water
Recorded feature	**Basis for attribution of HMS score**
Reinforcement	Presence: bank or bed, partial or whole
Resectioning	Presence: bank or bed, partial or whole
Two-stage bank modification	Presence
Embankment	Presence
Poaching of bank	Presence (livestock or humans)
Set-back embankment	Presence
Two-stage channel	Presence
Plant management	Evidence of weed-cutting or bank-mowing
Culvert	Presence (major)
Dam, weir, ford	Presence (minor, intermediate or major)
Bridges	Presence (minor, intermediate or major)
Enhancements	E.g. presence of groynes (minor, intermediate or major)
Flow control	Site partially (<33%) or extensively (> 33%) affected
Realignment of channel	Site partially (<33%) or extensively (> 33%) affected

(64–256 mm), gravel and pebbles (2–64 mm), sand, silt and clay (<2 mm) and particulate organic matter (POM). Sample contents were placed in a plastic flask and fixed *in situ* using 4% formaldehyde.

Physico-chemical readings of temperature, conductivity, pH and dissolved oxygen were taken with hand-held electronic field probes, depth was measured using the graduated handle of a hand-net and water velocity was estimated using a moulinette flowmeter.

In the laboratory, the benthic macroinvertebrate samples were washed, sieved, sorted and identified using a low-power stereo microscope. All individuals were picked out of the sample although subsampling was used if more than 200 individuals of a given taxon were present in the sample. Macroinvertebrates were identified to genus or beyond whenever possible. However, four taxa were not identified beyond family pending confirmation of identification.

Data analyses

This study aimed at determining the relative contribution of two explanatory variable groups (EVG), namely environmental variables and human-impact-related variables (hereafter referred to as 'pressures') driving change in the benthic macroinvertebrate community over the three spatial scales of habitat, reach and basin. This would allow the most appropriate levels of intervention to be identified for implementing

DOI: 10.1002/aqc

compensatory measures as part of the dam construction programme. Here, the 'habitat' spatial level is defined as the features recorded at the RHS spot-check where the macroinvertebrate sample was taken, 'reach' as the 500-m stretch covered by the RHS methodology, and 'basin' as the cumulative drainage area upstream of the RHS reach.

In order to identify gradients of habitat type and habitat quality in the Odelouca study area, selected RHS data (HQA and HMS scores, elements contributing to the HMS and HQA scores, valley and channel-form, distance to source and catchment drainage area) were analysed by hierarchical clustering using Bray–Curtis dissimilarity and Group Average clustering methods (PRIMER 5. 2. 9, using standardized and log-transformed data). Differences between macroinvertebrate assemblages and habitat parameters of the derived clusters of sites were assessed by comparing selected metrics (calculated using the ASTERISC software version 3.0, downloaded from the AQEM website (http://www.aqem.de) using ANOVA (for normally distributed data) or the Kruskal–Wallis test (for non-normally distributed data) following testing for normal distribution (Kolmogorov–Smirnov test) and equal variance (Levene Median test) ('SigmaStat for Windows', Jandel Scientific). The Student–Newman–Keuls Method (ANOVA) or the Dunn's Method (Kruskal–Wallis test) were used to identify which cluster(s) differed significantly by pairwise comparisons of all possible pairs of groups ($P < 0.05$).

The data sets were analysed by canonical correspondence analyses (CCA) and partial canonical correspondence analyses (pCCA) using CANOCO version 4.5 (ter Braak, 1988, 1990), a constrained eigenvalue ordination for relating multivariate ecological data matrices. Macroinvertebrate abundance values of the 84 taxa used in the analyses were log-transformed ($\ln(Ay + B)$). Species occurring at only a single site were excluded from the analyses. The CANOCO manual forward selection procedure was applied to 64 variables divided into two EVGs of environmental variables and pressure variables for each defined spatial scale (Table 2). A cut-off point 0.10 was used (Magnan *et al*., 1994; Aguiar *et al*., 2002) in order to retain only the variables explaining significant amounts of variation in the data sets.

In order to assess how variation was distributed throughout the data set total variation in the macroinvertebrate data matrix was partitioned into four components (ter Braak, 1990; Borcard *et al*., 1992) for each spatial level using the following method: (i) a CCA was carried out on selected environmental variables, (ii) a CCA was carried out on selected pressure variables, (iii) a pCCA was carried out on environmental variables, excluding the influence of pressure variables, and (iv) a pCCA was carried out on pressure variables, excluding the influence of environmental variables.

The separate components of variation, expressed as a percentage of the total variation in the species data, were derived by dividing the canonical eigenvalues of a particular CCA or pCCA by the total inertia (total variation) — namely the sum of all the eigenvalues of a correspondence analysis of the macroinvertebrate abundance matrix.

Based on the procedure set down above partitioned variation values for each spatial scale were derived in the following way: variation explained by environmental variables alone was given by step 3 and variation explained by pressure variables alone was given by step 4. Total explained variation was derived from the sum of steps $1 + 4$ or $2 + 3$; variation shared by both environmental variables and pressure variables was derived by subtracting step 3 from 1 or 4 from 2. Unexplained variation was derived by subtracting the total variation (expressed as a percentage) from 100% (Borcard *et al*., 1992). For each CCA or pCCA analysis, a Monte Carlo test (999 permutations) on both the first axis eigenvalue and the sum of all canonical eigenvalues) was used to evaluate the significance of the effects under analysis (ter Braak, 1990).

RESULTS

A total of 41 985 individuals from 112 taxa (family and genus) were sorted and identified. The samples were dominated by Diptera (Table 3), in particular chironomids and simuliids (44.98% and 15.95% of the total

DOI: 10.1002/aqc

Table 2. A list of the variables and their spatial scale of occurrence considered in this study. Variables marked with an asterisk were retained for subsequent CCA and pCCA analyses following forward selection. EVG=explanatory variable groups

EVG	Variable	Unit/ expression	Abbreviation	Data source
Habitat				
E	Conductivity	$mS\,m^{-1}$	COND	macroinvertebrate sample site measurement
E	*pH	Sorensen scale	PH	macroinvertebrate sample site measurement
E	Dissolved oxygen	$Mg\,L^{-1}$	DO	macroinvertebrate sample site measurement
E	Water velocity	$m\,s^{-1}$	WVEL	macroinvertebrate sample site measurement
E	*Boulders or stones	Classified 0–4	BOLSTON	macroinvertebrate sample site measurement (%)
E	Gravel	Classified 0–4	GRAV	macroinvertebrate sample site assessment (%)
E	Sand/silt/clay/POM	Classified 0–4	SASIC	macroinvertebrate sample site assessment (% coverage)
E	Macrophytes and algae	Classified 0–4	MACROP	macroinvertebrate sample site assessment (%)
E	*Mean depth	m	DEPT	macroinvertebrate sample site assessment
E	*Bank-top land use natural	m	BTNT10	macroinvertebrate sample site assessment (10 m)
P	*Bank modification	Presence/absence 0/1	BNK_MOD	macroinvertebrate sample site assessment
P	Bank poached	Presence/absence 0/1	POAC	macroinvertebrate sample site assessment
P	Channel modification	Presence/absence 0/1	CH_MOD	macroinvertebrate sample site assessment
P	Retention impact	Presence/absence 0/1	RET_PRES	macroinvertebrate sample site assessment
P	Bank-top land use urban	Presence/absence 0/1	BT_UR10	macroinvertebrate sample site assessment (10 m)
P	Bank-top land use agriculture	Presence/absence 0/1	BT_AG10	macroinvertebrate sample site assessment (10 m width)
P	Bank-top land use pasture	Presence/absence 0/1	BT_RP10	macroinvertebrate sample site assessment (10 m)
P	Bank-top land use forestry	Presence/absence 0/1	BT_FR10	macroinvertebrate sample site assessment (10 m)
Reach				
E	*Altitude	m	ALT	GIS data
E	*Distance to source	km	DIST_S	GIS data
E	HQA bank features	Count derived score	HQA_BF	RHS field data (count along 500-m reach)
E	HQA bank vegetation structure	Count derived score	HQA_BKVG	RHS field data (count along 500-m reach)
E	*HQA channel vegetation	Count derived score	HQA_CHVG	RHS field data (count along 500-m reach)
E	*HQA trees	Count derived score	HQA_TR	RHS field data (count along 500-m reach)
E	HQA associated features	Count derived score	HQA_ASS	RHS field data (count along 500-m reach)
E	*Number of riffles	Count	RIFF	RHS field data (count along 500-m reach)

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Table 2. *continued*

EVG	Variable	Unit/ expression	Abbreviation	Data source
E	Number of pools	Count	POOL	RHS field data (count along 500-m reach)
E	Number of bars	Count	BARS	RHS field data (count along 500-m reach)
E	Bank-full width	m	BKFULL	RHS field data
E	Water width	m	WT_WDT	RHS field data
E	Average bank-top height	m	AVBKTP	RHS field data
E	Riparian gallery width	m	WDTRIP	RHS field data
E	Number of side channels	Count	SUBCH	RHS field data (wet or dry)
E	Vegetation in channel	Classified 0–2	CH_VEG	RHS field data
E	Land use seminatural	Classified 0–4	LU250_NAT	250-m buffer on each bank — GIS (%)
E	Land use scrub	Classified 0–4	LU250_SCR	250-m buffer on each bank — GIS (%)
E	Riparian quality	Classified 1–5	RIP_QUAL	250-m buffer on each bank — GIS (%)
P	Habitat modification score	Derived score	HMS	RHS software
P	Bank resectioned	Count derived score	BK_RS	RHS field data (count along 500-m reach)
P	Bank reinforced	Count derived score	BK_RI	RHS field data (count along 500-m reach)
P	Bank poached	Count derived score	BK_PC	RHS field data (count along 500-m reach)
P	Bank embanked	Count derived score	BK_EM	RHS field data (count along 500-m reach)
P	Channel dammed	Classified 0–4	CH_DA	RHS field data (count along 500-m reach)
P	Ford	Classified 0–4	FORD	RHS field data (count along 500-m reach)
P	Tipped debris	Classified 0–4	TD	RHS field data (count along 500-m reach)
P	Land use — urban	Classified 0–4	LU250_UR	250-m buffer on each bank — GIS (%)
P	*Land use — agriculture	Classified 0–4	LU250_AG	250-m buffer on each bank — GIS (%)
P	Land use — forestry	Classified 0–4	LU250_FO	250-m buffer on each bank — GIS (%)
P	Land use — roads	Classified 0–4	LU250_RO	250-m buffer on each bank — GIS (%)
P	*Land use — bare	Classified 0–4	LU250_BA	250-m buffer on each bank — GIS (%)
Basin				
E	Valley form	Classified 0–4	VALFRM	GIS data
E	Channel form	Classified 1–2	CHAN_F	GIS/RHS data
E	*Drainage area	km^2	DRAIN	GIS data (nested value)
E	Precipitation	$mm\,km^{-2}$	PREC	GIS data
E	*Temperature	$^{\circ}C\,km^{-2}$	TEMP	GIS data
E	*Geology turbidites	Classified 0–4	GEOLT_C	GIS data (%)
E	*Geologia sinaitos	Classified 0–4	GEOLS_C	GIS data (%)
P	*Urban area	% catchment area	URB_A	GIS data (%)
P	Monocultures	% catchment area	MONO_A	GIS data (%)
P	*Agriculture	% catchment area	AGRI_A	GIS data (%)

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Table 2. *continued*

EVG	Variable	Unit/ expression	Abbreviation	Data source
P	Roads (km)	km	ROAD	GIS data (cumulative km per unit area) GIS data (cumulative)
P	No. organic discharges	Count	ORG_D	Count
P	No. industrial discharges	Count	IND_D	Count

Table 3. Summary data of the benthic macroinvertebrate samples

	No. taxa	Range of abundance	Total	%	Mean abundance ± SD	No. of sites (%)
Tricladida	1	0–1760	2369	5.64	78.93 (±321.89)	30 (36.67%)
Gastropoda	8	0–312	1399	3.33	46.37 (±86)	28 (76.67%)
Bivalvia	1	0–17	31	0.07	1.00 (±3.56)	24 (10%)
Oligochaeta	6	0–544	2940	7.00	97.80 (±160.25)	11 (80%)
Hirudinea	3	0–3	16	0.04	0.43 (±1.01)	26 (20%)
Hydracarina	1	0–1	2	0.00	0.03 (±0.18)	23 (3.33%)
Ostracoda	1	0–80	122	0.29	4.03 (±15.05)	28 (16.67%)
Maxillopoda	1	0–3	5	0.01	0.13 (±0.57)	24 (6.67%)
Isopoda	1	0–1	2	0.00	0.03 (±0.18)	22 (3.33%)
Decapoda	2	0–197	798	1.90	26.53 (±40.96)	20 (80%)
Collembola	1	0–3	4	0.01	0.10 (±0.55)	5 (3.33%)
Ephemeroptera	14	0–772	4625	11.02	153.70 (±193.17)	9 (93.33%)
Plecoptera	6	0–146	1252	2.98	41.53 (±44.79)	3 (93.33%)
Odonata (Zygoptera)	2	0–14	52	0.12	1.67 (±3.68)	6 (30%)
Odonata (Anisoptera)	5	0–41	224	0.53	7.30 (±11.71)	2 (66.67%)
Hemiptera (Heteroptera)	5	0–5	12	0.03	0.23 (±0.97)	2 (6.67%)
Coleoptera	18	0–61	322	0.77	10.13 (±16.48)	1 (73.33%)
Trichoptera	19	0–1266	2034	4.84	67.17 (±236.17)	1 (86.67%)
Diptera	16	0–3932	25 776	61.39	858.67 (±1223.59)	1 (100%)

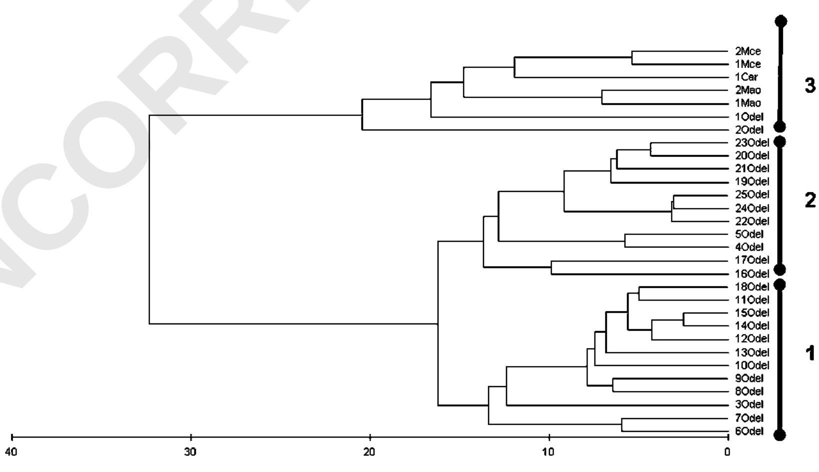

Figure 2. Dendrogram of hierarchical cluster analysis (Bray–Curtis dissimilarity and Group Average Clustering) of Odelouca sites, based upon drainage basin and habitat quality or modification parameters.

Table 4. Summary data of selected metrics for the clusters of Odelouca sampling sites. (GrSc=grazer/scrapers, Sh=shredders, GC=gatherers/collectors, FF=filter feeders, Pr=predators; %EPT=percentage Ephemeroptera/Plecoptera/Trichoptera)

		HQA				HMS	Benthic macroinvertebrate metrics								
		Channel vegetation	Trees	Associated features	Score	Score	No. of genera	BMWP (Spain)	Shannon–Wiener	%GrSc	% Sh	% GC	% FF	% Pr	%EPT
Cluster 1	max.	7	19	5	66	6	31	125	2.12	33.60	24.43	63.78	45.35	34.86	79.68
	min.	0	7	0	26	0	8	27	0.99	6.09	0.14	15.08	2.95	5.75	2.88
	mean	3.17	12.17	3.75	48.83	1.08	16.25	80.83	1.73	15.44	6.87	35.71	12.38	16.29	37.99
	SD	2.04	4.30	2.26	11.23	2.15	6.08	34.67	0.31	7.79	6.49	15.08	13.49	9.46	25.61
Cluster 2	max.	8	19	5	55	37	24	119	2.14	24.21	5.74	84.79	58.50	42.13	41.82
	min.	0	0	0	29	4	6	9	0.68	4.49	0.00	16.66	0.15	3.40	0.00
	mean	5.36	5.00	1.82	41.36	17.45	16.73	66.55	1.30	16.36	1.32	35.09	25.59	11.30	13.80
	SD	3.01	5.18	2.52	7.74	10.11	5.35	30.17	0.43	5.93	1.77	19.64	17.17	10.92	11.23
Cluster 3	max.	6	19	5	59	4	41	180	2.23	35.32	14.30	66.90	42.19	17.73	74.80
	min.	1	8	0	46	0	19	106	1.33	5.73	0.08	18.02	4.77	7.78	21.55
	mean	4.14	11.57	3.57	53.14	1.71	26.43	132.57	1.81	24.33	3.30	35.17	13.90	12.71	42.52
	SD	1.57	3.51	2.44	4.88	1.70	7.48	25.65	0.38	10.71	4.90	16.40	12.91	3.48	24.26

Table 5. Results of the ANOVA/Kruskal-Wallace tests and pairwise analyses where significant differences between clusters were found for selected parameters and metrics (GrSc=grazers scrapers, Sh=shredders, GC=gatherers/collectors, FF=filter feeders, Pr=predators; %EPT=percentage Ephemeroptera/Plecoptera/Trichoptera; H=Kruskal-Wallis test, F=ANOVA)

	HQA score	HMS score	HQA trees	BMWP (Spain)	Diversity (*H*)	EPT taxa	Evenness	% functional feeding groups				
								Sh	GrSc	GC	FF	Pr
Test Statistic $p =$	$F = 4.14$ 0.0269	$H = 20.7$ 0.0001	$H = 13.2$ 0.0014	$H = 13.0$ 0.0015	$F = 5.24$ 0.0129	$F = 7.2$ 0.0036	$F = 4.27$ 0.0259	$H = 9.40$ 0.0091	$F = 4.04$ 0.0308	$H = 0.801$ 0.6699	$H = 3.70$ 0.1569	$F = 2.06$ 0.149
Significantly different clusters	3 *vs* 2	2 *vs* 1	3 *vs* 2	1 *vs* 2	3 *vs* 2	1 *vs* 2	1 *vs* 2	1 *vs* 2	3 *vs* 1			
		2 *vs* 3	1 *vs* 2		1 *vs* 2	3 *vs* 2			3 *vs* 2			

Table 6. Variables retained for analyses by the manual forward selection process and listed in order of their inclusion in each model, together with the additional variance each variable explains at the time it was included (lambda-*A*). The significance (*p*-value) of the variable at that time of inclusion is also given (Monte Carlo test, 999 permutations). The Variance Inflation Factor (VIF) expresses the degree of collinearity among the retained variables

	Variable	EVG	λA	p	Variance explained		VIF	Correlation canonical axes (interset)		Canonical coefficients	
					Selected variables	All variables		Axis 1	Axis 2	Axis 1	Axis 2
Habitat	pH	e	0.18	0.002	0.18		1.694	0.858	−0.068	0.825	0.395
($n = 5$)	BTNT_10	e	0.14	0.002	0.33		1.743	0.564	−0.606	0.123	−1.086
	BOLSTON	e	0.11	0.01	0.43		1.176	−0.438	−0.302	−0.219	−0.587
	DEPT	e	0.09	0.04	0.53	0.92	1.117	0.099	0.271	0.240	0.169
	BNK_MOD	p	0.11	0.04	0.11	0.58	1.000	—	—	—	—
Reach	RIFF	e	0.2	0.001	0.2		4.238	−0.141	0.877	0.669	0.696
($n = 7$)	HQA_CHVG	e	0.18	0.001	0.38		1.653	−0.718	−0.371	−1.122	−0.144
	DIST_S	e	0.12	0.001	0.5		4.014	0.063	−0.699	1.050	−0.126
	ALT	e	0.1	0.018	0.6		1.627	0.338	0.113	0.532	−0.033
	HQA_TR	e	0.09	0.036	0.69	1.47	2.276	0.057	0.720	−0.456	0.235
	LU250_BA	p	0.17	0.002	0.17		1.140	0.775	−0.331	0.706	−0.801
	LU250_AG	p	0.09	0.036	0.69	1.04	1.140	0.659	0.464	0.503	0.942
Basin	TEMP	e	0.21	0.001	0.21		5.809	−0.896	0.098	−1.924	0.706
($n = 8$)	DRAIN	e	0.16	0.001	0.38		3.776	−0.055	0.167	1.518	6.127
	GEOLS_C	e	0.14	0.001	0.51		6.776	0.497	−0.297	−2.079	−18.027
	GEOLT_C	e	0.12	0.004	0.63	0.89	10.428	−0.545	0.167	−2.592	−19.429
	ORG_D	p	0.17	0.001	0.35		7.350	0.680	−0.558	2.359	0.684
	ROAD	p	0.12	0.005	0.47		9.786	0.274	−0.820	−1.958	−1.814
	URB_A	p	0.11	0.018	0.46		2.905	0.235	0.452	−0.628	−0.301
	AGR_A	p	0.1	0.024	0.56	0.64	1.577	0.199	−0.066	0.084	0.516

Dipteran abundance respectively). Ephemeroptera (11.02%) and Oligochaeta (7%) were also relatively well represented. The most diverse higher taxonomic group was the Trichoptera (19 genera), followed by the Coleoptera (18 genera) and the Diptera (16 genera). Dipteran taxa occurred most frequently across the sampling sites (100%), followed by ephemeropteran and plecopteran taxa (both 93.33%). Taxa occurring only at a single site (and excluded from ordination analyses) were Collembola, Hydracarina and Isopoda.

The hierarchical classification analysis clearly reveals three groups of sites (Figure 2) based on their physical character and the RHS recorded habitat quality and modification parameters (Tables 4 and 5). Sites on the main channel (cluster 1) and along the tributaries (cluster 3) with low levels of human intervention and higher habitat quality, in particular the presence of stands of riparian vegetation, are clearly distinct from degraded sites along the main channel (cluster 2).

Of the 11 sites comprising cluster 1, 10 (90.9%) are situated in the reservoir inundation area between the town of São Marcos da Serra and the dam. Levels of human intervention in this area are low and riparian woody stands are relatively intact, reflected in the lower average HMS score and the higher average value of the HQA trees and HQA associated features (Tables 4 and 5). This is also evident for the cluster 3 sites, which mainly comprise sites along the tributaries. Macroinvertebrate communities at these sites are more diverse, indicate higher ecological quality, and contain a higher percentage of shredder species and EPT taxa. Shredder taxa such Psychomyiidae, *Lymnaea*, *Lype*, and *Serratella, Capnioneura* and *Tipula* are associated with these sites, as well as predator taxa such as *Atherix*, *Clinocera* and *Platycneumus*.

Cluster 2 sites, mostly situated downstream of the partially built dam (81.8%), are subject to higher levels of human intervention made evident by the high average HMS value. Riparian vegetation is highly

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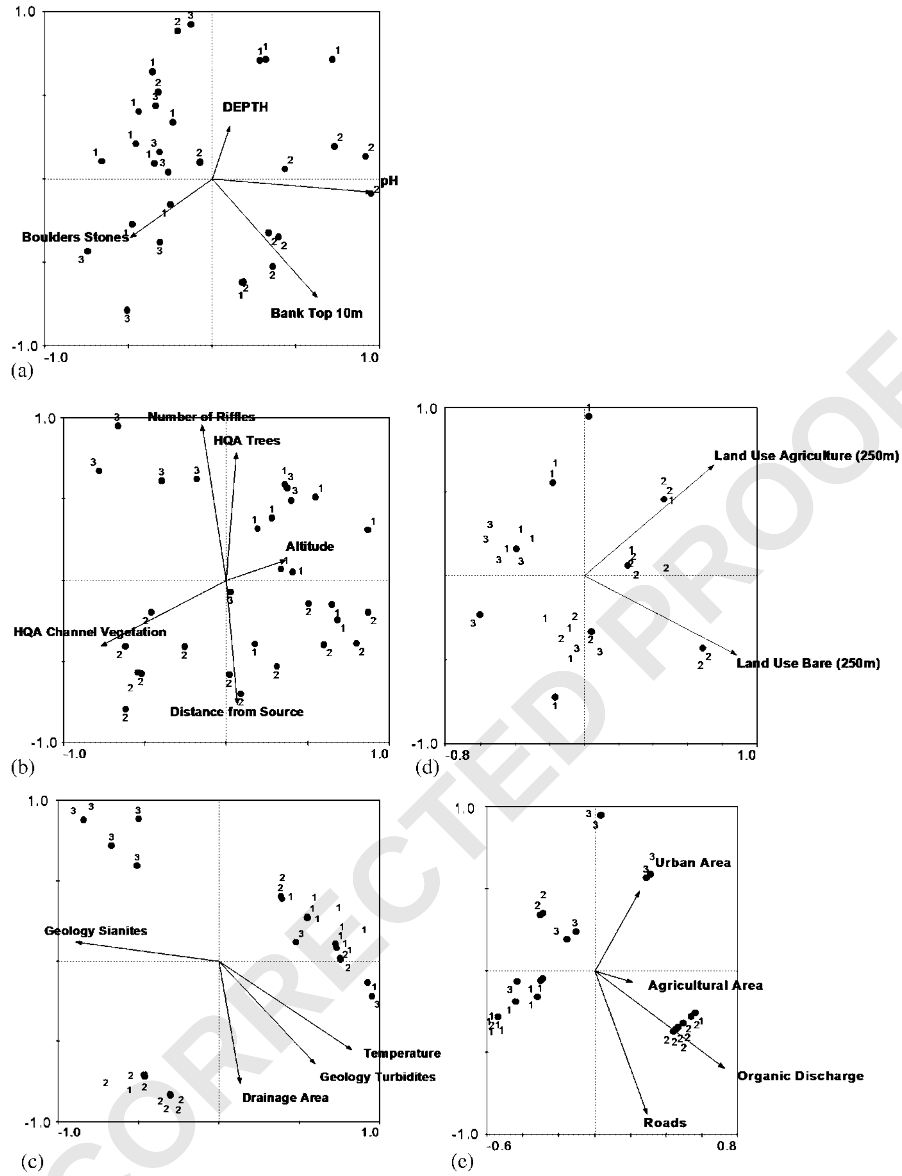

Figure 3. Ordination plots of the pCCA analyses using selected environmental and pressure variables over three spatial levels. (a) Habitat-level environmental variables. (b) Reach-level environmental variables. (c) Basin-level environmental variables. (d) Reach-level pressure variables. (e) Basin-level pressure variables.

degraded or even absent at these sites, which are mostly situated in agricultural areas. The increased insolation and diffuse agricultural runoff results in extensive stands of aquatic vegetation (principally *Ranunculus*, often covered with *Cladophora*), reflected in the higher average HQA channel vegetation score. Fewer, essentially non-specialist species or species with adaptations (the ability to respire atmospheric oxygen, physiological tolerance to higher temperatures, poorer water quality, a preference for reduced flow regimes) occupy these degraded habitats such as scraper/grazers *Bythinia*, *Gyraulus*, *Rhithrogena* and *Stenelmis* as well as the highly adaptable and mobile predator *Sympetrum*.

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The manual forward selection procedure retained 20 (31.25%) of the initial 64 variables for subsequent CCA and pCCA analyses (Table 6). The number of retained environmental explanatory variables ($n = 13$) is almost twice that of retained explanatory pressure variables ($n = 7$). With the exception of the highest spatial scale, where the number of selected variables per EVG is equal (four per group), the number of environmental variables per spatial scale far exceeds the number of pressure variables.

The cumulative percentage of variance of the species data extracted from the first ordination and second axes by the selected habitat scale environmental variables is 36.8% and 69.2% respectively. Together, the third and fourth axes account for 30.6% of the percentage variance of habitat level species–environment variance. The interset correlations of environmental variables with the axes (Table 6) show a strong positive physico-chemical gradient (pH) along axis 1 of the ordination plot and a gradient related to physical parameters related to substrate and bank-top vegetation (BTNT10, BOLSTON) along the second axis.

A pH gradient is clearly evident along axis 1; most cluster 2 sites have higher pH values and are distributed in the right-hand quadrant of the ordination plot (Figure 3(a)). These sites, situated downstream of the dam, are shallow, wide and unshaded with dense stands of aquatic vegetation. The lowest pH values were recorded among the cluster 3 sites which are also shallower with coarser substrates, hence their positions in the lower left quadrant of the ordination plot. A weaker gradient related to bank top habitat quality and the two instream variables is discernible along axis 2. Sites distributed to the left of the ordination plot tend to be less disturbed, characterized by stands of natural or semi-natural vegetation within the 10-m stretch of bank top where macroinvertebrate samples were taken.

At reach scale the cumulative percentage of variance of the species–environment relation explained by axis 1 and axis 2 is 30.8% and 61.1% respectively, and 29.5% by axes 3 and 4. The interset correlations between the axes and environmental variables reveal distinct changes in the macroinvertebrate assemblages related to strong longitudinal changes in the environmental quality of the reaches in the study area, reflected in the strong separation of cluster 2 sites from cluster 1 and cluster 3 sites. HQA_CHVG is strongly correlated with axis 1 and RIFF, HQA_TR and DIST_S with axis 2 of the ordination plot (Figure 3(b)).

Cluster 1 and cluster 3 sites are characterized by greater numbers of riffles and more intact stands of riparian vegetation, highlighting their habitat heterogeneity and integrity. At basin scale (species–environment relation variance = 43.9% axis 1; axis 2 = 27.2%, 28.9% = axes 3 and 4) interset correlations reveal a strong correlation between axis 1 and TEMP and a weaker correlation with GEOLT_C. Correlation coefficients of the explanatory variables with axis 2 are all weak (Table 6). The clusters form almost exclusive groups in the ordination space, indicating that the selected environmental variables at this spatial scale exert a strong influence on the macroinvertebrate assemblages. Cluster 3 sites (predominantly tributaries) are strongly separated from sites on the principal course (Figure 3(c)) by virtue of their smaller size, lower temperature (diminished insolation resulting from their situation in steeper, narrower valleys which receive less insolation as well as extensive shading by riparian vegetation) and the sianitic geology of the area in which they are situated. Cluster 2 sites form a distinct group in the ordination space owing to their position further down the drainage area of the catchment, increased temperature and predominantly turbite geology. Cluster 1 sites also form a distinct group, to the right of the ordination plot.

The single selected habitat level pressure variable (only 5.14% of the total explanatory variation, 100% variance of the species data extracted from ordination axis 1) essentially separates sites with or without modified bank profiles. The reach level selected land-use variables (LU250_BA, LU250_AG, species–environment relation variance: axis 1 = 75%, axis 2 = 25%) along axis 1 separates macroinvertebrate assemblages at sites with natural/semi natural land-use (clusters 1 and 3) to the left of the ordination space, whilst predominantly cluster 2 sites on the right-hand side of the ordination plot represent macroinvertebrate assemblages taken at sites with no surrounding vegetation or with agriculture in the immediately surrounding area (Figure 3(d)).

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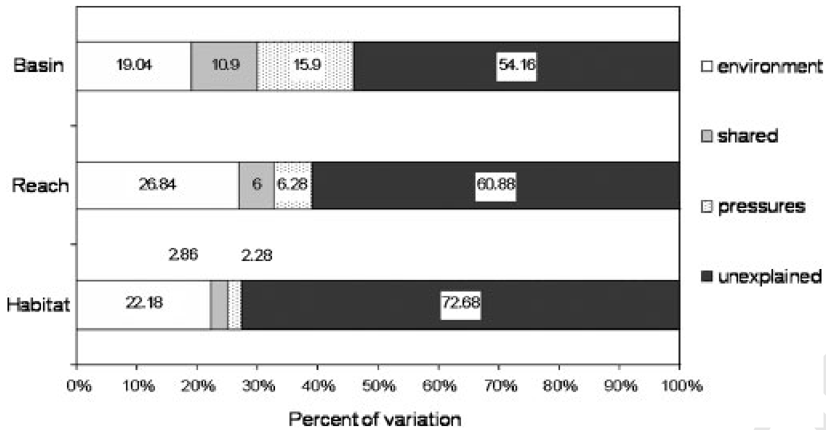

Figure 4. Distribution of partitioned variation over the spatial scales of habitat, reach and basin in the Odelouca macroinvertebrate data.

Basin-scale pressure variables are important anthropogenic drivers of macroinvertebrate community change in the study area (variance of the species–environment relation along axis 1 = 37.1%, axis 2 = 30.7%), indicated by the strong separation of the sites in the derived clusters and the interset correlation coefficients which reveal a strong gradient related to organic discharge along axis 1 and the cumulative effect of various urban infrastructures along axis 2. Cluster 2 sites form a strongly distinct group in the ordination space (Figure 3(e)) indicating that the macroinvertebrate assemblages are strongly subject to the cumulative effect of organic discharge, agricultural activities and impacts resulting from the presence of roads. The effect of these impacts is undoubtedly exacerbated by the severely reduced instream flow downstream of the dam coupled with abstraction for agriculture in the lower catchment. Relatively unaffected cluster 1 and some cluster 3 sites form a distinct group in the lower left quadrant of the ordination plot. However, a small group of cluster 3 sites, situated along the Monchique and Monchicão are clearly affected by urban runoff, organic discharge and abstraction.

Partitioning of variance provides an accurate estimate of the spread of shared and pure variance in the species data set described by environment/pressure variables, permits the interaction between the separate sets of variables to be assessed, and provides a visual guide to where the greatest amount of variance resides amongst the spatial scales under study (Borcard *et al*., 1992). The Odelouca macroinvertebrate data (Figure 4) show that environmental and pressure variables account for greater amounts of variation (pure and shared) with increasing spatial scale (habitat 27.32%, reach 39.12% and basin 45.84%). Variation due to environmental factors was highly significant across all spatial scales: habitat 22.18% ($p = 0.001$, 999 permutations under full model); reach 26.84% ($p = 0.001$) and basin 19.04% ($p = 0.001$).

Less variation is explained by the pure 'pressure' component, although the contribution of these effects increases with increasing spatial scale (habitat 2.28%, reach 6.28%, basin 15.9%) and is significant only at the highest spatial level of the basin ($p = 0.008$, 999 permutations) indicating that large-scale human intervention in the basin is an important driver of change in the lotic macroinvertebrate communities. Shared variation is low across all spatial levels demonstrating that a large amount of the explained variation is compartmentalized exclusively into one of the two EVGs, a consequence of the fact that the variables under analysis (environment *vs* human impacts) tend to be mutually exclusive, especially within the same spatial scale under analysis. Unexplained variation is high across all three spatial levels, in particular at the level of habitat. This can be attributed to overlooked or non-measured factors (factors not taken into account in this study), the use of single spot measurements taken at sample sites, or to a large amount of stochastic variation (Borcard *et al*., 1992).

DOI: 10.1002/aqc

DISCUSSION

Rivers are hierarchically structured ecosystems with highly heterogeneous communities of lotic biotic elements (Heino *et al*., 2004). Many studies show that key environmental variables at reach and habitat scales are important determinants of freshwater community function and structure (Allan *et al*., 1997; Davies *et al*., 2000; Allan, 2004b; Heino *et al*., 2004) but that they are influenced by factors operating at higher spatial levels as well as historical events (Frissell *et al*., 1986; Rickleffs, 1987; Malmqvist, 2002). The results of this study in a southern European Mediterranean river system support this paradigm.

Classification analysis of the study sites based on parameters related to physical aspects of the watercourse, drainage basin, habitat quality and the degree of human influence identified distinct clusters of sites in the study area. Suites of traditionally used metrics, such as biological and diversity indices, percentage feeding groups and percentage of pollution-sensitive taxa successfully identified statistically significant differences between macroinvertebrate assemblages at sites with high habitat quality and those with degraded habitats. The use of a diverse suite of metrics provides an ample source of data for detecting change in the macroinvertebrate assemblages in this Mediterranean system. Recent studies on the use of taxonomic distinctness measures (Average Taxonomic Distinctness, Variation in Taxonomic Distinctness and Total Taxonomic Distinctness) to assess human pressures on a Mediterranean system of the Iberian Peninsula were not very successful (Abellán *et al*., 2006).

Significantly more diverse assemblages, with a higher proportion of shredder species and EPT taxa (normally associated with good ecological status) were clearly associated with sites on the Odelouca with good habitat quality (namely, features indicative of flow and channel substrate heterogeneity) and intact stands of riparian vegetation with important associated features such as woody debris, submerged roots and leaf litter. Although shredder species tend to be less well represented in Meditteranean systems (Aguiar *et al*., 2002) because of the sclerophyllous nature of the leaves from riparian vegetation (as a source of CPOM) and the effect of seasonal scouring reducing leaf litter retention (Gasith and Resh, 1999), their clear association with sites of good ecological quality in the study is an important metric of Mediterranean habitat integrity and CPOM dynamics (Cummins, 2002).

CA and pCCA clearly show that selected environmental variables across the spatial scales considered in this study are important drivers of change in macroinvertebrate assemblage structure and function. A spatially nested hierarchical pattern (Sandin and Johnson, 2004) is evident with lower-scale, principally environmental, processes being affected by both environmental and human factors such as geology and land-use patterns at higher spatial levels (Frissell *et al*., 1986; Allan *et al*., 1997; Poff, 1997). Although some studies state that local scale factors are more important in determining the structure and function of macroinvertebrate assemblages (Boyero, 2003; Rios and Bailey, 2006), these results clearly show that factors at the highest spatial level such as geology, basin area, temperature and changes in land use are also fundamental. These large-scale landscape features exert a 'domino effect' upon multiple environmental processes at lower spatial scales (Allan *et al*., 1997) that induce change in the macroinvertebrate assemblages.

Similarly to the findings of Sandin and Johnson (2004), macroinvertebrate communities responded to a chemical gradient (in this case pH) and instream physical variables related to substrate at habitat level. However, the influence of pH on community structure and function is a result of factors operating at higher spatial scales. Background levels of pH, associated with undisturbed environmental conditions are related to catchment geology (an extremely important basin level determinant of macroinvertebrate community structure), climate and instream biological processes typical of relatively undisturbed sites (semi-natural land use, decomposition processes, photosynthesis and respiration processes). Where riparian vegetation is highly degraded or absent through human intervention on the floodplain, decreased shading of the channel, increased siltation, pollutant and nutrient transport due to reduced buffering capacity (Naiman and Décamps, 1997) result in profound physico-chemical and biological alterations in the lotic processes and

DOI: 10.1002/aqc

biotic complexity and function (Rios and Bailey, 2006). The analyses clearly indicate that most of the degraded sites in the Odelouca basin are situated downstream of the dam, an area subject to the highest levels of human intervention in the basin. Already affected by an extremely reduced flow regime due to dam retention, the cumulative effect of urban and agricultural impacts and physical alteration of the banks and channel, most of these sites are characterized at habitat level by dense stands of aquatic vegetation. The higher pH levels at these degraded sites are a result of the photosynthetic activity of these aquatic plant stands and its effect upon instream physico-chemical processes.

Environmental factors recorded by RHS at reach scale such as the presence and complexity of riparian galleries, habitat and flow heterogeneity, are particularly important drivers of macroinvertbrate assemblage change in the Odelouca study area that clearly describe longitudinal changes in land use, habitat quality and the macroinvertebrate community. The selected variables and their influence on the macroinvertebrate fauna are clearly affected by factors related to land use at the highest spatial level used in this study. The findings at this spatial level complement the results of the classification analysis, i.e. sites in the mid-section of the Odelouca and the tributaries (clusters 1 and 3 respectively), found to be more trophically and functionally diverse, are characterized by predominantly natural/semi-natural land use, minimal habitat modification, greater habitat heterogeneity and relatively intact riparian galleries vegetation compared with sites downstream of the dam. Assessment of the extent and diversity of natural important features (channel substrate, channel features, side channels and backwaters, bank features, bank vegetation complexity and land use) and the extremely low levels of human intervention contribute to the enhanced biological integrity of these sites, indicating their suitability as candidate sites for the implementation of compensatory measures following completion of the Odelouca dam.

The influence of pressure variables on macroinvertebrate assemblages is significant only at higher spatial scales, supporting the observation that 'human actions at the landscape scale are a principal threat to the ecological integrity of river ecosystems, impacting habitat, water quality, and the biota via numerous and complex pathways' (Allan, 2004b). Agricultural activity is the principal pressure acting upon the Odelouca system, especially at sites situated below the dam, made evident by the strong response of macroinvertebrate assemblages to agriculture-related reach- and basin-scale parameters. The effect of urbanization and associated infrastructures is also important at this scale. The multiple impacts of agricultural activity are spread over several spatial scales from the clearance of natural bank-side vegetation and riparian galleries, bank reinforcement, damming, abstraction, the application of pesticides and fertilizers to the clearance of indigenous vegetation. These impacts reduce habitat connectivity, aggravate sediment and contaminant runoff, induce disturbance in natural flow patterns and lead to a decline in water quality. The impact of these factors is particularly marked in Mediterranean systems since competition for water is high in these naturally water-stressed systems (Gasith and Resh, 1999).

The continuing divergence between ecosystem and societal needs concerning freshwater resources (Poff *et al*., 2003) is reflected in the continued global decline in aquatic systems as a result of unrelenting human intervention across all spatial scales (Karr and Schlosser, 1978). This serious dilemma has prompted the implementation of restoration, rehabilitation and compensatory measures as a means of recovering ecosystem integrity, although their efficacy has been questioned (Karr and Schlosser, 1978; Kershner, 1997; Dobson and Cariss, 1999; Booth *et al*., 2004; Cortes, 2004; Harrison *et al*., 2004; Moerke and Lamberti, 2004). The attainment of 'good' ecological status for all European Union Member State surface waters by 2015 as stipulated by the Water Framework Directive also implies far-reaching restoration initiatives for many European rivers and streams in the near future.

Although the Odelouca is a typical non-equilibrium, disturbance-driven Mediterranean river, it is also, like most Mediterranean river systems, highly regulated as a result of the historically high demand for water from an intrinsically water-limited system that suffers predictable but extreme seasonal patterns of drying and flooding (Gasith and Resh, 1999). Compensatory and restoration objectives for the Odelouca must be self-sustaining, allowing manageable change driven by (albeit) controlled natural events to occur within the

DOI: 10.1002/aqc

system, thereby allowing chains of key abiotic and biotic processes and events to confer habitat function, complexity and heterogeneity (Hughes *et al*., 2001). Alterations operating upon the basin under study must also be taken into account (Kershner, 1997).

The results of this study clearly show that compensatory measures for the Odelouca must extend across several spatial scales, comprising site-, reach- and basin-level initiatives. Rehabilitation efforts concentrated at lower spatial scales alone have been shown to have limited success (Harrison *et al*., 2000; Moerke and Lamberti, 2004), calling for greater emphasis on the study and recognition of processes interacting within and across several spatial scales (Hughes *et al*., 2001; Wohl *et al*., 2005). The fact that the Odelouca will become a highly regulated system with a profoundly altered flow regime must also be considered, since hydrological factors such as flood events are fundamental to riparian and instream habitat structure and function (Hughes *et al*., 2001). Historically, Mediterranean systems such as the Odelouca have been subject to high levels of agricultural activities, resulting in extremely fragmented and degraded habitats (Aguiar and Ferreira, 2005). Fortunately, the Odelouca system possesses considerable stretches that serve as benchmark sites for developing and implementing compensatory measures along both the main channel and the tributaries.

The presence of riparian vegetation, and habitat features directly and indirectly associated with this ecotone (channel shading, CPOM input and dynamics including leaf retention, large woody debris, channel and flow heterogeneity, macroinvertebrate trophic and functional complexity) are clearly vital abiotic and biotic factors to be considered for compensatory measures. The role of this ecotone as a buffer against agricultural sediments is also important (Karr and Schlosser, 1978; Naiman and Décamps, 1997) A combined approach (Sandin and Johnson, 2004) will be necessary, taking into account the assessment and selection of reaches that can be managed in such a way as to reintroduce complex bank-side, riparian and instream structures. Flow requirements — particularly the occurrence of flood events—are perhaps the single most important instream environmental factor shaping these disturbance-driven systems. Flood events create flow and substrate heterogeneity (scouring and sedimentation events) fundamental to riparian seed dispersal and recruitment (Dixon, 2003) and habitat heterogeneity (pools, riffles, leaf banks retained by large woody debris) which favour more trophically complex benthic macroinvertebrate and fish assemblages (Pearson *et al*., 1992; Laasonen *et al*., 1998; Lee *et al*., 2001).

Development and implementation of riparian buffer guidelines (Wenger, 1999) for the Odelouca must take into account the appropriate width, the vegetation type and the degree of complexity necessary for restoring ecological integrity to predefined levels suitable for a Mediterranean river system. Long-term biomonitoring will also be necessary in order to assess whether the implemented compensation measures have been successful and, if necessary, to introduce alterations and improvements to rehabilitation methods. This study has shown that the RHS methodology has successfully provided essential information, not only on Mediterranean system habitat quality but also on the identification of key environmental characteristics. Such characteristics can be used as sentinels of change in the macroinvertebrate community, contributing to the development and implementation of effective compensation measures at the appropriate spatial levels of intervention.

ACKNOWLEDGEMENT

Many thanks to Luis Lopes for his indispensable and constant support in the field.

REFERENCES

Abellán P, Bilton DT, Millán A, Sánchez-Fernandez D, Ramsay PM. 2006. Can taxonomic distinctness assess anthropogenic impacts in inland waters? A case from a Mediterranean river basin. *Freshwater Biology* **51**: 1744–1756.

Aguiar FC, Ferreira MT. 2005. Human-disturbed landscapes: effects on composition and integrity of riparian woody vegetation in the Tagus River basin, Portugal. *Environmental Conservation* **32**: 1–12.

Aguiar FC, Ferreira MT, Pinto P. 2002. Relative influence of environmental variables on macroinvertebrate assemblages from an Iberian basin. *Journal of the North American Benthological Society* **21**: 43–53.

Allan D, Erickson D, Fay J. 1997. The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology* **37**: 149–161.

Allan JD. 2004a. Influence of land use and landscape setting on the ecological status of rivers. *Limnetica* **23**: 187–198.

Allan JD. 2004b. Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology Evolution and Systematics* **35**: 257–284.

Bonada N, Zamora Muñoz C, Rieradevall M, Prat N. 2005. Ecological and historical filters constraining spatial caddisfly distribution in Mediterranean rivers. *Freshwater Biology* **50**: 781–797.

Booth DB, Karr JR, Schauman S, Konrad CP, Morley SA, Larson MG, Burges SJ. 2004. Reviving urban streams: land use, hydrology, biology, and human behaviour. *Journal of the American Resources Association* **03187**: 1351–1364.

Borcard D, Legendre P, Drapeau P. 1992. Partialling out the spatial component of ecological variation. *Ecology* **73**: 1045–1055.

Boyero L. 2003. Multiscale patterns of spatial variation in stream macroinvertebrate communities. *Ecological Research* **18**: 365–379.

Coelho MM, Bogutskaya NG, Rodrigues JA, Collares-Pereira MJ. 1998. *Leuciscus torgalensis* and *L. aradensis*, two new cyprinids for Portuguese fresh waters. *Journal of Fish Biology* **52**: 937–950.

Coelho MM, Mesquita N, Collares-Pereira MJ. 2005. *Chondrostoma almacai*, a new cyprinid species from the southwest of Portugal, Iberian Peninsula. *Folia Zoologica* **54**: 201–212.

Cortes RMV. 2004. *Requalificação de Cursos de Água*. Instituto da Água.

Cummins KW. 2002. Riparian-stream linkage paradigm. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* **28**: 49–58.

Davies NM, Norris RH, Thoms MC. 2000. Prediction and assessment of local stream habitat features using large-scale catchment characteristics. *Freshwater Biology* **45**: 343–369.

Dixon MD. 2003. Effects of flow pattern on riparian seedling recruitment on sandbars in the Wisconsin River, Wisconsin, USA. *Wetlands* **22**: 125–139.

Dobson M, Cariss H. 1999. Restoration of afforested upland streams — what are we trying to achieve? *Aquatic Conservation*: *Marine and Freshwater Ecosystems* **9**: 133–139.

Frissell CA, Liss WJ, Warren CE, Hurley MD. 1986. A hierarchical framework for stream habitat classification — viewing streams in a basin context. *Environmental Management* **10**: 199–214.

Gasith A, Resh VH. 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology Evolution and Systematics* **30**: 51–81.

Harrison SSC, Harris IT, Croeze A, Wiggers R. 2000. The influence of bankside vegetation on the distribution of aquatic insects. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* **27**: 1480–1484.

Harrison SSC, Pretty JL, Shepherd D, Hildrew AG, Smith C, Hey RD. 2004. The effect of instream rehabilitation structures in macroinvertebrates in lowland rivers. *Journal of Applied Ecology* **41**: 1140–1154.

Hawkins CP, Norris RH. 2000. Performance of different lanscape classifications for aquatic bioassessments: introduction to the series. *Journal of the North American Benthological Society* **19**: 367–369.

Heino J, Louhi P, Muotka T. 2004. Identifying the scales of variability in stream macroinvertebrate abundance, functional composition and assemblage structure. *Freshwater Biology* **49**: 1230–1239.

Hughes FMR, Adams WM, Muller E, Nilsson C, Richards KS, Barsoum N, Decamps H, Foussadier R, Girel J, Guilloy H, Hayes A, Johansson M, Lambs L, Patou G, Peiry J-L, Perrow M, Vauteir F, Winfield M. 2001. The importance of different scale processes for the restoration of floodplain woodlands. *Regulated Rivers*: *Research and Management* **17**: 325–345.

Hughes SJ. 2006. Temporal and spatial distribution patterns of larval trichoptera in Madeiran Streams. *Hydrobiologia* **553**: 27–41.

Karr JR, Chu EW. 2000. Sustaining living rivers. *Hydrobiologia* **422**: 1–14.

Karr JR, Schlosser IJ. 1978. Water resources and the land–water interface. *Science* **201**: 229–234.

Kershner JL. 1997. Setting riparian/aquatic restoration objectives within a basin context. *Restoration Ecology* **5**: 15–24.

Laasonen P, Muotka T, Kivijärvi I. 1998. Recovery of macroinvertebrate communities from stream habitat restoration. *Aquatic Conservation: Marine and Freshwater Ecosystems* **8**: 101–113.

Lee KE, Goldstein RM, Hanson PE. 2001. Relation between fish communities and riparian zone conditions at two spatial scales. *Journal of the North American Benthological Society* **37**: 1465–1473.

Magalhães MF, Beja P, Canas C, Collares-Pereira MJ. 2002. Functional heterogeneity of dry-season fish refugia across a Mediterranean catchment: the role of habitat and predation. *Freshwater Biology* **47**: 1919–1934.

Magnan PMA, Rodriguez P, Legendre P, Lacasse S. 1994. Dietary variation in a freshwater fish species: relative contributions of biotic interactions, abiotic factors and spatial structure. *Canadian Journal of Fisheries and Aquatic Sciences* **51**: 2856–2865.

Malmqvist B. 2002. Aquatic invertebrates in riverine landscapes. *Freshwater Biology* **47**: 679–694.

Moerke AH, Lamberti GA. 2004. Restoring stream ecosystems: lessons from a Midwestern State. *Restoration Ecology* **12**: 327–334.

Naiman RJ, Décamps H. 1997. The ecology of interfaces: riparian zones. *Annual Review of Ecology and Systematics* **28**: 621–658.

Newbold JD, Elwood JW, O'Neil RV, Van Winkle W. 1981. Measuring nutrient spiralling in streams. *Canadian Journal of Fisheries and Aquatic Sciences* **38**: 860–863.

Pearson TN, Li HW, Lamberti GA. 1992. Influence of habitat complexity on resistance to flooding and resilience of stream fish assemblages. *Transactions of the American Fisheries Society* **121**: 427–436.

Poff NL. 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. *Journal of the North American Benthological Society* **16**: 391–409.

Poff NL, Allan JD, Palmer MA, Hart DD, Richter BD, Arthington AH, Rogers KH, Meyer JL, Stanford JA. 2003. River flows and water wars: emerging science for environmental decision making. *Frontiers in Ecology and the Environment* **1**: 298–306.

Raven PJ, Fox PJA, Everard M, Holmes NTH, Dawson FD. 1997. River habitat survey: a new system for classifying rivers according to their habitat quality. In *Freshwater Quality: Defining the Indefinable*?, Boon PJ, Howell DL (eds). The Stationery Office: Edinburgh; 215–234.

Rickleffs RE. 1987. Community diversity: relative role of local and regional processes. *Science* **235**: 167–171.

Rios SL, Bailey RC. 2006. Relationship between riparian vegetation and stream benthic communities at three spatial scales. *Hydrobiologia* **553**: 153–160.

Sandin L, Johnson RK. 2004. Local, landscape and regional factors structuring benthic macroinvertebrate assemblages in Swedish streams. *Landscape Ecology* **19**: 501–514.

Ter Braak CJF. 1988. CANOCO — a FORTRAN program for canonical community ordination by [partial][detrended][canonical] correspondence analysis, principal components analysis and redundancy analysis (version 2.1).

ter Braak CJF. 1990. Interpreting canonical correlation analysis through biplots of structural correlations and weights. *Psychometrika* **55**: 519–531.

Turak E, Flack LK, Norris RH, Simpson J, Waddell N. 1999. Assessment of river condition at a large spatial scale using predictive models. *Freshwater Biology* **41**: 283–298.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 130–137.

Wenger S. 1999. A review of the scientific literature on riparian buffer width, extent and vegetation. Review Office of Public Service & Outreach, Institute of Ecology, University of Georgia, Athens, GA.

Wohl E, Angermeier PL, Bledsoe B, Kondolf GM, MacDonnell L, Merritt DM, Palmer MA, Poff NL, Tarboton D. 2005. River restoration. *Water Resources Research* **41**, DOI:10.1029/2005WR003985.

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