



# The lentic–lotic character of Mediterranean rivers and its importance to aquatic invertebrate communities

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**Abstract** Hydromorphological features are crucial in structuring habitats for freshwater organisms. The quantification of these variables is often performed through accurate measuring or detailed estimation, but their assessment is not always feasible for river management purposes. Economic and time constraints often lead to difficulty in creating simple summaries of collected data for practical use. The Lentic–lotic River Descriptor (LRD) was developed to identify the character of a river site in terms of local hydraulic conditions. Information about the presence of flow types, channel substrates, in-stream vegetation, organic debris and artificial features is included in its calculation. The main aim of this paper is to investigate whether the lentic–lotic character of a river site, as summarized with the LRD descriptor, is relevant to aquatic invertebrate communities in nearly natural river sites. Invertebrate data were collected with multi-habitat, proportional sampling and hydromorphological information was gained by applying the CARAVAGGIO method (river habitat survey technique) in the field. The dataset was generated from High or Good ecological status river sites located in Mediterranean areas of Italy. Correspondence Analysis was performed to relate the invertebrate community structure to a set of catchment-scale, reach-scale and chemical environmental variables. The results of the multivariate analysis indicate that LRD provides a

persuasive explanation of the most important axis of variation in benthic data. This paper also presents the optimal LRD range for a set of invertebrate taxa, accompanied by a short discussion of their potential use in conservation issues.

**Keywords** Mediterranean rivers · Hydraulic habitat · LIFE index · WFD · Climate change

## Introduction

Hydraulic and morphological features are crucial in structuring the habitats of aquatic organisms in rivers (Statzner and Higler 1986). Water depth (Brooks et al. 2005), velocity (Gore et al. 2001), turbulence, shear stress (Hynes 1970; Mérigoux and Dolédec 2004; Brooks et al. 2005) and flow types (Urbanič et al. 2005) are among the major hydraulic parameters affecting biota distributions, along with channel substrate that obviously affects invertebrate, fish and macrophyte distributions (e.g., Cummins and Lauff 1969; Brookes 1988). The relationship between such habitat variables and the presence or abundance of a variety of aquatic organisms has already been identified and described (e.g., Brooks et al. 2005).

Most studies dealing with river ecosystems quantify habitat variables by means of accurate measurements or detailed estimation (Mérigoux and Dolédec 2004; Brooks et al. 2005; Syrovátka et al. 2009). Nonetheless, their assessment is not always feasible for river management purposes, due to economic or time constraints, or the difficulty of adequately summarizing the gathered information for practical use (Jowett 2003). Physical variables such as flow conditions, depth and substrate type have assumed a central role in river management practices (Newson et al.

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1998a). Now that pollution, which used to be the overriding stressor, has been widely resolved, the effects of habitat degradation have become much more obvious (Feld 2004). A renewed accent is being placed on describing (Syróvátka et al. 2009) or modelling (Dolédec et al. 2007) the hydraulic preferences of benthic macroinvertebrates. It should be stressed, however, that although the value of such information is currently accepted, no simple means for its comprehensive quantification is yet available for routine use in the monitoring, assessment and management of rivers.

Some complex protocols have been developed that need accurate in-field measuring that estimate hydraulic habitat availability and the subsequent occurrence of predicted taxa based on predictive models. Among them, PHABSIM (Physical Habitat Simulation System; Bovee et al. 1988), HABSCORE (Milner et al. 1998) and ESTIMHAB (Lamouroux and Capra 2002) are species-oriented in-stream habitat models that need accurate local calibration. These methods do not focus on obtaining a general description of the hydraulic habitat based on a large range of variables at the site scale, but usually estimate selected combinations of habitat features in detail. For their correct application, considerable effort is required, necessitating the use of measuring devices in the field. In contrast, some standard hydromorphological methods widely applied and based on visual assessments, such as the River Habitat Survey (RHS) method (Raven et al. 1998), are available. These methods, however, although aimed at reflecting habitat suitability, do not provide specific tools for the quantification of reach scale hydraulic conditions. The significance of hydromorphology, including hydromorphological regime, in supporting the interpretation of biological communities and in the establishment of management plans for European river ecosystems is now recognized and authoritatively stated by the Water Framework Directive (WFD; European Commission 2000).

Within this context, the European Committee for Standardization (CEN) is defining a framework for hydromorphological river assessment and interpretation across Europe. A first standard (CEN 2004) was approved and CEN is currently working on a broadly applicable scoring system for quantifying hydromorphological degradation (CEN 2008), without a special focus on local hydrological aspects. While flow-related aspects are indisputably relevant in all river systems, they are expected to be crucial in studies of the Mediterranean area (Sabater et al. 2008).

Nonetheless, it might be argued that the measure of habitat variables (e.g., substrate types, water depth, Reynolds Number) in detail (Syróvátka et al. 2009) is needed for a scientifically sound interpretation of biological response because summary and simple descriptors based on visual assessment may not prove suitable at that range. The

outline and selection of cost-effective descriptors and indices is advocated for the interpretation of biological responses to habitat variability, especially in terms of local hydraulic conditions (Newson et al. 1998b). In this regard, the concept and use of flow type categories (Padmore 1998) represents relevant progress in visually assessing the hydraulic character of a river section at the micro and meso-scale (Newson et al. 1998b). With this in mind, an index, which identifies the character of a river site in terms of its local hydraulic conditions, was presented based on visual habitat assessment in the field: the Lentic–lotic River Descriptor (Buffagni 2004). This descriptor was originally applied at the Italian scale (Buffagni 2004; Bona et al. 2008) and successively employed at a European scale (Erba et al. 2006; Buffagni et al. 2009) with the main aim of exploring the overall response of aquatic communities to different sources of perturbation or natural variability.

Our main goal here is to provide a detailed explanation of the Lentic–lotic River Descriptor (LRD) focusing on the method by which this descriptor can be calculated. As a complementary tool, the authors provide support for LRD relevancy and meaning for aquatic invertebrate communities. In relation to this last aspect, an example of the possible use of LRD information will be provided, presenting the optimal LRD range for a set of invertebrate taxa occurring in Mediterranean rivers, with a special focus on Ephemeroptera.

## Methods

### Study area

The rivers included in the study are typical Mediterranean rivers and are located in Sardinia (22 samples) and the Northern (12 samples), Central (11 samples) and Southern Italian Apennines (19 samples) (Fig. 1). The area selected for study, i.e., across Italy, includes a wide geographical and hydrological range so that the potential differences between river sites, in terms of local hydraulics, can be observed and quantified based on a variety of natural conditions. Further criteria for the selection of sampling areas and sites follow the statements of the EU research projects AQEM (Hering et al. 2004) and STAR (Furse et al. 2006). In each of the four geographical areas examined, nearly natural sites (reference sites) were selected (Buffagni et al. 2001; Nijboer et al. 2004), together with sites that, in the worst cases, are classified with a Good status, to exclude any variability linked with anthropogenic pressures. The site/sample ecological status classification for the selection of sites to be included in the analysis (i.e., from reference to good status) was obtained by means of a multivariate analysis performed in previous investigations (Buffagni et al. 2001, 2004) or according to biological and

**Fig. 1** Geographical distribution of study sites in Italy



chemical methods formally in use in Italy (Buffagni, unpublished data). A summary description of the abiotic conditions observed in the sampling sites is reported in Appendix Table 5 and further details can be found elsewhere (Buffagni et al. 2001; Buffagni 2004). With regard to physio-chemical data, high natural variability was observed in the Sardinian dataset, even though Moderate to Bad ecological quality sites were excluded from the analysis. This variability seems related to the intermittent character of the Sardinian rivers studied (Buffagni 2004; Sangiorgio et al. 2007).

#### Data collection

##### *Benthic macroinvertebrates*

For the collection of macroinvertebrates, a ‘multi-habitat’ sampling procedure was used derived from Barbour et al. (1999). Single sample units were collected according to the occurrence of different substrate types that included biotic microhabitats (Hering et al. 2004) and then combined in a site sample. Only riffle areas (Buffagni et al. 2001, 2004) were considered for the allocation of single sampling units. Invertebrates were quantitatively collected at 0.5 m<sup>2</sup> in Northern and Southern Apennines sites and 1 m<sup>2</sup> in Central Apennines and Sardinian sites. The abundance data were corrected to standardize density across areas prior to data

analysis. The data used were collected between 2000 and 2004, and one to three sampling seasons (spring, autumn or winter and summer) were considered for each area.

The taxa identification level reached varied in relation to the purpose of the analysis. On the one hand, the choice of using different taxonomical levels was linked to the availability of adequate knowledge concerning the larval identification to species for many invertebrates in Italy (Buffagni et al. 2003). On the other hand, not using the species level complies with the need to avoid the fact that differences in taxa composition resulting solely from biogeographical trends can excessively affect the results of the analysis. The compromise adopted in order not to lose too much taxonomical information was the use of a different taxonomic resolution, i.e., identification level, for different taxa groups. Reducing the number of taxa as compared to the number of samples also supports the removal of unwarranted noise in multivariate analysis. Genus was reached for Irudinea, Mollusca, Odonata and Plecoptera. Family was considered for Trichoptera, Diptera, Oligocata, Crustacea. For Ephemeroptera, the level was defined according to the Operational Unit concept (OU; Buffagni 1997). OUs are characterized by an intermediate level of identification that falls between genus and species and are adopted to preserve as much ecological information as possible, without the need for identification at the species level. OUs are defined on the basis of the common morpho-

taxonomic characteristics of species (Buffagni 1997). The number of Ephemeroptera OUs for Italy is 35 compared with a total of 110 reported species. The outline of Operational Units was only available for Ephemeroptera and taxonomic experts have yet to define this level for other benthic groups. When possible, species level was used for the definition of optimum LRD values of example taxa.

The LIFE index is commonly used for flow evaluation and is based on the association of given taxa to given flow conditions (Extence et al. 1999). This index was originally developed to characterize the benthic community in terms of long or mid-term hydrological conditions (Extence et al. 1999) and is solely based on biotic information. The different taxa scores are derived from documented relationships between flow conditions and the occurrence of macroinvertebrate taxa. Low scores are assigned to taxa considered rheophilic, while higher scores are assigned to limnophilic ones. The flow score is then combined with abundance categories. The final score is obtained by dividing the sum of the score obtained for each taxon by the total number of taxa. This index was used to support the interpretation of the benthic community structure with regard to lentic–lotic characteristics. For the calculation of LIFE, the software ASTERICS 3.1.1 (AQEM European stream assessment program: <http://www.aqem.de>; <http://www.fliessgewaesser-bewertung.de>) was employed.

#### *Hydromorphological features and environmental data*

The CARAVAGGIO method (Buffagni and Kemp 2002; Buffagni et al. 2005) was applied to gather the hydromorphological information. It is a procedure partly based on the UK River Habitat Survey (RHS) method (Raven et al. 1998). This method, as opposed to the UK method, includes the collection of additional data regarding substrate, flow type, channel and water width, and describes the characteristics of a secondary channel whenever it is present (Buffagni and Kemp 2002; Buffagni et al. 2005). The method required the collection of data from a 500 m long river stretch, using 10 equally spaced transects (*spot-checks*). Some features were recorded at spot-checks only, but a general description of the whole 500 m was provided by the *sweep-up* investigation. The CARAVAGGIO method was applied to best characterize the adjacent upstream reach where invertebrates were collected (i.e., the macroinvertebrate area was located 50 m upstream of the downstream end of the CARAVAGGIO survey area) and was repeated in all the seasons that biological samples were collected.

Hereafter, the adjective lentic is used to refer to rivers predominantly characterized by still or slow flowing waters, while lotic is used to indicate rivers dominated by fast and/or turbulent flowing areas.

For each sample, the altitude, distance from source, catchment area, slope of the thalweg, water width, channel

width, instantaneous temperature, and mean July and January temperatures were recorded. In addition, the characteristics of each sample were identified in terms of water velocity, depth and Froude number by averaging the values observed for the 10 sampling units where invertebrates were collected.

The characteristics of each sample were noted in terms of physio-chemical (Appendix Table 5) and hydromorphological features. The following water quality variables were measured: O<sub>2</sub>%, BOD<sub>5</sub>, *Escherichia coli*, N–NH<sub>4</sub>, N–NO<sub>3</sub>, P–PO<sub>4</sub> and TP. From the data obtained through the application of the CARAVAGGIO method, among others, two indices were calculated, the Habitat Modification Score (HMS) and the Habitat Quality Assessment (HQA) (Raven et al. 1998). The HMS is an index that increases with the presence of artificial features in the river while the HQA gives a measure of habitat richness and diversification, which increases according to a growth in habitat diversity.

#### *Assessment of the lentic–lotic character of a river site*

The local hydraulic conditions, *sensu lato*, were calculated by means of the Lentic–lotic River Descriptor (LRD; Buffagni 2004). LRD considers information regarding the presence and variety of flow types, channel substrates, in-stream vegetation, organic debris and artificial features. All these characteristics are separately considered in the main channel and wherever present in the secondary one. Features considered indicative for lotic conditions receive negative scores, while lentic features receive positive ones.

Table 1 shows the scoring system for the different features that are considered in the computation of LRD. The LRD scoring system presented here refers to data collected with the CARAVAGGIO or RHS methods, but this approach can be easily applied to datasets compiled using other methods. In more detail, the descriptor was developed based on expert opinion by limiting feature scores within fixed ranges, and in turn validated for its relevance in the interpretation of ecological quality status classification with external datasets (e.g., Erba et al. 2006; Bona et al. 2008; Buffagni et al. 2009). Scores were also assigned taking into account the way in which different features were recorded in the field with the RHS or CARAVAGGIO methods, e.g., spot-check versus sweep up sections considering their potential variability to avoid the over-estimation of extreme values (Buffagni 2004). As an example, flow types (see below) collected at the spot-check level only, excluding dry flow conditions, range individually from  $-2$  to  $+2$  (see Table 1). This means that at the site level they can globally range from  $-30$  to  $+30$  ( $+80$ , including dry flow), i.e., sum of the values for the 10 spot-checks. On the other hand, the flow type scores recorded for the sweep-up section only range between  $-17.5$  and  $+10$  ( $+24$ ).

**Table 1** Features as recorded for the CARAVAGGIO and RHS methods and related scores considered for the calculation of the Lentic–lotic River Descriptor (LRD)

Spot-checks							
Code	Description	Category	Feature	Score			Notes
				Primary	Secondary <sup>a</sup>		
F	Flow type	Lentic	DR	8	–		To be repeated in main and secondary channel
			NP	2	1		
		Lotic	CH, SM, UP	0	0		
			RP	–0.5	–0.25		
			UW	–1	–0.5		
S	Channel substrate	Lentic	BW, CF, FF	–2	–1		
			CL, SI, SA	1	0.5		
		Lotic	GP, BE	0	0		
			CO, BO	–1	–0.5		
D	Maximum water depth	Artificial	AR	0	0		
		Deep	>75 cm		1.0		
			25 ≤ x ≤ 75 cm		0.5		
V <sup>b</sup>	Channel vegetation types/ Organic debris	Lentic	Shallow	<25 cm	0.0		
			Extension			<33%	≥33% (or W)
		Lotic	Emergent reeds/sedges/ rushes/grasses; floating-leaved (rooted); free-floating	1	3		
			Organic matter (CPOM/FPOM)	1	3		
			Liverworts/mosses/lichens	–1	–3		
MH	F	is the sum of primary and secondary flow type scores of a spot-check					
	S	is the sum of primary and secondary substrate type scores of a spot-check					
	D	is the score for maximum water depth of each spot-check					
	V	is the channel vegetation type/organic debris score of a spot-check					
Sweep-up							
Code	Description	Category	Feature	Score			Notes
				Occurrence (# features)			
SWC	Flow type	Lentic	DR	16	24	24	To be repeated in main and secondary channel
			NP	4	6	10	
			CH, SM, UP	0	0	0	
		Lotic	RP	–1	–1.5	–2.5	
			UW	–2	–3	–5	
			BW, CF, FF	–4	–6	–10	
Bars	Lotic	Every recorded bar	–0.5 (Max. –5)				
SWSa	Artificial features	Lentic	Weir/sluice, bridge, culvert,	2	1	0	Common to main and secondary channel
			Deflector, ford	1	1	1	
		Extension			<33%	≥33% (or W)	
SWSn	Features of special interest	Lentic	Is water impounded by weirs/sluices?	3	7		
		Lotic	Debris dam(s)	1	3		
			Natural water fall(s) >5 m high	–3	–5		
			Natural water fall(s) <5 m high	–1	–3		

<sup>a</sup> Secondary features for substrate and flow score 50% of primary features

<sup>b</sup> Max V: + 3 for each Spot-check

For single feature description see Raven et al. (1998) and Buffagni and Kemp (2002)

Flow type acronyms: DR dry, NP no perceptible flow, CH chute, SM smooth, UP upwelling, UW unbroken standing waves, BW broken standing waves, CF chaotic flow, FF free fall. Channel substrate acronyms: CL clay, SI silt, SA sand, GP gravel/pebble, BE bedrock, CO cobble, BO boulders, AR artificial

In general terms, at the spot-check level, four categories were considered: flow (F), substrate (S), maximum water depth (D) and channel vegetation (V). Within each of these categories different features can be recorded, each possibly producing a different score (see Table 1) according to their

lentic or lotic characteristics. Two records of flow and substrate type are carried out in each channel, as main or secondary, according to their relative extension, i.e., the main flow occupies the largest area of the channel as compared to that of other flows. The secondary flow (or

substrate) features always score half of that of the primary one, apart from dry (DR), which cannot be recorded as a secondary flow. Each feature listed in the above-mentioned categories can be observed in the primary or secondary channel and can be thus catalogued accordingly.

If the hydraulic conditions observed were caused by the presence of adjacent artificial structures, e.g., weirs, they are recorded as ‘artificial’. If this is the case, the whole transect (spot-check) is recorded as ‘artificial’ and all the features associated with the transect will contribute to the ‘artificial’ LRD sub-score (LRDa). At the sweep-up level, the categories include (see Table 1): flow types and bars (SWC), which have to be considered separately in terms of main and secondary channels and artificial features (SWS<sub>a</sub>) and features of special interest (SWS<sub>n</sub>). The latter two, not being considered for main and secondary channels. All these aspects are included in the calculation of the final LRD score. Table 2 presents the formula for the calculation of the LRD descriptor, which incorporates four different steps.

1. A micro-habitat score (MH) is calculated at the spot-check level, considering only the information from flows (F), substrates (S), depth (D) and in-channel vegetation (V). In this computation, the scores obtained from ‘natural’ spot-checks are kept separate from the scores derived from ‘artificial’ ones (see above). The computation is also run separately on main and secondary channels. Thus, four different MH scores are obtained for each spot-check: MH<sub>aCHI</sub>, MH<sub>nCHI</sub>, MH<sub>aCHII</sub>, MH<sub>nCHII</sub> (see Tables 1, 2 for acronyms). Each of the four sub-scores is derived from the sum of the single scores of the different features.
2. A final spot-check score—which includes both main and secondary channels—is derived by the sum of

each MH score multiplied by the water width of the respective channel. This value is then divided by total water width.

3. The 10 spot-check scores obtained according to step 2 must be added to the scores of the sweep-up analysis (SWC and SWS), separately for natural and artificial features. In this step, LRD is scaled-up to the whole 500 m reach (including sweep-up and spot-checks), keeping the artificial and natural sub-scores separate.
4. The site LRD value is obtained as the sum of LRD artificial and LRD natural.

The values of LRD can vary between  $<-75$ , for extremely lotic conditions, and  $+100$ , for extremely lentic ones. To facilitate an easier interpretation of the results, the range of LRD values was divided into classes. Five equally sized classes were defined between  $-50$  and  $+50$ . Two additional classes were added: ‘extremely lotic’, for the most negative values, with  $LRD \leq -50$ , and ‘extremely lentic’, for the most positive ones, with  $LRD \geq +50$  (Table 3). LRD was especially designed to be derived from CARAVAGGIO data and can be automatically calculated by using a software developed for CARAVAGGIO or RHS data storage and analysis (CARAVAGGIOsoft: Di Pasquale and Buffagni 2006), based on software initially proposed within the STAR project (Furse et al. 2006).

#### Data analysis

#### Multivariate analysis

To evaluate the main patterns in biotic distribution, a multivariate ordination technique was run on the 64

**Table 2** Formulae for the calculation of the Lentic–lotic River Descriptor (LRD) and sub-indices

Step	Formula
Total computation	
1	MH <sub>a/n, CHI</sub> Each sum has to be calculated separately for main and secondary channels, artificial and natural features.
2	LRD <sub>n</sub> [SC <sub>x</sub> ] Natural LRD of the $x$ Spotcheck ( $x = 1-10$ ) LRDa [SC <sub>x</sub> ] Artificial LRD of the $x$ Spotcheck ( $x = 1-10$ )
3	LRD <sub>n</sub> Natural LRD sub-index LRDa Artificial LRD sub-index
4	LRDt Total LRD

MH, SWC, SVS: see Table 1

CHI main channel (I), secondary channel (II), a/n artificial/natural,  $x$  number of the spot-check (1–10),  $ww$  water width

**Table 3** Lentic–lotic River Descriptor (LRD) classes and boundaries

Class	Name	Value	LRD	Value
1 <sup>+</sup>	Extremely lotic		LRD	<−50
1	Very lotic	−50≤	LRD	<−30
2	Lotic	−30≤	LRD	<−10
3	Intermediate	−10≤	LRD	<10
4	Lentic	10≤	LRD	<30
5	Very lentic	30≤	LRD	<50
5 <sup>+</sup>	Extremely lentic		LRD	≥50

invertebrate samples collected from 49 sites. Due to the unsatisfactory comprehension of the interactions between environmental variables and macroinvertebrate distribution in Mediterranean rivers, an indirect ordination method of analysis seemed the most appropriate (Peeters et al. 1994) to explain the major variation in community composition. A Detrended Correspondence Analysis (DCA; Hill and Gauch 1980) was first run on the invertebrate data to calculate the length of the variation gradient (Rabeni and Doisy 2000). As the length of the gradient along the first axis was higher than 3, a unimodal method was applied (Ter Braak and Prentice 1988) and a Correspondence Analysis (CA; Ter Braak and Smilauer 1997) was run to search for the main variation gradients in the benthic data. The analysis was performed by the computer program CANOCO, version 4.0 (Ter Braak and Smilauer 1997). Species data were log-transformed:  $\ln(x + 1)$  and a bi-plot scaling option was selected, with scaling focus on inter-species distances. Pearson correlation coefficients (Legendre and Legendre 1998) of the sample scores with environmental water quality variables jointly with the LIFE index and CARAVAGGIO indices (i.e., LRD, HMS and HQA) were computed to explain the CA gradients. Correlations and box and whisker graphs were obtained by using STATISTICA software v. 5.0 (Statsoft, Inc. 1995).

#### *Calculation of optimum LRD values for benthic taxa*

To identify invertebrate taxa with respect to their response to the lentic–lotic character of a river site, the LRD weighted average—in order to determine the optimum value of a species (Ter Braak and Prentice 1988)—was calculated for selected benthic taxa. The weighted standard deviation of optimum values was also calculated (Heckett and Filliben 1996). Due to the variability that can be observed within the same family, only taxa at least identified to genus level were considered for LRD weighted average calculation.

## Results

### Interpretation of the Correspondence Analysis

The results of the Correspondence Analysis (CA) are reported in Table 4. Table 4 also shows Pearson correlation coefficients between CA axes and environmental variables, plus the biotic metric LIFE. The explained variation in the first axis is comparatively low ( $\approx 10\%$ ), presumably as a result of the bio-geographical differences between the studied areas. The highest correlation is observed with the LRD descriptor (0.80), which is the variable that best correlates to CA axis 1 among the whole set of environmental variables. Water velocity ( $-0.71$ ) correlates well to axis 1, even though it shows a lower value when compared to LRD. It should be noted that water velocity was measured in correspondence to the points where invertebrate samples were collected. As far as the LIFE index is concerned, this biotic metric correlates well to the first axis (0.89).

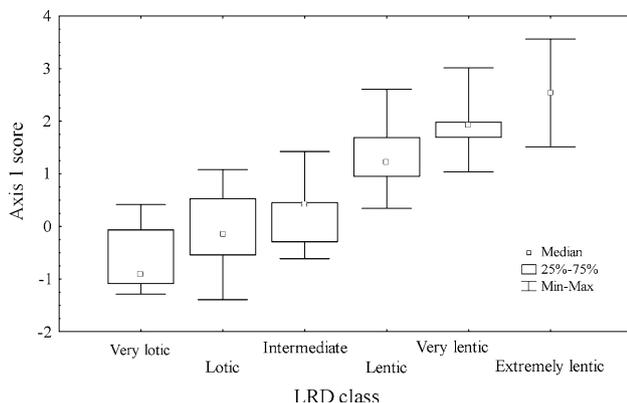
CA axis 1, i.e., the most important gradient in terms of invertebrate community variation, is then primarily related to the lentic–lotic character of river sites. This is related to seasonal trends (e.g., water temperature, January mean air temperature) and partly to stream type differentiation among the four investigated areas that, when combined, reflect a geographical and climatic gradient.

Using box and whisker plots, the relationship is shown between LRD classes (Table 3) and the scores obtained by individual invertebrate samples on the first CA axis (Fig. 2). Increasing CA values correspond to increasing LRD classes, i.e., lotic to lentic gradient. In particular, median CA site score values distinctly increase from one class to the next, confirming the interpretation of the first CA axis in terms of lentic–lotic character. The largest overlap is observed between ‘lotic’ and ‘intermediate’ LRD classes. No samples corresponded to the ‘extremely lotic’ class, so this class is not presented in the figure.

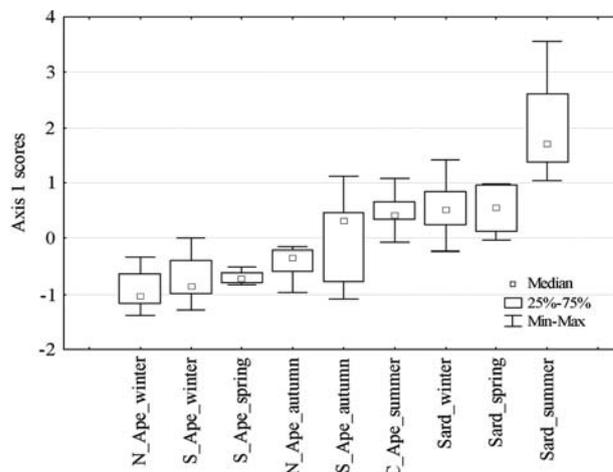
Looking at the ordination of samples in more general terms, the first axis roughly mirrors the gradient between the four geographical areas in which the samples were collected, while accounting for seasonal variation (Fig. 3). To graphically present the variation of axis 1 scores, samples were grouped according to season and geographical area. Groups were then ordered in Fig. 3 according to the increasing median values of axis 1 scores. Sardinian summer samples were positioned at one extreme of the graph: the positive end. The other two Sardinian seasons were similar to samples from the Central Apennines. Southern Apennines autumn samples vary between positive and negative scores. The rest of the samples of the Southern and Northern Apennines produced negative scores.

**Table 4** CA axes eigenvalues and Pearson correlation coefficients (reported if significant and  $> 0.3$ ) for selected environmental variables and LIFE metric

		Axis				Total inertia
		1	2	3	4	
	Eigenvalues	0.373	0.24	0.2	0.18	3.64
	Species–environment correlation	0.998	0.98	0.97	0.97	
	Cumulative percentage variance of species data	10.3	16.9	22.3	27.1	
	of species–environment relation	11.5	18.3	24.1	28.3	
Environmental variable		Correlation coefficients				
Descriptive	Season (numerical code)	0.55		0.33	−0.31	
	Altitude of the site (m)	−0.45		0.49		
	Mean annual rainfall (mm)	−0.59		0.45		
	Mean air temperature of July (°C)	0.54		−0.49		
	Mean air temperature of January (°C)	0.68		−0.46		
	Water temperature (°C)	0.71			−0.39	
	Cl <sup>−</sup> (mg l <sup>−1</sup> )	0.67				
Water Quality	Hardness (mg l <sup>−1</sup> CaCO <sub>3</sub> )	0.53	−0.41			
	HQA (Habitat Quality Assessment)	−0.62			0.34	
	Conductivity (μS/cm)	0.47				
	O <sub>2</sub> saturation (%)	−0.36				
	BOD <sub>5</sub> (O <sub>2</sub> mg l <sup>−1</sup> )	0.52		−0.48		
Local hydraulics and lentic–lotic character	Log discharge (m <sup>3</sup> s <sup>−1</sup> )	−0.54			0.32	
	Mean water velocity (cm s <sup>−1</sup> )	−0.71				
	LRD (Site score)	0.80				
Biotic metric	LIFE	−0.89				

**Fig. 2** Variation of CA first axis scores according to LRD classes

The first CA axis expresses a gradient between taxa preferring fast flowing and turbulent waters, e.g., *Chloroperla* sp., *Siphonoperla* sp., *Rhithrogena hybrida* Gr., *Dinocras* sp., thus, producing negative scores and taxa mainly linked to lentic river characteristics, e.g., *Nepa* sp., *Notonecta* sp., the Dixidae and Glossomatidae producing positive scores.

**Fig. 3** Variation of CA first axis scores according to geographical areas and seasons. *Sard* Sardinia, *C\_Ape* Central Apennines, *N\_Ape* Northern Apennines, *S\_Ape* Southern Apennines

In the other three CA axes presented, the correlation coefficients for all the analyzed variables are lower than those observed for the first axis, and their interpretation is not as simple as that for axis one. The second axis is

difficult to interpret (Table 4), and shows the highest correlation to hardness, thus being possibly linked to river type differences. The third axis is related to altitude of site and source and to BOD<sub>5</sub>, perhaps expressing an overall water quality gradient. The fourth axis is again difficult to interpret, being weakly correlated to parameters indicating seasonal conditions.

#### Invertebrates and the lentic–lotic character of rivers

The relation between the LIFE index and the LRD descriptor was also studied. The regression between the LIFE index and the LRD descriptor shows a highly significant linear distribution ( $R^2 = 0.49$ ;  $P \leq 0.001$ ; Fig. 4), with the highest LIFE values corresponding to strongly negative LRD values.

In Fig. 5, the optimum values are shown for the LRD descriptor of selected macroinvertebrate taxa. Some insect taxa belonging to the Plecoptera order, e.g., *Dinocras* sp. and *Protonemura* sp., resulted as typical of lotic sites characterized by markedly negative LRD values. Different Ephemeroptera taxa are able to colonize lentic or lotic habitats, with respect to the ecological specialization of species. For example, *Rhithrogena semicolorata* (Curtis, 1834), *Baetis alpinus* (Pictet, 1843), *B. melanonyx* (Pictet, 1843) and *Epeorus sylvicola* (Pictet, 1865) resulted as typical of lotic habitats with an optimum LRD value between  $-30$  and  $-25$ , while *Procladius bifidus* (Bengtsson, 1912), *Baetis muticus* (Linné, 1758) and *B. buceratus* (Eaton, 1870) were mainly found at sites with weakly positive LRD values (optimum values between 0 and 10). Taxa such as *Physella acuta* (Draparnaud, 1805) and *Notonecta* sp. were confined to lentic or extremely lentic sites, with an optimum value higher than 15 and 45, respectively. The taxon showing the highest standard deviation—demonstrating the potential to colonize a wide lentic–lotic range—was *Baetis muticus*. *Notonecta* sp. also

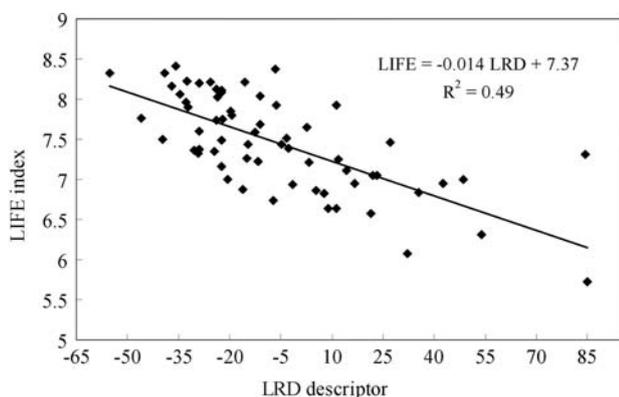
showed a high standard deviation but it was confined to very lentic conditions.

## Discussion

### Hydraulic habitat and LRD

The Lentic–lotic River Descriptor, devised as a summary indicator for describing hydraulic habitats in rivers, was found to be the abiotic variable that best correlated to the first multivariate axis expressing major invertebrate community variation (see Table 4; Fig. 2). This result is in line with Buffagni et al. (2009), who recognized that LRD is an important factor in explaining the variability of quality metrics based on the benthic invertebrate community in 11 datasets ranging from lowland to alpine streams across Europe. LRD relevancy for biological invertebrate and diatom communities, as indicated by this paper and elsewhere (Bona et al. 2008; Buffagni et al. 2009), attests to the efficacy of the LRD theoretical scoring system. Moreover, LRD results were very important for invertebrate communities when only High/Good status samples (e.g., present results) as well as the whole quality gradient across river sites (Buffagni et al. 2009) are considered.

An explanation of its success in clarifying the habitat gradient relevant to aquatic fauna can be found in the theoretical base of its calculation. For example, flow velocity, substrate type and water depth are all directly or indirectly considered. The information on flow types—(*sensu* Padmore 1998)—is incorporated in the descriptor as a surrogate for flow velocity and turbulence. Flow types proved to be a successful way of integrating information on flow velocity, depth and turbulence (Newson et al. 1998a, 1998b; Padmore et al. 1999) being easily recorded in the field (Padmore et al. 1999). Furthermore, flow types relate well to the distribution of aquatic biocoenosis (Buffagni et al. 2000; Harper et al. 2000). Syrovátka et al. (2009), studying in detail the hydraulic micro-habitat for Chironomidae and Oligochaeta species, included many parameters in the analysis, such as Froude and Reynolds numbers, roughness shear velocity and inferred boundary Reynolds number. At the micro-habitat scale, current velocity at 0.8 depth (from bottom) was the best explanatory variable and Froude number was found to be very useful in discriminating between the four observed chironomid community types (Syrovátka et al. 2009). In addition, our study showed that flow velocity was one of the variables most correlated to the first axis, even though it is less correlated than LRD, which refers to a wider spatial scale than flow velocity measures. This indirectly confirms the reliability of flow types, which are an estimation of surface water turbulence (Padmore



**Fig. 4** Linear regression between LRD descriptor and LIFE index ( $R^2 = 0.49$ ;  $***P \leq 0.001$ )

**Fig. 5** Preferences of selected taxa for the lentic–lotic character (LRD) of river sites. Medians (*circles*) and standard deviation (*lines*) of LRD are weighted by taxa abundance. Taxa shown here were identified to species or genus level, were present in at least 7 sites and had abundance usually higher than 150 specimens. For some large-sized or endemic taxa, values are shown even if they were derived based on a smaller number of specimens



1998), in simply recording hydraulic habitat for benthic invertebrates.

In spite of the measurement of flow velocities in correspondence to biological sampling, the calculation of LRD is obtained jointly in the collection of a huge range of information that the use of the CARAVAGGIO and RHS methods provide for WFD requirements in the support of biological data interpretation (e.g., Erba et al. 2006). The short time needed in the field for their application, makes these methods relevant in many water management activities (Raven et al. 2000). The LRD with its easily automated computation procedure (Di Pasquale and Buffagni 2006), which also facilitates the calculation of other

environmental indices (e.g., HQA, HMS), can be used for many other water management applications and habitat conservation concerns. Obviously, when compared with the direct measurement of water velocity, which seems to roughly express the same kind of information in high quality sites, the use of LRD will prove more or less appropriate depending on the aims of the study. When the study is very focused at a local scale on the estimation of the quantitative relationship for individual taxa and detailed in-field measurement of flow velocity is possible and cost effective, this measure can be proficiently included in the study protocol. On the other hand, whenever RHS-similar surveys are planned, e.g., for large scale

studies and routine monitoring, the derivation of LRD, HQA and HMS scores can be very useful for management purposes, especially in the interpretation of biological responses to acting pressures and overall site conditions in terms of habitat features. In all intermediate cases, the usefulness of deriving summarized information, instead of, or to combine with detailed flow measurements, should be evaluated according to the aims of the investigation.

Even though the LRD was designed to represent the hydraulic conditions of different stream types (from Alpine to temporary Mediterranean rivers) in devising LRD calculation formulae, comparatively higher (positive) scores were assigned to low flow conditions than to high flow ones, which usually correspond to negative scores (see Table 1 for single scores). This criterion is linked to the fact that in low flow conditions in the Mediterranean region, portions of the river channel can become partly or totally dry, representing dramatic stress for aquatic communities (Lake 2003).

For the LRD calculation, substrate types were attributed to categories ranging from ‘lotic’, characterized by coarse substrate, to ‘lentic’, characterized by fine or organic substrates (Church 1994; Harper and Everard 1998; Newson et al. 1998b).

The maximum water depth of each section scored as a lentic feature when higher than 75 (25) cm, in relation to its potential association with the dominance of low turbulence flows, such as not perceptible and smooth flow types. Thus, deeper areas take on a positive lentic score, because they are less easily colonized by aquatic species, especially by rheophilic benthic and fish taxa (Jowett and Richardson 1995).

As far as vegetation types are concerned—as recorded for the CARAVAGGIO and RHS methods—emergent reeds, floating-leaves and free-floating are all attributed to the lentic category because their presence influences the flow regime or is clearly related to slow flow conditions. In general terms, they increase the flow resistance and sedimentation of fine particulate matter, reducing flow velocity and generally producing a lentic condition (Pitlo and Dawson 1990; Harper and Everard 1998). In intermittent and regulated rivers, these phenomena are often enhanced by the absence of high flow periods that are able to limit macrophyte growth (Eschner 1983; Johnson 2000). The presence of organic debris (CPOM and FPOM) is considered an indicator of lentic areas because low flows—usually associated with rather lentic conditions—facilitate the sedimentation processes of organic matter (Bañuelos et al. 2004; Lemly and Hilderbrand 2000). Mosses were included in the lotic category because they are often associated with transport units and turbulent flows (Kemp et al. 1999).

In the LRD computation, the presence of deposition bars is selected as a weak indicator of lotic areas because a decreased slope and depth close to the bank could induce local turbulent flows. These channel form features can offer a habitat for rheophilic species because of increased re-oxygenation levels. These levels are not found in deeper areas that are often dislocated in the central part of the channel and/or in channel sections where bars are not present.

The presence of artificial features, e.g., weirs, bridges, culverts, is recognized as often being responsible for impoundment phenomena (Petts 1984). Impoundments generally produce areas of slow moving flow upstream, cause silt and sand sedimentation, facilitate macrophyte growth (Ogbeibu and Oribhabor 2002) and mainly increase the lentic character of the affected areas. Positive scores are thus assigned to these features.

In general terms, even though LRD is designed to be directly calculated from data collected using CARAVAGGIO (Buffagni et al. 2005) and RHS (Raven et al. 1998) methods, the same overall principles and scoring system (see Table 1) can be adopted to summarize information gained with other methods or be presented in datasets of a different shape or origin. Buffagni et al. (2009) calculated a simplified version for the computation of LRD based on RHS survey forms (Raven et al. 1998), barely modifying the diagnostic power of the descriptor described here. Since 1994, 17000 surveys have been carried out by the UK Environmental Agency with RHS and a massive database is now available in that country (Environment Agency 1997; <http://www.environment-agency.gov.uk>). The RHS protocol has also been applied in a number of European countries and worldwide. Recently, during the EU co-funded STAR project, the application of RHS was widely adopted by 13 European Member States, in a total of 263 sites (Davy-Bowker and Furse 2006; Szoszkiewicz et al. 2006). Further applications have been made in Poland with more than 600 sites studied (Szoszkiewicz, unpublished data) and outside of Europe (Manel et al. 1999). Thus, LRD can be potentially computed for all these RHS datasets, supporting both the analysis of historical series and that of recent changes in local hydraulic conditions.

The lentic–lotic character illustrates the observed ratio between lentic and lotic in-channel habitats, resulting from the interaction between channel morphology, discharge and sediment transport. Single variables, such as water velocity, water depth and Froude number, even though they contribute to the character of local hydraulic conditions, appear to be less relevant in this study than the overall lentic–lotic character of a site. A lentic–lotic calculation using LRD is able to provide a holistic picture of habitat

conditions and identify the local hydraulic aspects that are particularly important to invertebrate communities.

#### Invertebrates and the lentic–lotic character of rivers

The LIFE and LRD indices supply a complementary reading of the lentic–lotic character of rivers. In fact, the LIFE index is focused on biotic communities while the LRD descriptor illustrates hydromorphological features. It is also worth noting the difference in scale applied by the two indices. The LIFE index is usually based on information gathered along a river stretch 20–50 m long, while LRD focuses on habitat information collected from a river stretch of 500 m long. The existence of a highly significant relationship between these two indices (see Fig. 4) is of particular interest because of the independence of methods used to collect data. Furthermore, such a relationship supports the efficiency of both the LIFE index, on the biological side, and the LRD descriptor, on the hydromorphological side, in quantifying the lentic–lotic character of river sites.

In this study, we have presented the optimal range for selected benthic taxa in lentic or lotic conditions. These results must be considered as preliminary because a larger dataset will be investigated in the future to report in a more comprehensive way on the auto-ecological preferences of invertebrate species in relation to the lentic–lotic character of sites. However, the usefulness of some results of the study is apparent, with a special emphasis on Ephemeroptera taxa, for the information gained for, e.g., some endemic species. In fact, the lentic–lotic character of a site summarizes a large number of habitat features related to local hydraulics, climate and river typology and can thus be used—together with indicators for other relevant aspects—to assess the potential of a site in hosting animal or plant species.

Most Plecoptera taxa were found to prefer lotic sites. As indicated in the results of previous studies, they are usually limited to the well-oxygenated and cool waters (e.g., Earle 2004), often linked to lotic conditions. The Ephemeroptera species *Rhithrogena semicolorata*, *Baetis alpinus* and *B. melanonyx* present very lotic LRD optimum values. This result is consistent with the fact that they are usually described as typical headwater species (e.g., Guerold et al. 2000) exhibiting clear preferences for turbulent and high velocity water stretches (Bauernfeind and Moog 2000, Dolédec et al. 2007). *Baetis lutheri* Müller-Liebenau 1967 is described as a reophilic species (Müller-Liebenau 1969; Sowa 1975) usually inhabiting cobble habitats with fast and turbulent current (Belfiore 1983) and presents here an optimum value for LRD of ca. –23, typical of sites characterized by the predominance of riffle areas with turbulent flows. *Baetis fuscatus* (Linné,

1761), *Caenis luctuosa* (Burmeister, 1839) and *Centropilum luteolum* (Müller, 1776) present LRD optimum values of –7, –4 and 0, respectively. These findings support other research results in which *B. fuscatus* is considered a limno-reophilic species (Müller-Liebenau 1969; Sowa 1975; Buffagni and Desio 1994), *C. luteolum* and *C. luctuosa* limnephilic species (Bournaud et al. 1987; Elliott et al. 1988; Mobes-Hansen and Waringer 1998; Extence et al. 1999; Haybach et al. 2003). A potamophilic species such as *Baetis buceratus* (Derka 1998) presents a positive LRD optimum value (+5.7), corresponding to sites where lentic habitats tend to prevail with smooth flow conditions.

The Odonata *Orthetrum sp.* (+9.3) and the Gastropoda *Physella acuta* (+18.0) are described as taxa that exhibit a general preference for lentic areas (Coimbra et al. 1996) and present a very positive LRD optimum value, generally corresponding to low flow conditions and sites with a dominance of pool habitats.

The information gained is of particular importance for river management practices and habitat conservation because taxa preferring ‘extreme’ values of LRD and with a narrow range of preference are supposed to be more sensitive than less specialized taxa to changes in river flow and local hydraulic conditions (Dolédec et al. 2007). This could become a central factor in conservation issues in a global climate change scenario. The species, whose conservation status may be affected by climatic variation and habitat reduction with regard to the lentic–lotic character of rivers are as follows: *Baetis cyrneus* Thomas and Gazagnes, 1984, *Ecdyonurus belfiorei* Haybach and Thomas, 2001, *Serratella spinosa* (Ikonomov, 1961), *Habroleptoides pauliana* (Grandi, 1959) (Ephemeroptera) and *Thyrenoleuctra zavattarii* (Consiglio, 1956) (Plecoptera). These species with negative LRD preferences may be more affected by climate changes than other species that present similar optimum values of LRD because they are inhabiting rivers in which a reduction in lotic habitats is more severe in relation to climate variation. Such considerations may be useful in defining the correct action required in the management of Mediterranean river ecosystems.

#### Potential for practical application

This paper has demonstrated that the lentic–lotic character of river reaches, expressed by means of the LRD descriptor, is closely related to the structure of aquatic invertebrate communities across Mediterranean rivers.

Some of the main potential uses of lentic–lotic indicators are summarized below, grouped for ease of interpretation into two main categories (direct and indirect).

- Direct—(1) Monitoring long-term changes in river habitat, due to large-scale, e.g., climate-related, as well as smaller scale alterations, e.g., water abstraction and morphological modifications; (2) Quantification of the distance from expected reference conditions, in terms of flow-related, habitat features; (3) Estimation of ecologically acceptable flows in highly dynamic rivers. (4) Supports the outline of river typologies, and (5) Assessing seasonal differences in habitat structure.
- Indirect—(1) For conservation issues, definition of lentic–lotic preferences of aquatic taxa to protect or enhance river habitats to support the safeguard of, e.g., endangered, rare, endemic, flag or umbrella species; (2) Using lentic–lotic information to interpret biotic communities' response to environmental change (natural or due to man-induced activities), and (3) Including correction factors, when needed, in assessment systems for the evaluation of the ecological quality of rivers, where results might be susceptible to lentic–lotic character variation.

As far as the direct application of lentic–lotic indicators is concerned, the first three points above can be unequivocally related to the implementation of WFD (European Commission 2000), the achievement of which is, to some extent, linked to the availability of cost-effective and scientifically sound indicators. Furthermore, the confounding effect of flow-related factors in the evaluation of ecological quality using benthic invertebrates, clearly depicted by LRD (Buffagni et al. 2009), should be taken into account in the implementation of WFD compliant methods and in the development of water quality management plans.

In general, and focusing on global change, variations in precipitation regimes and river discharge dynamics are to be expected (IPCC 2007). This will affect local hydraulics and flow type assemblages, modifying micro-habitat structures. The overall structure of rivers will be affected and their lentic–lotic character will change accordingly. As a consequence, macroinvertebrate communities will also be thoroughly modified and the availability of widely applicable indicators able to shed light on the link between hydraulic habitat and biotic response will prove highly valuable.

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## Appendix

**Table 5** Variability of the environmental parameters in the considered dataset

Category	Variable	Mean	Standard deviation	Min.	Max.
Descriptive	Source distance (Km)	17.3	13.1	3.0	60.9
	Altitude of the site (m)	350	169	30	620
	Catchment area (Km <sup>2</sup> )	166	380	7	2147
	Slope (%)	1.8	1.5	0.3	5.5
	Total channel width (m)	20.3	23.9	1.9	124.7
	Wetted channel width (m)	8.3	7.6	0.3	34.8
	Mean air temperature of July (°C)	22.3	1.7	20.0	25.0
	Mean air temperature of January (°C)	4.2	2.4	2.5	7.5
	Air temperature range (°C)	18.0	1.8	15.0	20.0
	Mean annual rainfall (mm)	979	287	500	1600
	Hardness (mg l <sup>-1</sup> CaCO <sub>3</sub> )	22.1	28.3	0.3	113.1
	Cl <sup>-</sup> (mg l <sup>-1</sup> )	37.8	61.2	3.8	321.0
	Water temperature (°C)	16.1	5.9	4.1	28.8
	HMS (Habitat Modification Score)	13.1	19.1	0.0	84.0
	HQA (Habitat Quality Assessment)	57.7	9.1	28.0	77.0
	Water quality	pH	7.9	0.3	7.0
Conductivity (µS/cm)		435	291	2	1686
O <sub>2</sub> saturation (%)		94.4	19.5	11.3	127.5
BOD <sub>5</sub> (O <sub>2</sub> mg l <sup>-1</sup> )		2.9	2.8	0.2	10.6
N-NH <sub>4</sub> (mg l <sup>-1</sup> )		0.0	0.1	0.0	0.6
N-NO <sub>3</sub> (mg l <sup>-1</sup> )		0.7	1.1	0.0	7.1
P-PO <sub>4</sub> (µg l <sup>-2</sup> )		84	184	0	1120
TP (µg l <sup>-3</sup> )		56.3	111.4	0.0	722.0
<i>E. coli</i> (CFU/100 ml)		2,064	9,299	0	63,000
Local hydraulics and lentic–lotic character		Discharge (m <sup>3</sup> s <sup>-1</sup> )	1.1	2.7	0.0
	Mean water depth (cm)	11.3	7.3	0.1	25.9
	Mean water velocity (m s <sup>-1</sup> )	0.4	0.2	0.0	1.2
	Froude number	0.1	0.2	0.0	0.8
	LRD (Total site score)	-6.2	28.8	-55.0	85.0

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