J. Limnol., 68(1): 92-105, 2009

Does the lentic-lotic character of rivers affect invertebrate metrics used in the assessment of ecological quality?

Andrea BUFFAGNI*, David G. ARMANINI and Stefania ERBA

CNR-IRSA, Water Research Institute, Via della Mornera, 25, 20047 Brugherio (MI), Italy
*e-mail corresponding author: buffagni@irsa.cnr.it

ABSTRACT

The importance of local hydraulic conditions on the structuring of freshwater biotic communities is widely recognized by the scientific community. In spite of this, most current methods based upon invertebrates do not take this factor into account in their assessment of ecological quality. The aim of this paper is to investigate the influence of local hydraulic conditions on invertebrate community metrics and to estimate their potential weight in the evaluation of river water quality. The dataset used consisted of 130 stream sites located in four broad European geographical contexts: Alps, Central mountains, Mediterranean mountains and Lowland streams. Using River Habitat Survey data, the river hydromorphology was evaluated by means of the Lentic-lotic River Descriptor and the Habitat Modification Score. To quantify the level of water pollution, a synoptic Organic Pollution Descriptor was calculated. For their established, wide applicability, STAR Intercalibration Common Metrics and index were selected as biological quality indices. Significant relationships between selected environmental variables and biological metrics devoted to the evaluation of ecological quality were obtained by means of Partial Least Squares regression analysis. The lentic-lotic character was the most significant factor affecting invertebrate communities in the Mediterranean mountains, even if it is a relevant factor for most quality metrics also in the Alpine and Central mountain rivers. Therefore, this character should be taken into account when assessing ecological quality of rivers because it can greatly affect the assignment of ecological status.

Key words: aquatic invertebrates, ecological quality, WFD, organic pollution, morphological impairment

1. INTRODUCTION

The importance of local hydraulic conditions in influencing the freshwater biotic community is largely recognized by the scientific community. Many authors, at different spatial scales and in varying degrees of detail, have demonstrated the relationships between, e.g., current velocity and discharge (Chutter 1969; Extence *et al*. 1999), turbulence (Hynes 1970), hydraulic conditions (Statzner *et al*. 1988; Brooks 1990; Dolédec *et al*. 2007), shear stress (Cummins 1962; Cummins & Lauff 1969) and invertebrate taxa. In spite of this, most current methods based on invertebrates in the assessment of ecological quality and status do not take such relationships into account, even though they can have a strong impact on structuring communities and may exert a great influence on the evaluation of river quality (Roy *et al*. 2003; Sheldon 2005). In fact, in rivers where local hydraulic conditions are highly variable over space and time, the water quality evaluation and the classification of ecological status will be subject to the 'disturbance' of flow-related natural factors (Sheldon 2005). These factors will affect the response of biological methods and metrics, for example, to water pollution. The quantification of such disturbance, however, is not straightforward because of the difficulty in describing in a complete and simple way the overall lentic-lotic character of a river site. Recently, we proposed a comprehensive approach to evaluate this character based on the quantification and weighting of, e.g., flow types, macrophytes and substrate types (Buffagni 2004; Erba *et al*. 2006; see session 2.2).

Due to the need for a timely implementation of the Water Framework Directive (WFD; EC/2000/60), most European countries have begun the process of revising and adapting their assessment systems, so that quality evaluations are performed according to the new directive. The WFD currently aims at reaching good ecological status for all European water bodies by 2015 and sets out a number of strict recommendations for this to be achieved. In a quite complex overall picture, uncertainty in biological quality evaluations plays a relevant role because it can create an unbalanced allocation of environmental or economic resources. For this reason, any study aimed at providing direct evidence of the influence of natural factors on quality evaluations could have a relevant impact on river management practices and monitoring planning. In this context, the WFD attests to the priority of contemplating the hydromorphological features of rivers both in the selection of pristine or semi-natural sites (i.e., WFD 'reference sites') and as a support in the interpretation of biological information when classifying river quality and defining restoration measures. Where local hydraulic dissimilarities are relevant, new assessment methods should then integrate information on the extent of the natural variability of lentic-lotic features, so that quality judgments

Tab. 1 Summary description of datasets used in the present paper, which were collected for the EU AQEM and STAR projects. Main stressor codes: M, degradation in stream morphology; O, organic pollution; G, general degradation (i.e.. a main stressor cannot be identified). Season codes: 1, spring; 2, summer; 3, autumn; 4, winter. Quality class codes are from 1 (bad status) to 5 (high status).

River category	Country	AQEM/STAR code	Stream type description	Altitude (m)	No. of samples	Dominant stressor	Season	Quality class
Alps	Austria	A06	Small-sized crystalline streams of the ridges of the Central Alps	200-800	9	M	2	1-5
	Italy	I01	Small-sized streams in mountainous areas in the Alps (siliceous)	>800	11	M	3	1-5
	Italy	I05	Small-sized streams in the southern calcareous Alps	>800	10	M	1,2	1-5
Central mountains	Austria	A05	Central Sub-alpine Mountains	200-800	8	M	2	1-5
	Czech Republic	C04	Central Sub-alpine Mountains	200-800	14	M	2	1-5
	Czech Republic	C05	Small-sized streams in the Central sub-alpine Mountains	200-800	10	G	2	2-5
	Slovak Republic	V01	Small sizes, siliceous mountains streams in the West Carpathians	200-800	23	O	3	1-5
Mediterranean mountains	Italy	I02	Small-sized streams in lower mountainous areas of the Southern Apennines (Italy)	200-800	11	G	3	1-5
	Italy	I06	Small-sized calcareous streams in the Central Apennines	200-800	11	G	2	3-5
	Italy	I03	Mid-sized streams in lower mountainous areas of the Northern Apennines	200-800	11	M	3	1-5
Lowland streams	Italy	I04	Small source streams in the Po Valley	<200	12	G	3.4	1-5

are not influenced by natural differences in hydromorphology between sites and seasons.

The aim of the present paper is to investigate the influence of local hydraulic conditions on a selection of invertebrate community metrics commonly used in the assessment of ecological quality and estimate their potential influence on the evaluation of river water quality. In addition, the implications on water quality assessment in different geographical contexts across Europe is illustrated, together with an overall quantification of the lentic-lotic gradient observed in various riverine contexts.

2. METHODS

2.1. Dataset

The dataset used for the present work mainly originated from the EU co-funded AQEM (The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates) and STAR (Standardisation of River Classifications) projects (Hering *et al*. 2004; Furse *et al*. 2006). From the complete AQEM and STAR database some data were excluded for two reasons: a) biological data did not always match with abiotic data; b) no ecological quality classification of samples or no 'high status' samples (sensu WFD) were available. A sub-set of 130 samples, for which invertebrate and environmental data were collected in a highly comparable way, was thus available to investigate the relationship between biological quality metrics and the lentic-lotic character of rivers. To avoid increasing internal variability of specific datasets where more seasons were investigated, data to be analyzed were harmonized by selecting only one sample for each site (from summer or autumn season) out of the full set of available data. For two Italian datasets, sampling began at the end of one season and ended at the beginning of the following season. Four geographical contexts/river categories were covered by the study, taking into account the typological grouping performed by Verdonschot (2006): the Alps, Central European mountains, Mediterranean mountains and Lowland streams. In particular, to investigate the relationship between biological data and lentic-lotic character, information from Austria, Czech Republic, Italy and Slovak Republic was considered. The datasets selected are described in table 1 with information on stream type, dominant stressors, season, and quality classes covered. Detailed information on such datasets can be found in Hering *et al*. (2004) and Furse *et al*. (2006).

2.2. Hydromorphological descriptors

To characterize hydromorphological features of river sites during the STAR project and, partly, the AQEM project, the River Habitat Survey protocol (RHS: Raven *et al*. 1998; Furse *et al*. 2006) was applied.

Based on the RHS protocol, the morphological impact (Habitat Modification Score, HMS) was estimated for each site. To derive the HMS index, scores were assigned according to Raven *et al*. (1998). To calculate HMS, a different score is given to each morphological modification (e.g., bank re-sectioning, number of weirs, bridges), according to the estimated importance of the impact type and to the extent of its presence. HMS is thus the sum of all the individual scores. The HMS score was normalized here by subtracting to the maximum value observed in the overall dataset (HMS=96) the site score and then by dividing by the same maximum value in order to obtain an index that ranges from 0 to 1. In the present paper, increasing values of the normalized HMS score indicate an increase in morphological quality.

The Lentic-lotic River Descriptor (LRD: Buffagni 2004), was used to define the local hydraulic character of a site. The LRD is an abiotic index devoted to the evaluation of the local hydraulic condition of streams, derived mainly by giving scores to various habitat features in relation to their ability to indicate the lotic or lentic character of the site. LRD negative values represent rivers with a predominantly lotic character, positive values are reached when lentic habitats dominate. The LRD was developed on the basis of hydromorphological data collected with the CARAVAGGIO method (Buffagni *et al*. 2005), which represents an implementation of the UK River Habitat Survey method (RHS: Raven *et al*. 1998), focusing especially on and adapted to Mediterranean rivers (Buffagni & Kemp 2002; Buffagni *et al*. 2005). Because the CARAVAGGIO method allows the recording of a higher number of hydromorphological features with respect to RHS, it was necessary to adjust the calculation of LRD to the features recorded with both methods. The features considered in the LRD version adapted to the UK RHS form are presented in table 2. Only the key features needed for LRD estimate are summarized here, while the formula for its calculation can be found elsewhere (Buffagni *et al*. 2004; unpublished). One important feature is the flow type: at laminar or null flow a positive score is assigned, while to turbulent flows negative values correspond. A particularly high value is given to dry conditions because they interrupt the longitudinal connectivity of the stream, potentially causing great changes in the benthic community. Substrate types receive a score in relation to granulometry, with small-sized substrata considered to be mainly associated with lentic features while larger ones achieve a negative score, somewhat providing a memory of high-flow events. Other scoring features are vegetation types and bars. Artificial features such as bridges or weirs obtain positive values, because they are considered in relation to the impoundment phenomena and flow impedance they can create. Because the presence of such artificial structures is considered as invariant in, for example, a year-long time scale, the variation that can be observed in LRD values at a river site will depend on the dynamic features only i.e. discharge and natural channel features (e.g., substrate, macrophytes), which are often driven by climate characteristics. Increasing values of LRD indicate an increase of lentic conditions. For selected data analyses (i.e., PLS analysis session 2.5.), in order to produce an index ranging from 0 to 1, the LRD site scores were normalized by adding the absolute value of the most negative value observed in the overall dataset (LRD = -51.50) and by dividing each LRD site score by the new maximum value obtained (LRD = 94.5).

Tab. 2. Habitat features considered for the calculation of the Lentic-lotic River Descriptor (LRD) in its simplified version adapted to the UK River Habitat Survey protocol.

	UK RHS field form Section	Habitat feature
spot-heck	E	Channel substrate & flow-type occurrence
	G	Channel Vegetation types
sweep-up	C&K	Bars & flow-type occurrence
	D	Artificial features
	M	Waterfalls & debris dam
	N	Channel choked with vegetation

2.3. Chemical descriptors

It is not the aim of this paper to investigate the response of invertebrate metrics to individual chemical variables. On the contrary, an overall evaluation of the community response to major synoptic factors is being undertaken: those which can mostly affect invertebrate taxa because they integrate various aspects of hydromorphology and pollution. Thus, to describe river sites in terms of water (organic) pollution, the Organic Pollution Descriptor (OPD) (Erba *et al*. 2007) was used. This descriptor is inspired by and conceptually similar to the one described in the Italian legislation (D.Lgs. 152/99; see Erba *et al*. 2007). The variables considered here are: Oxygen saturation deficit [%], chloride [mg L^{-1}], BOD5 [mg L^{-1}], ammonium [mg L^{-1}], nitrite [mg L^{-1}], nitrate [mg L^{-1}], ortho-phosphate [µg L^{-1}], total phosporous [µg L^{-1}], COD [mg L^{-1}], *Escherichia coli* [UFC/100 mL]. A score is assigned to each chemical variable available in the dataset. The score assigned, ranging from 0 to 1, is based on class boundaries obtained as multiples of the 75^{th} percentile of values found at reference sites (sensu WFD). The multiplicative factor of the 75^{th} percentile of reference sites used to define boundaries in OPD is increased by two times per each class of scores. The scores obtained from each chemical parameter are then averaged to obtain the final index value. Increasing value of OPD indicates an increase in water quality. The response of individual invertebrate metrics to single chemical pollutants can be found in Erba *et al*. (2007).

2.4. Benthic invertebrate sampling - Biotic metrics and indices

Invertebrates were sampled following the AQEM and STAR sampling protocols (AQEM consortium 2002; Hering *et al*. 2004; Furse *et al*. 2006). Both protocols focus on a multi-habitat scheme, where habitats are sampled in proportion to their presence within a reach (25-50 m). Sampling was usually performed with a Surber net (area 0.05 to 0.01 m^2; mesh size 0.5 mm). A full sample consists of 20 sampling units from the different microhabitat types occurring in at least 5% of the sampling sites.

A few biotic indices were calculated to assess the response of benthic communities to the river quality gradients and to the lentic-lotic character range experienced within each dataset: the STAR ICM metrics and index (Buffagni *et al*. 2007) were selected (see below). Those metrics were preferred for their wide applicability at an European scale, which was demonstrated for WFD Intercalibration purposes (Buffagni & Furse 2006). The identification level required to calculate these metrics and index is the family, which seems the most suitable level to perform a comparison at such a large geographical scale (Verdonschot 2006). These metrics are clustered into three groups, providing information on three major response areas:

- Tolerance: ASPT (Armitage *et al*. 1983);
- Abundance/Habitat: Log_{10} (Sel_EPTD +1) (based on Sel_EPTD, Buffagni *et al*. 2004); 1-GOLD, (Pinto *et al*. 2004), Total number of Families (N_families, e.g., Hering *et al*. 2004); number of EPT Families (EPT_taxa, e.g., Lenat 1983);
- Diversity: Shannon - Wiener diversity index (SHAN, Shannon 1948).

After their normalization by the median value of reference sites' samples or the 75th percentile of high status sites, the metrics were combined into the STAR_ICMi. The normalization was performed separately for each of the stream types defined during the AQEM and STAR projects (Hering *et al*. 2004; Furse *et al*. 2006) and included in the present study. A different weight is attributed to the metrics within each group, giving greater importance to the metrics based on the whole community (Buffagni *et al*. 2007). To obtain the final multi-metric score, the same weight is attributed to each of the three metric groups (0.333). To calculate these metrics and indices the ICMeasy software 1.2 (Buffagni & Belfiore 2006), was used.

2.5. Data analysis

The variability of the quality and environmental descriptors within each of the four considered river categories was assessed by means of box & whisker plots obtained with the Statistica 5.0 software (StatSoft Inc. 2004).

Partial least squares of latent structures (PLS) regressions were performed using SIMCA 11.0 (Umetri 2005) to define which environmental factors were more influential on invertebrate communities. For the purpose of the present paper, the main advantage offered by PLS modelling is that this analysis offers powerful diagnostic tools to obtain information on the model structure and of the contribution and sign of each predictor. A cross validation statistical test (CV, Wold 1978; Eriksson *et al*. 1995) was performed to select the number of significant components, which provides Q^2 (i.e. the cross-validated R^2). For models with less than 100 cases, like in the datasets tested here, the significance limit is set to $Q^2 > 0.05$ (Eriksson *et al*. 1995). Thus, components with Q^2 lower than this limit were excluded because they were classified as not significant. Once a PLS model has been calculated, different tools are available to construe its meaning e.g., weights, loadings, coefficients. Scaled and centred coefficients were taken into account to obtain information on the positive or negative contribution of each predictor (Eriksson *et al*. 1995). The range described by the confidence interval (95% limit) of the coefficients was considered to test their significance: only if the confidence intervals did not pass through zero, were they considered significant (Gauchi & Chagnon 2001). Another tool to construe PLS meaning is the Variable Influence on Projection (VIP), which gives information about the importance of each X-variable taking into account the amount of Y-variance explained by each latent variable (Wold 1995). Terms with VIP larger than 1 are the most relevant for explaining Y and values higher than 0.7 are considered important (Eriksson *et al*. 1999; Lepori *et al*. 2005). The VIP score was selected here as a useful indicator because it is considered the most condensed way to express the results of a PLS model (Eriksson *et al*. 1995). Furthermore, the VIP value is extremely useful for the objectives of this paper because, as it is dimensionless (Yao *et al*. 2004), it is possible, by means of simple sums, to compare the results of different models. The confidence interval (95% limit) of the VIP was considered, as explained for the coefficients, to test the significance.

3. RESULTS

3.1. Lentic-lotic variability

The variability of LRD values within each of the four considered geographic contexts is shown in figure 1. The highest variability is observed in the Mediterranean mountains. They present very lentic as well as very

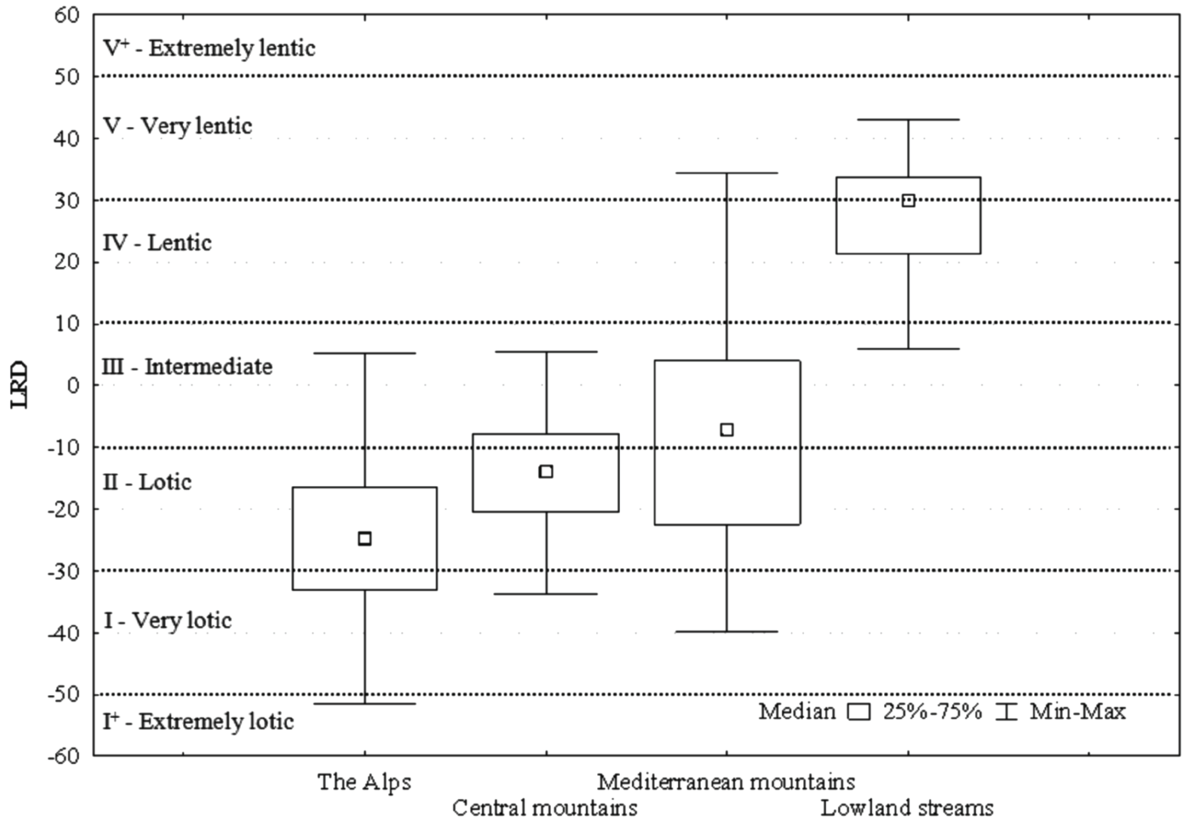

Fig. 1. Variability of the Lentic-lotic River Descriptor (LRD) in the four considered geographic areas with indication of the name and limits of LRD classes.

lotic LRD values with a quite wide 25th - 75th percentile range, which covers intermediate to lotic situations. The lowest LRD variability is observed in the Central Mountain rivers dataset. The lowest median value (*ca* -22) was observed in the Alps, while the highest LRD median value was observed for the Lowland streams dataset (*ca* +30).

Sites belonging to the Alps show a median value in the lotic class, while minimum value belongs to the extremely lotic class. Maximum value is a slightly positive value of the intermediate class. Central mountains present a lotic median value, with minimum and maximum values ranging from very lotic conditions, dominated by fast flowing areas, up to intermediate ones. Sites in the Mediterranean mountains show a median value corresponding to intermediate lentic-lotic conditions, while minimum and maximum values range between very lentic and very lotic conditions. Thus, Mediterranean mountains present a stretched gradient in hydraulic conditions, varying from net dominance of riffle or fast flowing areas up to the clear dominance of stagnant conditions. Lowland streams present a lentic median value commonly characterized by the dominance of pool areas with fine substrate, standing or low flowing water, presence of lentic vegetation and organic detritus. The median value is at the boundary between lentic and very lentic conditions. The range between minimum and maximum values varies from intermediate conditions up to very lentic ones characterized by standing water, fine substrate and the disappearance of riffle areas.

3.2. Organic pollution and Morphological degradation variability

The variability of organic pollution and morphological degradation variables within each of the four considered geographical contexts is shown in figure 2.

The highest chemical degradation was observed in the Central mountain area (OPD minimum value *ca* 0.1; median value *ca* 0.72). In the Central mountains the OPD gradient is wide both in terms of minimum and maximum values and height of the box. In this dataset both pristine and heavily degraded sites in terms of organic pollution are present. The lowest chemical degradation was observed in the Alps, where the highest OPD median value (*ca* 0.95) was observed and, for this parameter, all sites resulted in pristine or high quality conditions. In the Mediterranean mountains, even if the OPD median value corresponds to nearly good quality sites, a wide range of organic degradation was observed with values from a minimum of *ca* 0.2 up to pristine (*ca* 1) ones. In the Lowland streams the organic pollution

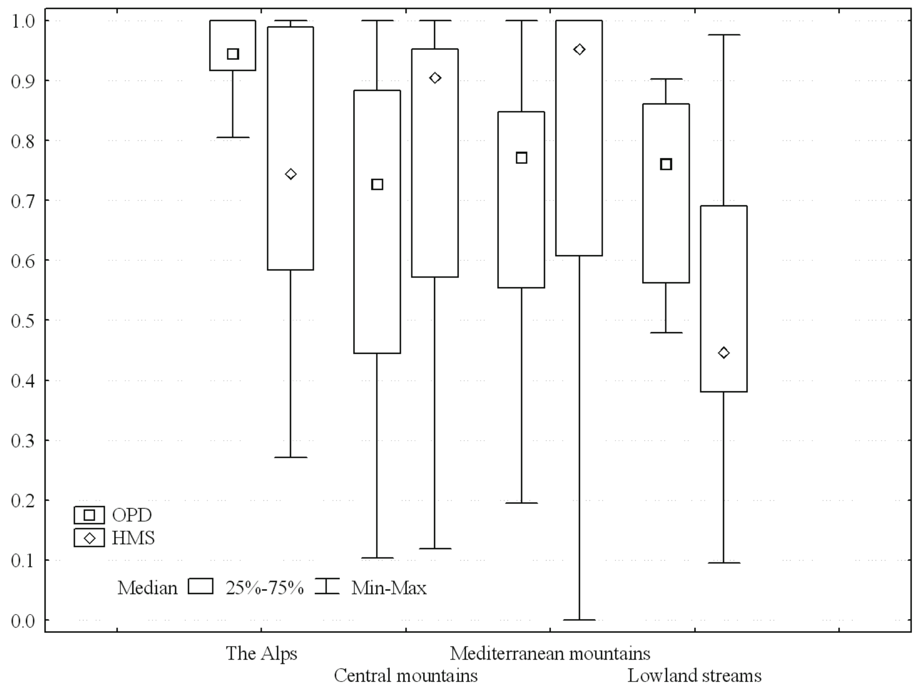

Fig. 2. Variability of Organic pollution (OPD) and Morphological impairment (HMS) in the four considered geographic areas.

ranged between moderate to pristine conditions with a good quality median value, and a box with values ranging from *ca* 0.55 to *ca* 0.85.

The highest morphological impairment variability was observed in the Alps. Sites in the Alps showed unmodified as well as altered sites described by HMS values with a wide 25^{th} - 75^{th} percentile range, covering intermediate to pristine conditions. In the Lowland streams the 25^{th} - 75^{th} percentile range comprised mainly modified streams and the lowest HMS median value was observed (*ca* 0.45). The highest median value was observed in the Mediterranean mountains (HMS equal to *ca* 0.95), corresponding to unmodified riverine sites and in general the gradient observed in Mediterranean rivers is similar to that of the Central mountains.

3.3. Biological quality metrics and lentic-lotic character

The results of the PLS regression models for rivers in the Alpine area are presented in table 3. In the Alps, the amount of biological variability explained ranges between 0.12 and 0.42 R^2Y values. Except for 1-GOLD, Shannon diversity and the Number of Families, all models were significant and showed a single component. All VIP values higher than 0.7 resulted significant except for OPD in the ASPT model and for HMS in the EPT taxa model. In general terms, morphological impairment was the most relevant factor in all significant models except for EPT taxa, for which organic pollution proved to be the most important. The lentic-lotic character of river sites affected all the considered metrics, being important and significant in all significant models. As far as the sign of the regression coefficients is concerned (Tab. 4), a decrease in morphological impairment corresponded to an increase in metric value, while a decrease in the lentic condition of sites corresponded to an increase in the metrics value. No clear trend could be seen for organic pollution in terms of coefficient signs, probably due to the generally good water quality of river sites.

In Central mountain rivers the amount of biological variability explained (Tab. 3) showed R^2Y values between 0.19 and 0.42. For all models only one component was identified and except for Selected EPTD all were significant. In general terms, organic pollution was the most relevant factor in all significant models except those for Shannon diversity and Number of Families, in which morphological impairment proved to be more important. Despite the short gradient of LRD observed here, the lentic-lotic character of river sites affected many of the considered metrics and resulted as significant in most cases. Looking at the coefficient signs (Tab. 4), the increase of both quality indicators caused an increase of the metrics value in all significant models, corresponding to better conditions in the benthic community, while an increase in the lentic condition of sites corresponded to a decrease in the metrics value.

In the Mediterranean mountains the amount of biological variability explained ranges from 0.23 to 0.32 in terms of R^2Y values. All regression models except that for the Total Number of Families are significant and generally show at least two significant factors (Tab. 3). As in the previous areas, only one component was identified for all the models. The lentic-lotic character was

Tab. 3. Summary of PLS regression models and VIP values between environmental factors and biological metrics in the four river categories (ns: non significant model). VIP values that are either lower than 0.7 (i.e. not important) or not significant are reported in italic.

Alps						Central mountains					
	R^2Y	Q^2	VIP				R^2Y	Q^2	VIP		
Metrics			OPD	HMS	LRD	Metrics			OPD	HMS	LRD
ASPT	0.42	0.34	*0.74*	1.32	0.84	ASPT	0.42	0.38	1.29	0.94	*0.67*
EPT_taxa	0.34	0.20	1.13	*0.76*	1.07	EPT_taxa	0.41	0.37	1.23	0.87	0.86
1-GOLD	ns	<0.05				1-GOLD	0.25	0.16	1.14	0.92	0.92
SHAN	ns	<0.05				SHAN	0.21	0.13	0.83	1.11	1.04
Log_EPTD	0.12	0.06	0.97	1.16	0.85	Log_EPTD	ns	<0.05			
N_families	ns	<0.05				N_families	0.19	0.14	0.99	1.28	*0.62*
STAR_ICMi	0.30	0.18	*0.52*	1.35	0.96	STAR_ICMi	0.42	0.38	1.18	1.00	0.79
Mediterranean mountains						**Lowland streams**					
	R^2Y	Q^2	VIP				R^2Y	Q^2	VIP		
Metrics			OPD	HMS	LRD	Metrics			OPD	HMS	LRD
ASPT	0.30	0.24	1.04	*0.68*	1.20	ASPT	0.50	0.42	1.30	1.14	*0.12*
EPT_taxa	0.23	0.10	*0.23*	1.18	1.25	EPT_taxa	0.52	0.46	1.26	1.18	*0.19*
1-GOLD	0.34	0.21	1.66	*0.15*	*0.46*	1-GOLD	0.63	0.44	1.36	1.07	*0.05*
SHAN	0.27	0.15	0.78	0.78	1.33	SHAN	0.55	0.42	1.17	1.12	*0.61*
Log_EPTD	0.31	0.21	*0.24*	1.14	1.28	Log_EPTD	0.48	0.40	1.24	1.21	*0.09*
N_families	ns	<0.05				N_families	0.56	0.43	1.27	1.14	*0.30*
STAR_ICMi	0.32	0.20	*0.66*	1.03	1.22	STAR_ICMi	0.62	0.55	1.28	1.17	*0.07*

Tab. 4. Intercept and scaled and centred coefficients of PLS regression models between environmental factors and biological metrics in the four river categories are reported. Not significant coefficients are reported in italic.

Alps					Central mountains				
Metrics	b	OPD	HMS	LRD	Metrics	b	OPD	HMS	LRD
ASPT	5.92	*-0.23*	0.41	-0.26	ASPT	4.72	0.38	0.20	*-0.28*
EPT_taxa	3.90	-0.33	*0.22*	-0.31	EPT_taxa	2.72	0.36	0.25	-0.25
1-GOLD					1-GOLD	3.14	0.25	0.20	-0.21
SHAN					SHAN	3.42	0.17	0.23	-0.22
Log_EPTD	4.80	0.16	0.19	-0.14	Log_EPTD				
N_families					N_families	3.98	0.20	0.25	*0.12*
STAR_ICMi	7.67	*-0.13*	0.34	-0.24	STAR_ICMi	6.50	0.34	0.29	-0.23
Mediterranean mountains					**Lowland streams**				
Metrics	b	OPD	HMS	LRD	Metrics	b	OPD	HMS	LRD
ASPT	7.82	0.26	*0.17*	-0.29	ASPT	2.20	0.42	0.37	*-0.04*
EPT_taxa	3.36	*0.05*	0.25	-0.27	EPT_taxa	1.06	0.40	0.37	*0.00*
1-GOLD	4.32	0.51	*0.05*	*-0.14*	1-GOLD	1.59	0.49	0.39	*-0.02*
SHAN	5.70	0.18	0.18	-0.31	SHAN	1.48	0.38	0.36	*0.20*
Log_EPTD	3.35	*0.06*	0.28	-0.32	Log_EPTD	1.04	0.39	0.38	*-0.03*
N_families					N_families	1.76	0.42	0.37	*0.10*
STAR_ICMi	6.29	*0.16*	0.25	-0.30	STAR_ICMi	1.72	0.45	0.41	*0.03*

the most relevant factor in all significant models of quality metrics, except for 1-GOLD. Organic pollution resulted as the only significant key factor for 1-GOLD. Morphological degradation was never the most relevant factor but was a significant variable in determining metrics variation in all models but those for 1-GOLD and ASPT. A similar pattern to that of Central mountain rivers was found with regard to regression coefficients (Tab. 4).

In the lowland streams, the amount of biological variability explained is high, with R^2Y values included between 0.48 and 0.63, and all regression models were significant showing one component only. Both organic pollution and morphological impairment yielded significant relationships in all quality metrics. The lentic-lotic character resulted unimportant in this stream type. As far as the sign of the regression coefficients is concerned (Tab. 4), the situation is the same as explained earlier for the two previous river categories. Because of the reduced size of the considered dataset, further data should be analyzed for this stream category to extend conclusions to other stream types e.g. showing a higher hydrological variability, and geographical contexts.

3.4. Response of invertebrate metrics to environmental factors

As far as R^2Y values are concerned, it is worth mentioning that the STAR_ICM index generally showed the highest or the second highest value for almost all areas (Tab. 3).

For the four river categories, looking at the sum of the number of times VIP values were important (i.e., >0.7) and significant, some general remarks can be outlined (Tab. 5). The ASPT metric responded to the effects of the three environmental factors considered, primarily organic pollution and morphological impairment, but also to differences in the lentic-lotic condition of river sites. The number of EPT taxa was influenced by the three environmental variables considered. The 1-GOLD metric resulted as influenced mainly by organic pollution and less affected by both morphology and lentic-lotic character. The Shannon diversity index resulted as primarily influenced by morphological alteration and organic pollution, but also lentic-lotic character exerted a strong control on determining metrics variation. Selected EPTD taxa was influenced mainly by HMS, but also by OPD and LRD descriptors. The total number of Families seemed to be highly related to both chemical and morphological degradation descriptors (two datasets only), while it appeared neutral to changes in lentic-lotic conditions. Finally the STAR_ICM index mainly felt the effect of morphological impairment but also that of the LRD and OPD descriptors, as expected by looking at single metrics models. It is notable that the only metric not influenced by lentic-lotic characteristics is the total number of Families.

Tab. 5. Number of times that significant VIP values are higher than 0.7 for each biological metric, with detail on each environmental factor. The values shown refer to significant PLS regression models for the four areas all together (top). A summary of the ranking, i.e. relative importance, of each environmental factor in the same models is also presented (bottom).

		Environmental factor			
		Significant models	OPD (organic pollution)	HMS (degradation in stream morphology)	LRD (lentic-lotic character)
metrics	ASPT	4	3	3	2
	EPT_taxa	4	3	3	3
	1-GOLD	3	3	2	1
	SHAN	3	3	3	2
	Log_EPTD	3	2	3	2
	N_families	2	2	2	
	STAR_ICMi	4	2	4	3
	Total	23	18	20	13
ranking	1		13	5	5
	2		3	14	5
	3		2	1	3

In more general terms, organic pollution (here expressed as OPD) was the most important factor in determining the variation of metrics devoted to the detection of general river degradation. In fact, OPD resulted 13 times out of a total of 23 as the most relevant factor and, in total, relevant 18 times in influencing biological quality metrics. Morphological degradation (quantified here through HMS) was also a relevant factor in explaining metric variation and resulted as the main determining factor 5 times and, in total, 20 times as a relevant aspect. It is worth noting that the lentic-lotic character (here LRD) resulted as the first variable that determined metrics variation 5 times. Generally, lentic-lotic features significantly influenced the variation of quality metrics in 13 models out of a total of 23; close to 60% of cases.

4. DISCUSSION

4.1. Lentic-lotic variability and overall remarks on PLS models

Different geographic areas and river types or categories are expected to present dissimilarities in the hydrological regime because this is driven by climatic and basin-scale control factors (e.g., Monk *et al*. 2006). Hydrological conditions, can show severe seasonal and spatial variability, influencing the local hydraulic situation of riverine sites (Monk *et al*. 2006). Consequently, changes in the hydrological and hydraulic conditions of rivers can severely affect aquatic communities, whose structure and functioning will have to adapt accordingly (e.g., Dolédec *et al*. 2007; Milner *et al*. 2001).

As far as the river categories and areas considered in this study are concerned, it must be noted that, for similarly sized rivers, in the Alps the annual discharge is proportionally higher than at lower altitudes and is strongly influenced by ice and snow accumulation (Horton *et al*. 2006). This can result in a highly variable annual and inter-annual hydrological cycle with periodic floods and seasonal flow patterns strongly influencing the benthic assemblages (Jakob *et al*. 2003). Furthermore, climate and anthropic changes are expected to amplify and alter current conditions causing further changes in the annual hydrological cycle. In the Alpine rivers studied one of the main pressures acting on freshwater ecosystems is morphological impairment. In Austria, for example, Muhar *et al*. (2000) observed that only a small portion of river stretches resulted as hydromorphologically unaffected. Similar trends were also observed by Füreder *et al*. (2002) in Alpine streams in general. Thus the relationship observed in the present paper between biological response and environmental variables in Alpine rivers seems consistent with results recorded by other Authors. The fact that the percentage of variance explained is lower than 0.45 could be linked to the prevalence of processes and dynamics acting at a larger scale than that studied e.g. extreme hydrological events (Matthaei *et al*. 1997; Finn & Poff 2005). The significant relationships found between the lentic-lotic character of the studied sites and the biological metrics is probably due to the presence in the dataset of sites ranging from intermediate to extremely lotic conditions (see Fig. 1). It seems extremely interesting that, notwithstanding the overall adaptation of benthic taxa to lotic or very lotic conditions in the Alpine region, the

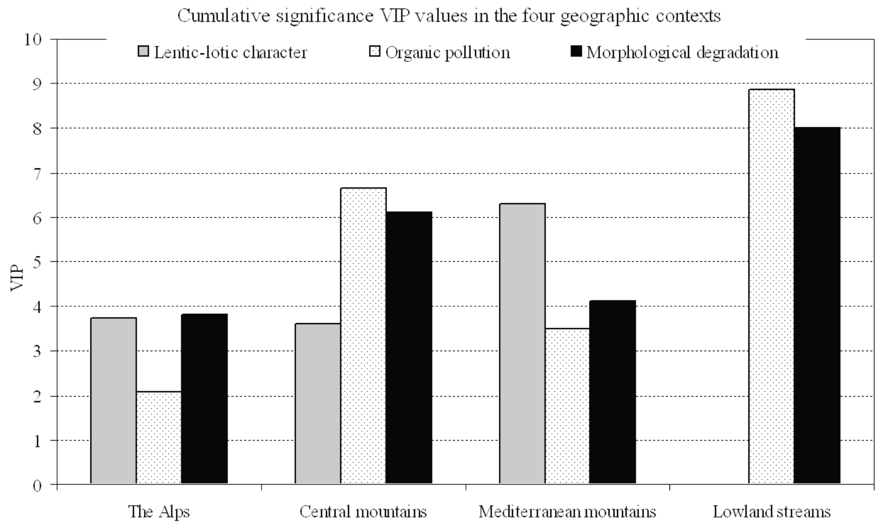

Fig. 3. Overall and relative importance of different disturbance and degradation factors in PLS regression models for biological metrics. Bars show cumulative VIP values, which resulted in the four geographic areas, for each of the considered environmental factors (Morphological degradation: HMS; Organic pollution: OPD; Lentic-lotic character: LRD). Not significant VIP were excluded.

lentic-lotic nature of the site has considerable influence on the invertebrate community.

In the Mediterranean region, rivers show a high natural flow variability, which can embrace droughts, and implies virtually strong seasonal differences of macroinvertebrate assemblages (Coimbra *et al.* 1996; Gasith & Resh 1999). Shifts from lotic to lentic conditions are observed annually in many rivers of the region (e.g., Pinto *et al.* 2004; Morais *et al.* 2004). Human activities often imply water abstraction (e.g., Prat & Munné 2000), frequently lengthening the low flow period of rivers. The resultant effects on the invertebrate community determine that existing approaches to assessing water quality, developed in other regions, may not be directly applicable (Graça & Coimbra 1998). In accordance with these authors, the results of the present paper call for extreme caution in using traditional approaches to assess ecological quality in Mediterranean mountain rivers. In effect, the lentic-lotic character resulted as the most important factor affecting invertebrate communities, even though organic pollution and morphological impairment acted strongly on the studied river sites.

In Central mountain rivers, the lentic-lotic character also proved to be an important factor, although organic pollution and morphological impairment had a greater effect on the biological metrics devoted to ecological assessment. This is probably due to the smaller hydraulic and hydrological variability of Central Mountain rivers compared to those of the Mediterranean area (Szoszkiewicz *et al.* 2006). It seems extremely interesting that, notwithstanding the reduced LRD variability that was observed, the lentic-lotic nature of the river site acts significantly on the invertebrate community.

The studied Lowland streams showed relatively constant and undisturbed hydrological features (e.g., Cotta-Ramusino *et al.* 1991), which included low turbulence (Szoszkiewicz *et al.* 2006) and uniform flow patterns, due to their prevalent spring character. In these streams, where the influence exerted by the lentic-lotic character on the benthic community was expected to be feeble, all PLS models were significant and explained a high proportion of biological variability. Indeed, the lentic-lotic character (i.e., through the LRD descriptor) did not significantly influence any quality metrics. Nonetheless, the restricted geographical range where the investigated sites are located i.e., all in Northern Italy, and the overall limited number of sites advocate for extreme caution in generalizing these conclusions. In fact, more investigations are needed prior to tranferring any assumption to other lowland river types.

An overall summary of the PLS results, obtained for each of the river categories, is shown in figure 3 where the cumulative VIP values for each area are shown, obtained by summing up only significant VIP values of significant models. Finally, organic pollution was an important factor in determining the variation of quality metrics. This result matched expectations, because organic pollution and general degradation were recognized as the main stressors for the majority of the considered datasets (Tab. 1; Hering *et al.* 2004; Furse *et al.* 2006). The lentic-lotic character of sites appears to act clearly as a confounding factor in three out of the four river categories studied, with the exception of Lowland streams, where the local hydraulic conditions were less relevant in determining benthic community composition. In particular, in the Mediterranean mountain rivers, lentic-lotic character was shown to be the most important factor influencing the structure of the benthic community and thus the assessment of ecological quality, as already recognized by Coimbra *et al*. (1996) for unpredictable Mediterranean rivers.

4.2. Relationships between biological indices and abiotic descriptors

In general terms, the shift of a site towards an increasingly lentic character generally leads to the disappearance of sensitive taxonomic groups or species, which are more frequently associated with lotic conditions (e.g., Pinto *et al*. 2004). Not surprisingly, the responsiveness of most of the studied quality metrics to the lentic-lotic character of river sites was evidently demonstrated, for three out of four of the river categories considered. Looking at the individual biological metrics considered in this paper, the ASPT was originally developed to assess water quality (Armitage *et al*. 1983), but it seems to also be highly influenced by hydrological parameters (Zamora-Munoz *et al*. 1995; Monk *et al*. 2006). In fact, many sensitive taxa e.g., Plecoptera, which receive a high score in the index calculation, are often rheophilic taxa and have high requirements concerning the level of dissolved oxygen (Extence & Ferguson 1989; Boulton 2003). When flow changes are associated with the increase of lentic conditions, these taxa tend to disappear (Boulton & Lake 1990; Morais *et al*. 2004). This obviously also influences the metric 'Number of EPT taxa', which constitute both a richness and a 'tolerance' metric and is often used to detect organic pollution (Verdonschot 2000). Erba *et al*. (2006) suggested that the number of EPT taxa can also be used to detect morphological impairment - confirming what was observed in the present paper - but the simultaneous effects of water quality and lentic-lotic character should be taken into account as well. 1-GOLD is a metric mainly devoted to the assessment of organic pollution (Pinto *et al*. 2004) and this is confirmed by the results presented here. Nonetheless, local hydraulic features influence this metric in central mountain rivers. The Shannon diversity index seems to respond to the variation of all the variables considered. The effects of morphological impairment linked to diminishing habitat heterogeneity and the incidence of organic pollution are important factors affecting this metric (Coimbra *et al*. 1996; Ward & Wiens 2001; Brunke 2002). In addition, unpredicted extreme flow conditions are known to potentially decrease the overall diversity of benthic communities (Cowell *et al*. 2004; Pinto *et al*. 2004) and thus act as a confounding factor in the use of this metric for assessing ecological quality. The Log_EPTD is a metric based on the abundance of selected taxa and is able to detect general degradation (Buffagni *et al*. 2007) as well as morphological impairment (Erba *et al*. 2006). This is partly confirmed by the results presented here. The number of Families, that significantly react to river alteration in two river categories only, seem not to be influenced by lentic-lotic character. In the Mediterranean rivers, the absence of a significant model for this metric can be connected to zoogeographical differences among the studied areas i.e., they are characterized by different levels of overall taxonomic richness. The STAR_ICM index is a multi-metric index obtained by combining all of the above commented metrics and was originally developed to assess general degradation of river sites for WFD intercalibration purposes (Buffagni *et al*. 2007). In comparison with individual metrics response, this index often shows a better performance in reacting to quality variables and frequently reaches the highest R^2 of Y in the PLS models i.e., higher predictability. Unsurprisingly, because some of the metrics that constitute the index are influenced by lentic-lotic character, the STAR_ICMi is also affected by the general hydraulic state of the sites.

4.3. The lentic-lotic character as a misleading factor in ecological quality evaluation

The Lentic-lotic River Descriptor (LRD) seems to be an important tool for improving understanding of biological metric variability but attention should also be directed to understanding the contribution of flow impedance phenomena in determining local hydraulic conditions. In the present paper it was observed that an increase in lentic conditions is associated with a decrease in quality metrics value, thus possibly causing an underestimation of ecological quality. In fact, if the presence of lentic conditions is due to natural processes, the obtained ecological status classification can be partly unsubstantiated. Nonetheless, when the alteration of the lentic-lotic character of a site is totally or partly due to a) water abstraction from upstream river stretches or b) the presence of local artificial works (e.g., weirs, dams, bridges) increasing flow impedance, the variation of biological metrics can reflect such alterations. In this case, it is then crucial, especially in the Mediterranean area, that the two components of the lentic-lotic character of a river site i.e. the natural one and that due to hydrological and morphological modifications, are assessed. In its full formulation the LRD descriptor (Buffagni 2004), which is based on the CARAVAGGIO survey method (Buffagni *et al*. 2005) can provide such a distinct estimation of the lentic-lotic character (Buffagni 2004), as far as the reach scale is considered. Also, it has to be noted that LRD and HMS, both being descriptors of hydromorphological aspects and relevant in determining a part of the observed metrics variation, can be somewhat correlated, because morphology can act directly and/or indirectly on local hydraulic conditions. Nevertheless, the two descriptors considered are actually focused on two different scales, HMS summarizing the whole site condition (i.e., instream, bankface and banktop), while the LRD is strictly an in-stream descriptor.

Tab. 6. Lentic-lotic character of river sites as a confounding factor for ecological status assignment.

	The Alps	Central Mountain rivers	Mediterr. Mountain rivers	Lowland streams
Lentic-lotic character as a confounding factor	Yes, important	Yes, important	Yes, very important	No evidence
Habitat-scale interpretation	Flow types very diversified over space and time; obvious riffle(step)/pool sequence; prevalence of lotic or very lotic conditions	Flow types diversified over space and time; obvious riffle/pool sequence; very lotic and intermediate conditions potentially present	Flow types very diversified over space and time; obvious riffle/pool sequence; very lotic and very lentic conditions potentially present	Homogeneous flow and weak riffle/pool sequence; prevalence of intermediate to lentic conditions
Effect on invertebrate community	Considerable part of community variance due to lentic-lotic variations and flow type diversification	Considerable part of community variance due to lentic-lotic variations and flow type diversification	Most part of community variance due to lentic-lotic variations and flow type diversification	Low community variance induced by lentic-lotic variation or flow type diversification
Other relevant factors acting on local hydraulics and assessment	Flushing flows and flooding events as main confounding factors	Flushing flows and flooding events; very low flow periods, only partly stated by lentic-lotic character	Flushing flows and flooding events; dry or very low flow periods, only partly stated by lentic-lotic character	-
Relation to organic pollution/morphological impairment	Morphological impairment and lentic-lotic character most important factors; organic pollution less important	Organic pollution most important factor; morphological impairment and lentic-lotic character often important as well	Lentic-lotic character more important than pollution and habitat degradation for invertebrates	Organic pollution most important factor; morphological impairment always important as well
Consequences on ecological quality assessment	Corrections to assessment systems certainly needed	Corrections to assessment systems certainly needed	Corrections to assessment systems unquestionably needed	Traditional approaches ok; no corrections for lentic-lotic needed
Potential integration to assessment methods (examples)	Riffle or pool specific sampling; habitat-specific sampling (e.g. only selected microhabitats); correction of biological metrics by flow type, turbulence or velocity assessed where sample units are collected; use of general regression models between lentic-lotic character and biological response; strict stream type-specific approach and type definition accounting for lentic-lotic character; actions above combined			-
Notes	-	-	For temporary rivers, not included in the present paper, an even larger influence of lentic-lotic character is expected	Data to be confirmed with larger datasets

When considering the lentic-lotic character of river sites as a misleading factor in the assessment of the ecological quality of European rivers, the situation is summarized in table 6.

4.4. Potential integration to existing assessment methods

From the experience gained in the study of European rivers, it seems reasonable to conclude that current monitoring methods should be improved and take into account flow-related conditions found at sites. Different strategies could be followed in order to refine assessment methods and/or systems (Tab. 6), most of which are presently in a revision phase to make them more suitable for WFD requirements. Firstly, in order to reduce the variability linked to local hydraulic features and to improve site comparability, the lentic-lotic character of sites should be taken into account when defining stream typologies and attributing river reaches to types (Buffagni *et al*. 2006; Erba *et al*. 2006). The assessment of ecological quality and status assignment in stream types with depleted internal lentic-lotic variability should reduce the jeopardy of misinterpretation of biological metric variation. Adaptation of sampling protocols might also be undertaken to reduce the influence of lentic-lotic variability on the invertebrate samples collected. This can be obtained, for instance, by positioning sample units in pool or riffle areas only (e.g., Brabec *et al*. 2004; Buffagni *et al*. 2004; Buffagni & Erba 2007), thus limiting the range of flow types covered by the integrative site sample used for the quality classification. To further increase sample uniformity in terms of flow types, micro- or meso-habitat specific samples might be assumed as the basis for metrics calculation (Buffagni *et al*. 2000; Dallas 2007). Alternatively, a correction of biological metrics by flow type, turbulence or velocity, assessed where single sample units are collected can be easily performed. It is worth mentioning, that in some European countries, especially in the UK, the visual assessment of flow types (Padmore 1997) is a routine part of the habitat and hydromorphological assessment of rivers at a National scale (e.g., Raven *et al*. 1998). An important advantage of identifying flow types i.e. compared to velocity measurements or turbulence estimation, is a shorter time frame, the non-obligation to use instruments in the field and its scientific soundness (e.g., Padmore 1997). The same information can be otherwise used to define for example, general regression models between lentic-lotic character and biological response in defined river types, so that an overall correction can be obtained at the site scale. In addition, the development of dedicated, type-specific multi-metric systems for the assessment of ecological quality, which include metrics devoted to quantifying the response or possibly indifferent to local hydraulics, can be contemplated (e.g., Morais *et al*. 2004; Pinto *et al*. 2004). All actions listed above, can be profitably combined to give a better account of the local peculiarity of stream systems.

In general terms, from the data analyzed in this paper, we can conclude that the lentic-lotic character of rivers should be taken into account in the assessment of ecological quality. In fact, even if local methods e.g. stream type adapted or stressor specific, might be less affected – also because they are often based on more in-depth taxonomic identification than Family – the disturbance due to the lentic-lotic character of the river still greatly concerns ecological status assignment. Townsend & Riley (1999) demanded further scientific investigation to define if current indices are robust enough with respect to detecting real changes in river health and avoiding the incorrect indication of changes. Thus, special attention should be given to quantifying the natural – and/or anthropic - range of local hydraulic conditions, so that the influence of the lentic-lotic character of streams on quality evaluation can be correctly estimated.

ACKNOWLEDGEMENTS

Authors would like to thank all AQEM and STAR partners for the use of the data. We thank members of CNR-IRSA staff who contributed to invertebrate sampling and identification, CARAVAGGIO or RHS application and chemical analysis. Also, many thanks to Jan Turvey for editing the English text. The EU research projects AQEM and STAR were co-funded by the European Commission, 5th Framework Program, Energy, Environment and Sustainable Development, Key Action Water, Contract no. EVK1-CT1999-00027 and Contract no. EVK1-CT2001-00089, respectively. The present paper is an outcome of the REBECCA project, that was co-funded by the European Commission, 6th Framework Programme, Contract No.: SSP1-CT-2003-502158.

REFERENCES

AQEM Consortium. 2002. *Manual for the application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive*. Version 1.0, February 2002.

Armitage, P.D., D. Moss, J.F. Wright & M.F. Furse. 1983. The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water Res*., 17: 333-347.

Boulton, A.J. 2003. Parallels and contrasts in the effects of drought on stream macroinvertebrate assemblages. *Freshwat. Biol*., 48: 1173-1185.

Boulton, A.J. & P.S. Lake. 1990. The ecology of two intermittent streams in Victoria, Australia. I. Multivariate analyses of physicochemical features. *Freshwat. Biol*. 24: 123-141.

Brabec, K., S. Zahrádková, D. Nějcová, P. Páril, J. Kokeš & J. Jarkovskỳ. 2004. Assessment of organic pollution effect considering differences between lotic and lentic stream habitats. *Hydrobiologia*, 516: 331-346.

Brooks, A. 1990. *Channelized Rivers Perspectives for Environmental Management*. Wiley, Chichester.

Brunke, M. 2002. Floodplains of a regulated southern alpine river (Brenno, Switzerland): ecological assessment and conservation options. *Aquat. Conserv*., 12: 583–599.

Buffagni, A. (Ed.) 2004. *Classificazione ecologica e carattere lentico-lotico in fiumi mediterranei*. Quad. Ist. Ric. Acque 122 Roma (IRSA): 175pp.

Buffagni, A. & J.L. Kemp. 2002. Looking beyond the shores of the United Kingdom: addenda for the application of River Habitat Survey in South European rivers. *J. Limnol*., 61: 199-214.

Buffagni, A. & C. Belfiore. 2006. ICMeasy 1.2: A Software for the Intercalibration Common Metrics and Index easy calculation. User Guide. *IRSA-CNR Notiziario dei Metodi Analitici*, Marzo 2007 (1): 101-114.

Buffagni, A. & M. Furse. 2006. Intercalibration and comparison – major results and conclusions from the STAR project. *Hydrobiologia*, 566: 357-364.

Buffagni, A. & S. Erba. 2007. Macroinvertebrati acquatici e Direttiva 2000/60/EC (WFD) - Parte A. Metodo di campionamento per i fiumi guadabili. *IRSA-CNR Notiziario dei Metodi Analitici*, Dicembre 2007 (1): 2-27.

Buffagni, A., G.A. Crosa, D.M. Harper & J.L. Kemp. 2000. Using macroinvertebrate species assemblages to identify river channel habitat units: an application of the functional habitats concept to a large, unpolluted Italian river (River Ticino, northern Italy). *Hydrobiologia*, 435: 213-225.

Buffagni, A., S. Erba, M. Cazzola & J.L. Kemp. 2004. The AQEM multimetric system for the southern Italian Apennines: assessing the impact of water quality and habitat degradation on pool macroinvertebrates in Mediterranean rivers. *Hydrobiologia*, 516: 313-329.

Buffagni, A., M. Ciampittiello & S. Erba. 2005. Il rilevamento idromorfologico e degli habitat fluviali nel contesto della Direttiva Europea sulle Acque (WFD): principi e schede di applicazione del metodo CARAVAGGIO. *IRSA-CNR Notiziario dei Metodi Analitici*, Dicembre 2005 (2): 32-46.

Buffagni, A., M. Munafò, F. Tornatore, I. Bonamini, A. Didomenicantonio, L. Mancini, A. Martinelli, G. Scanu & C. Sollazzo. 2006. Elementi di base per la definizione di una tipologia per i fiumi italiani in applicazione della Direttiva 2000/60/EC. *IRSA-CNR Notiziario dei Metodi Analitici*, Dicembre 2006(1): 2-19.

Buffagni, A., S. Erba & M.T. Furse. 2007. A simple procedure to harmonize class boundaries of assessment systems at the pan-European scale. *Environ. Sci. Policy,* 10: 709-724.

Chutter FM. 1969. The distribution of some stream invertebrates in relation to current speed. *Int. Rev. Ges. Hydrobiol*., 54: 413-422.

Coimbra, C.N., M.A.S. Graça & R.M. Cortes. 1996. The effects of a basic effluent on macroinvertebrate community structure in a temporary Mediterranean river. *Environ. Pollut*., 94: 301-307.

Cotta-Ramusino, M., G. Crosa & A. Buffagni. 1991. Microhabitat preferences of benthic fauna (Ephemeroptera) in plane-springs (Fontanili). *Ass. Theor. Appl. Limnol*., 24: 1626-1628.

Cowell, B.C., A.H. Remley & D.M Lynch. 2004. Seasonal changes in the distribution and abundance of benthic invertebrates in six headwater streams in central Florida. *Hydrobiologia*, 522: 99-115.

Cummins, K.W. 1962. An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. *Am. Midl. Nat*., 67: 477-504.

Cummins, K.W. & G.H. Lauff. 1969. The influence of substrate particle size on the microdistribution of stream macrobenthos. *Hydrobiologia*, 34: 145-181.

Dallas, H.F. 2007. The influence of biotope availability on macroinvertebrate assemblages in South African rivers: implications for aquatic bioassessment. *Freshwat. Biol*., 52: 370-380.

D.Lgs. 152/99. 1999. Decreto Legislativo 11 maggio 1999, n. 152. Disposizioni sulla tutela delle acque dall'inquinamento e recepimento della direttiva 91/271/CEE concernente il trattamento delle acque reflue urbane e della direttiva 91/676/CEE relativa alla protezione delle acque dall'inquinamento provocato dai nitrati provenienti da fonti agricole. *Suppl. Ord. n. 101/L alla Gazzetta Ufficiale 29 maggio 1999, no. 124*.

Dolédec, S., N. Lamouroux, U. Fuchs & S. Mérigoux. 2007. Modelling the hydraulic preferences of benthic macroinvertebrates in small European streams. *Freshwat. Biol*., 52: 145-164.

EC/2000/60. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities L327* (43): 1-72.

Erba, S., A. Buffagni, N. Holmes, M. O'Hare, P. Scarlett & A. Stenico. 2006. Testing River Habitat Survey features for the aims of the WFD hydro-morphological assessment: an overview from the STAR Project. *Hydrobiologia*, 566: 281-296.

Erba, S., M. Cazzola, D. Di Pasquale & A. Buffagni. 2007. Analysis of the response of invertebrate Intercalibration Common Metrics and STAR_ICMi to organic pollution. *REBECCA Final conference, Book of Abstract*, 48-50.

Eriksson, L., J.L.M. Hermen, E. Johansson, H.J.M. Verhaar & S. Wold. 1995. Multivariate Analysis of Aquatic Toxicity Data with PLS. *Aquat. Sci*. 57: 217-241.

Eriksson, L., E. Johansson, N. Kettaneh-Wold N. & S.Wold. 1999. *Introduction to Multi- and Megavariate Data Analysis Using Projection Methods (PCA & PLS)*. Umetrics AB, Umeå, Sweden: 490 pp.

Extence, C.A. & A.J.D. Ferguson. 1989. Aquatic invertebrate surveys as a water quality management tool in the Anglian Water region. *Regul. River*., 4: 139-146.

Extence, C.A., D.M. Balbi & R.P. Chadd. 1999. River flow indexing using benthic macroinvertebrates: a framework for setting hydrobiological objectives. *Regul. River*., 15: 543-574.

Finn, D.S. & N.L. Poff. 2005. Variability and convergence in benthic communities along the longitudinal gradients of four physically similar Rocky Mountain streams. *Freshwat. Biol*., 50: 243-261.

Füreder, L., C. Vacha, K. Amprosi, S. Bühler, C.M.E. Hansen, C. Moritz. 2002. Reference conditions of alpine streams: physical habitat and ecology. *Water Air Soil Poll: Focus*, 2: 275-294.

Furse, M.T., D. Hering, O. Moog, P.F.M. Verdonschot, L. Sandin, K. Brabec, K. Gritzalis, A. Buffagni, P. Pinto, N. Friberg, J. Murray-Bligh, J. Kokes, R. Alber, P. Usseglio-Polatera, P, Haase, R. Sweeting, B. Bis, K. Szoszkiewicz, H. Soszka, G. Springe, F. Sporka & I. Krno. 2006. The STAR project: context, objectives and approaches. *Hydrobiologia*, 566: 3-29.

Gasith, A. & V.H. Resh. 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annu. Rev. Ecol. Syst*., 30: 51-81.

Gauchi, J.P. & P. Chagnon. 2001. Comparison of selection methods of explanatory variables in PLS regression with application to manufacturing process data. *Chemometr. Intell. Lab*., 58: 171–193.

Graça, M.A.S. & C.N. Coimbra. 1998. The elaboration of indices to assess biological water quality. A case study. *Water Res*., 32: 380-392.

Hering, D., O. Moog, L. Sandin & P.F.M. Verdonschot. 2004. Overview and application of the AQEM assessment system. *Hydrobiologia*, 516, 1–20.

Horton, P., B. Schaefli, A. Mezghani, B. Hingray & A. Musy. 2006. Assessment of climate-change impacts on alpine discharge regimes with climate model uncertainty. *Hydrol. Processes*., 20: 2091-2109.

Hynes, H.B.N. 1970. *The ecology of running waters*. Toronto University Press.

Jakob, C., C.T. Robinson & U. Uehlinger. 2003. Longitudinal effects of experimental floods on stream benthos downstream from a large dam. *Aquatic Sciences - Research Across Boundaries*, 65: 223-231.

Lenat, D.R. 1983. Chironomid taxa richness: natural variation and use in pollution assessment. *Freshwater Invertebrate Biology*, 2: 192-198.

Lepori, F., D. Palm & B. Malmqvist. 2005. Effects of stream restoration on ecosystem functioning: detritus retentiveness and decomposition. *J. Appl. Ecol*., 42: 228-238.

Matthaei, C.D., U. Uehlinger & A. Frutiger. 1997. Response of benthic invertebrates to natural versus experimental disturbance in a Swiss prealpine river. *Freshwat. Biol*., 37: 61-77.

Milner, A., J.E. Brittain, E. Castella & G.E. Petts. 2001. Trends of macroinvertebrate community structure in glacierfed rivers in relation to environmental conditions: a synthesis. *Freshwat. Biol*., 46: 1833-1847.

Monk, W.A., P.J. Wood, D.M. Hannah, D.A. Wilson, C.A. Extence & R.P. Chadd. 2006. Flow variability and macroinvetebrate community response within riverine systems. *River Res. Appl*., 22: 595-615.

Morais, M., P. Pinto, P. Guilherme, J. Rosado & I. Antunes. 2004. Assessment of temporary streams: the robustness of metric and multimetric indices under different hydrological conditions. *Hydrobiologia*, 516: 229-249.

Muhar, S., M. Schwarz, S. Schmutz & M. Jungwirth. 2000. Identification of Rivers with High and Good Quality: Methodological Approach and Applications in Austria. *Hydrobiologia*, 422/423: 343-358.

Padmore, C.L. 1997. Biotopes and their hydraulics: a method for defining the physical component of freshwater quality. In: P.J. Boon & D.L. Howell (Eds), *Freshwater Quality: defining the indefinable?* SNH, Edinburgh: 251-257.

Pinto, P., J. Rosado, M. Morais & I. Antunes. 2004. Assessment methodology for Southern siliceous basins in Portugal. *Hydrobiologia*, 516: 191-214.

Prat, N. & A. Munnè. 2000. Water use and quality and stream flow in a Mediterranean stream. *Water Res*., 34, 3876-3881.

Raven, P.J., T.H. Holmes, F.H. Dawson, P.J.A. Fox, M. Everard, I.R. Fozzard & K.J. Rouen. 1998. *River Habitat Survey, the physical character of rivers and streams in the UK and Isle of man*. River Habitat Survey Report No.2, May 1998. The Environment Agency, Bristol: 84 pp.

Roy, A.H., A.D. Rosemond, M.J. Paul, D.S. Leigh & J.B. Wallace. 2003. Stream macroinvertebrate response to catchment urbanisation (Georgia, U.S.A.). *Freshwat. Biol*., 48: 329-346.

Shannon, C.E. 1948. A Mathematical Theory of Communication. *The Bell System Technical Journal*, 27: 623-656.

Sheldon, F. 2005. Incorporating Natural Variability into the Assessment of Ecological Health in Australian Dryland Rivers. *Hydrobiologia*, 552: 45-56.
StatSoft Inc. 2004. *STATISTICA (data analysis software system)*. Version 5.0. www.statsoft.com.
Statzner, B., J.A. Gore & V.H. Resh. 1988. Hydraulic stream ecology: observed patterns and potentional applications. *J. N. Am. Benthol. Soc.*, 7: 307-360.
Szoszkiewicz, K., A. Buffagni, J. Davy-Bowker, J. Lesny, B.H. Chojnicki, J. Zbierska, R. Staniszewski & T. Zgola. 2006. Occurrence and variability of River Habitat Survey features across Europe and the consequences for data collection and evaluation. *Hydrobiologia*, 566: 267-280.
Townsend, C.R. & R. Riley. 1999. Assessment of river integrity: Accounting for perturbation pathways in physical and ecological space. *Freshwat. Biol.*, 41: 393-406.
Umetri, 2005. *SIMCA-P 11.0 manual*. Umetrics, UMEA, Sweden.
Verdonschot, P.F.M. 2000. Integrated ecological assessment methods as a basis for sustainable catchment management. *Hydrobiologia*, 422/423: 389-412.
Verdonschot, P.F.M. 2006. Evaluation of the use of Water Framework Directive typology descriptors, reference sites, and spatial scale in macroinvertebrate stream typology. *Hydrobiologia*, 566: 39-58.
Yao, N., J.D. Floros & K. Seetharaman. 2004. Identification of important production variables affecting hard pretzel quality. *J. Food Qual*.: 222-244.
Ward, J.V. & J.A. Wiens. 2001. Ecotones of riverine ecosystems: Role and typology, spatio-temporal dynamics, and river regulation. *Ecohydrology and Hydrobiology*, 1: 25-36.
Wold, S. 1978. Cross-Validatory Estimation of the Number of Components in Factor and Principal Components Models. *Technometrics*, 20: 397-405.
Wold, S. 1995. PLS for multivariate linear modelling. QSAR.In: H. van de Waterbeemd (Ed.), *Chemometric Methods in Molecular Design Methods and Principles in Medicinal Chemistry*. Vol. 2. Verlag Chemie, Weinheim, Germany: 195-218.
Zamora-Muñoz, C., C.E. Sáinz-Cantero, A. Sanchez-Ortega & J. Alba-Tercedor. 1995. Are biological indices BMPW' and ASPT' and their significance regarding water quality seasonally dependent? Factors explaining their variations. *Water Res*., 29: 285-290.

Received: June 2008
Accepted: September 2008