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Assessment methodology for southern siliceous basins in Portugal

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Abstract

A sampling programme was developed in three stream types, of siliceous geology, from the south of Portugal (small and mid-sized lowland streams and small-sized median altitude streams). The samples were taken according to the AQEM site protocol procedure, keeping transport and depositional habitats samples separated. In each stream type, at least 13 sites were studied over a gradient of organic pollution (pre-classification). The benthic macroinvertebrates were identified to the lowest possible taxonomic level. A Detrended Correspondence Analysis of macroinvertebrate communities identified a gradient of organic pollution strongly related to the first axis. This ordination allowed the establishment of classes of organic pollution using the Kmeans software (post-classification). Metrics based on the macroinvertebrate communities (tolerance, richness, composition and trophic structure) were computed and tested for correlation with the gradient of organic pollution (first axis of DCA). Most of the selected metrics were able to discriminate the four quality classes (high, good, moderate and poor) of ecological status. A multimetric index, integrating ASPT′ index, Trichoptera families and percentage of Gasteropoda, Oligochaeta and Diptera, is proposed to assess the ecological status of Portuguese southern siliceous basins.

Introduction

Benthic macroinvertebrates have been used since the beginning of the last century as an important tool to evaluate the water quality of lotic ecosystems (Kolkwitz & Marsson, 1908). Generally, the biotic indices were based on the *taxa* tolerances to organic pollution (Alba Tercedor & Sanchez Ortega, 1988; Wright et al., 1993) and water quality assessment was only based on pollution level. However, a new concept of water monitoring was recently introduced according to the American experience (Barbour et al, 1996, 1998; Cairns, 2002; Reynolds, 1997), as a frame in the implementation of the Water Framework Directive in Europe (2000/60/CE). In this new concept, the target is ecological quality (based on the ecosystem), rather than water quality (based on human use). A special research effort must be made in order to define the reference situations for each stream type and in order to compare and classify the impaired sites according to deviations from reference. More local research must be undertaken to identify specific constraints affecting aquatic ecosystems from the different water bodies.

The hydrological regime is one of the principal constraints for biotic communities in the Portuguese southern streams as it is in most Mediterranean regions (Puig et al., 1991; Stanley et al., 1994; Bernardo & Alves, 1999; Gasith & Resh, 1999), influencing stream intermittency. In temporary streams, the summer superficial flow interruption is probably one of the most important environmental factors, affecting the structure of biological communities, inducing specific physiological and behavioural survival strategies (Deluchi, 1989; Gasith & Resh, 1999). The hyporheic zone is a permanent refuge for invertebrates (Williams, 1984; Delucchi, 1989). Boulton (1989) suggested that invertebrates develop life-cycle adaptation strategies, in order to survive under extreme summer conditions. During winter and spring, the unpredicted flood events are responsible for accentuated decrease in the diversity and abundance of stream assemblages (Resh et al., 1988). The specificity of those temporary streams needs the development of new assessment methodologies at a local scale. Concerning the Iberian Peninsula, the BMWP′ (Alba Tercedor & Sanchez Ortega, 1988) has been widely used as a

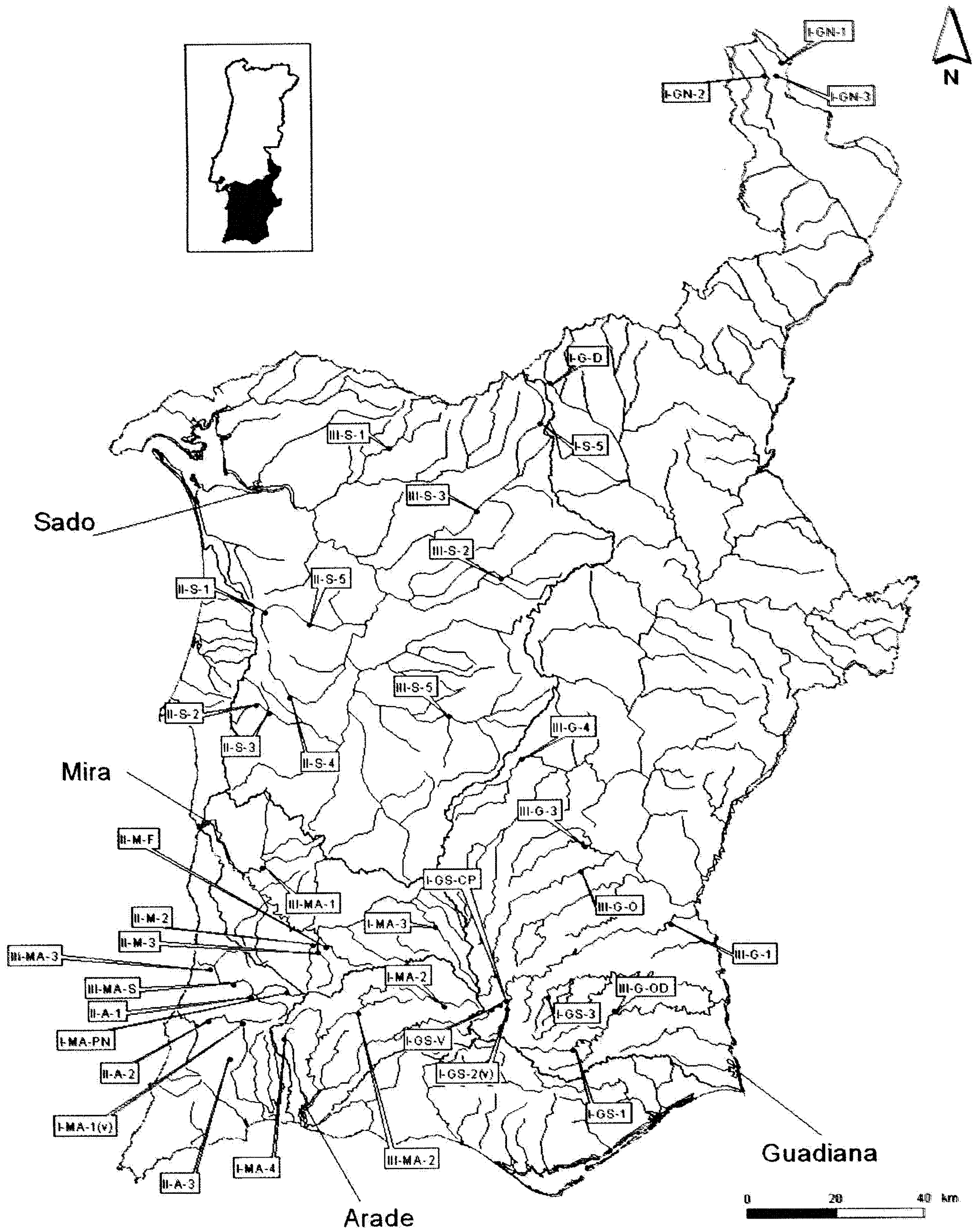

Figure 1. Localization of sampling sites.

common methodology to evaluate the biological water quality (Cortes et al., 2002). This metric is assumed to account for different tolerances of invertebrates to pollution (tolerance metric), but it does not measure other attributes of communities, such as species composition, richness and ecological preferences. The metric gives a score aimed at defining quality classes in the Iberian Peninsula, but these quality classes are not calculated in relation to reference situations of stream types according to the Water Framework Directive. Recently, Graça & Coimbra (1998) developed a methodology to evaluate the impact of mining activities in the south of Portugal. This study is also based on *taxa* tolerances.

Organic pollution represents the main impact pressure on southern Portuguese streams, with agriculture and cattle grazing as principal causes of pollution. This study aims to develop a multimetric approach to evaluate organic pollution in southern siliceous basins in Portugal. Three types of streams were selected based on system A typology of the Water Framework Directive (2000/60/CE) and, within each stream type, at least 13 sites were selected, according to the gradient of organic pollution.

Study area

In total, 39 collecting sites were established for the whole siliceous basins in South Portugal (Fig. 1) according to system A typology defined by the Water Framework Directive (2000/60/CE). Small median altitude basins (altitude between 200 and 800 m; drainage area up to 100 km^2), small lowland basins (altitude up to 200 m; drainage area up to 100 km^2) and medium lowland basins (altitude up to 200 m; drainage area between 100 and 1000 km^2) were selected for this study. For each stream type, at least 13 sites were established in relation to the existing organic pollution gradient: 3 of high quality; 3 of good quality; 3 of moderate quality; 2 of poor quality and 2 of bad quality. Due to the absence of historical monitoring data to support site selection, this was done by expert evaluation in the field (pre-classification). Whenever possible, replicas of each quality class were located in different basins. This procedure was adopted in order to take into account variability among basins, preventing data bias. From the 39 sampling sites, three were excluded: III-S-2 due to the absence of superficial flow during the sampling period; III-MA-2 disturbed by sand abstraction; III-GO disturbed by copper mining activities.

Methodology

Streams were sampled in spring 2000. During the sampling period, significant rainfall episodes occurred, disturbing the ecosystems (flood events of different magnitude). During flood events samples were stopped and sites were visited only two or three weeks after, when the system had recovered. The objective of this procedure was to reduce the effect of flood disturbances, preventing possible errors in assessment.

Water temperature, current velocity, pH, conductivity and dissolved oxygen were measured in the field with appropriate probes. Five litres of water were sampled and preserved in cold containers to analyse in the laboratory the following parameters: alkalinity, total hardness, chloride, biological oxygen demand, ammonium, nitrite, nitrate, total phosphate, and chlorophyll *a*. All these parameters were analysed according to Portuguese law, based on the American Public Health Association (1998).

A multihabitat procedure developed by AQEM consortium (2002) was adopted at each site to sample benthic macroinvertebrates. A total of 20 Surber samples (25 cm square side with a mesh size of 0.5 mm) were taken, in a reach of 100 m, covering different habitats. Surber numbers were sampled in a proportional number to each habitat area. Habitats represented by less than 5% of the total area were excluded. The samples were fixed in the field with alcohol 96%. Samples were kept separated per depositional and transport habitats.

In the laboratory, samples were sieved with two mesh sizes (1 mm and 0.25 mm). The coarse fraction (>1 mm) was completely sorted, and all the organisms preserved in alcohol 70%. The fine fraction (0.25–1.00 mm) was subsampled: successive known volumes of sample were extracted and sorted. This procedure stopped either when 500 organisms were counted or when relative frequencies of families tended to be stable. Whenever possible, sorted organisms were identified to the species level. In the absence of taxonomic keys for southern Iberian Peninsula fauna, an intermediated level of identification was attained (sub-family, genus or group). Prior to any treatment, a taxonomic adjustment was made according to the abundances of each taxonomical level and their ecological information (AQEM consortium, 2002). The objective of this procedure is to avoid the inclusion of taxa in different taxonomical levels. To prevent distortions on the multivariate analysis, caused by the most abundant *taxa*, the macroinvertebrate abundances were converted into 9 Preston classes.

To validate and correct pre-classification (study area section) multivariate techniques were performed. The aim was to identify the organic pollution gradient (ordination) and to establish new quality classes based only on macroinvertebrate communities (classification). A Detrended Correspondence Analysis (DCA) was done to prevent the arch effect. The identification of ecological gradients (first and second axes) along axes was made by Pearson correlations between environmental parameters and ordination coordinates.

The Kmeans 2 software (Legendre & Legendre, 1998) was used to perform a non hierarchical classification of sites in relation to their location along the organic pollution gradient (first DCA axis coordinates). The pseudo-F-statistic (Calinski-Harabasz, 1974 in Legendre & Legendre, 1998) was computed in order to evaluate the most suitable number of groups. This number was converted into no more than 5 quality classes. The new sites classification (post-classification) represents the starting point for developing the multimetric assessment tool.

Macroinvertebrate communities, at each site, were evaluated by a set of metrics: tolerance to organic pollution, richness, composition and trophic structure. Those metrics were computed by using the Atic software (AQEM consortium, 2002). Other metrics, based on the data structure of the macroinvertebrate community were also tested (Table 1). Pearson correlation and linear regressions between the pollution gradient defined by the first axis (independent variable for linear regression) and the studied metrics and indices (dependent variable for linear regression) were done in order to evaluated how metrics and indices explain the gradient detected by the first DCA axis. Selection of the most suitable metrics was made in four steps: in the first step, metrics with no significant correlation with the first DCA axis were excluded; in the second step, those where R^2 of linear regression with the first axis of the DCA was lower than 0.5, wererejected; in the third step, performing Box Cox graphics by the SPSS software (version 11.0), those which had power to discriminate all the quality classes were selected; in the last step were selected only those that, at least, allow discrimination between good and moderate classes. This last step was only adopted when no metric was selected by the previous step. In Box Cox graphic analysis, it was considered a good discrimination power when the percentile 25 of the upper class is not superimposed with the percentile 50 of the next class.

All the selected metrics were reduced to a range of variation from 0 to 1 (0 the worst score; 1 the best score) using the expression:

$$M' = \frac{M - \min M}{\max M - \min M},$$

M, raw metric value; min M, minimum metric value; max M, maximum metric value; M', metric value between 0 and 1.

When it appeared to be impossible to predict the maximum score of a given metric, such as EPT (Ephemeroptera, Plecoptera, Trichoptera taxa) and EPTO (Ephemeroptera, Plecoptera, Trichoptera, Odonata taxa), 110% of the highest score obtained was assumed as the maximum value. Concerning TRICF (number of Trichoptera families), the maximum possible score is 21, corresponding to the number of the existing Trichoptera families in the ecoregion 1 (Vieira Lanero, 2000). GOLD (percentage of Gasteropoda, Oligochaeta, Diptera) increases with the organic pollution in opposition to other metrics. To invert this pattern, GOLD was subtracted from one. Multimetric indices were developed combining

Table 1. New metrics tested. The development of these metrics was done according to the structure of the sampled macroinvertebrate communities.

Metric category	Short code	Description
Richness	PT	Number of Plecoptera and Trichoptera *taxa*
	EPTO	Number of Plecoptera, Ephemeroptera, Trichoptera and Odonata *taxa*
	PLECF	Number of Plecoptera families
	TRICF	Number of Trichoptera families
Composition	S1+2	Percentage of individuals with BMWP′ scores of 1 and 2
	S10+8	Percentage of individuals with BMWP′ scores of 10 and 8
	S10	Percentage of individuals with BMWP′ score of 10
	GOLD	Percentage of Gasteropoda, Oligochaeta and Diptera
Trophic composition	%pred.	Percentage of predators
	pred/T′	Ratio between predators anal the other trophic groups
	%fit	Percentage of phytofagous
	%she	Percentage of shredders
	%col	Percentage of collectors
	%fit/col	Ratio between the percentage of phytofagous and percentage of collectors

metrics of different categories (tolerance, richness and composition). The values of multimetric indices result from the mean of respective metrics. A similar procedure adopted for the metric selection was used to choose the most suitable multimetric indices.

Boundaries between quality classes were established in Box Cox graphics exploratory analysis, using as criterion the percentile 25.

The most suitable multimetric index was developed, based on total community (course fraction and fine fraction for both depositional and transport habitats). The resulting multimetric classification was applied to the total community, total communities of transport and deposition habitats separately, and the community present only in the coarse fraction (higher than 1 mm).

Sites were classified by the selected multimetric index (multimetric classification). Differences between multimetric scores of consecutive quality classes (both for post-classification and multimetric classification)

Table 2. Physical and chemical parameters of the water.

Site	Ammonium (mg l^{-1})	Nitrite (mg l^{-1})	Nitrate (mg l^{-1})	Total phosphate (µg l^{-1})	Oxygen saturation (%)	Chlorophyll (µg l^{-1})	pH-value
I-GN-1	0.005	0.001	2.320	25	95	–	7
I-GN-2	0.005	0.001	2.080	25	86	–	7.1
I-GN-3	0.02	0.001	5.470	25	84	–	7.3
I-MA-I	0.005	0.020	2.500	25	79	0.370	7
I-MA-2	0.02	0.002	0.003	25	72	0.570	7.2
I-MA-3	0.04	0.001	0.003	25	80	0.280	7.7
I-MA-PN	0.005	0.001	0.003	25	87	0.430	7
I-GS-1	0.005	0.003	0.003	–	96	0.430	8.2
I-GS-2	0.16	0.001	0.003	25	98	0.280	7.8
I-GS-3	0.12	0.001	0.003	25	93	0.210	8.1
I-GS-V	0.23	0.024	0.003	25	69	0.380	7.3
I-GS-CP	0.08	0.001	0.003	25	71	0.640	7.6
I-MA-4	1.45	0.231	11.060	1910	82	8.820	7.9
I-G-D	0.21	0.004	0.960	280	52	5.200	7.4
I-S-5	0.71	0.175	1.120	1930	13	1.560	7.6
II-S-1	0.005	0.005	1.710	25	105	0.430	8.4
II-S-2	0.005	0.121	23.130	990	74	8.540	7.7
II-S-3	0.02	0.001	4.660	25	87	1.710	7.9
II-M-2	0.02	0.002	0.003	80	91	0.250	7.3
II-M-F	0.005	0.002	0.003	25	100	0.270	7.6
II-M-3	0.005	0.002	0.003	25	91	0.660	7.5
II-A-1	0.005	0.003	0.370	25	89	0.210	7.8
II-A-2	0.03	0.003	2.270	25	96	0.320	7.4
II-A-3	0.005	0.004	0.030	1200	84	0.430	7.2
II-S-4	0.14	0.001	0.003	25	86	4.780	7.5
II-S-5	0.12	0.001	0.300	25	88	2.850	7.9
III-S-1	0.15	0.012	1.390	60	74	4.870	7.9
III-S-3	0.24	0.088	4.930	3990	89	15.930	8.2
III-MA-1	0.04	0.006	0.003	110	101	0.430	7.8
III-MA-3	0.07	0.005	1.100	50	78	0.130	7.2
III-MA-S	0.08	0.001	0.003	25	78	0.210	6.7
III-G-1	0.1	0.001	0.003	25	110	1.140	8.5
III-G-3	0.15	0.011	0.003	50	100	9.740	7.9
III-G-OD	0.15	0.001	0.003	25	79	0.270	7.7
III-G-4	0.02	0.006	0.910	25	62	8.980	8.3
III-S-5	0.06	0.031	2.640	160	62	4.270	7.9

Continued on p. 196

Table 2. Continued.

Site	Conductivity (μS cm^{-1})	Alkalinity (mmol l^{-1})	Total hard -ness (mmol l^{-1})	Chloride (mg l^{-1})	BOD$_5$ (mg l^{-1})	Mean depth (cm)	Mean current velocity (m s^{-1})
I-GN-1	120	0.85	0.60	4.71	3.5	19.30	0.418
I-GN-2	60	0.42	0.29	5.71	3.0	19.05	0.613
I-GN-3	70	0.39	0.28	6.70	3.0	9.10	0.318
I-MA-I	5.5	0.27	0.40	16.63	3.3	19.85	0.591
I-MA-2	200	0.47	0.53	25.31	2.4	30.15	0.259
I-MA-3	220	0.93	0.64	22.58	4.4	20.60	0.281
I-MA-PN	136	0.34	0.14	19.60	0.0	15.15	0.444
I-GS-1	200	0.54	0.62	22.08	6.9	19.50	0.273
I-GS-2	180	0.66	0.53	22.57	6.3	14.90	0.324
I-GS-3	140	0.74	0.49	17.20	8.4	13.65	0.157
I-GS-V	165	0.40	0.38	30.28	1.0	23.10	0.099
I-GS-CP	166	0.23	0.62	28.29	1.0	22.05	0.277
I-MA-4	330	1.11	0.82	34.49	3.5	10.85	0.418
I-G-D	530	3.14	1.70	40.94	1.8	35.75	0.026
I-S-5	900	4.45	3.08	89.83	5.0	41.25	0.202
II-S-1	340	1.56	0.91	38.71	1.6	31.00	0.149
II-S-2	660	1.84	1.60	72.21	14.6	21.80	0.155
II-S-3	660	1.16	1.45	111.42	5.6	56.10	0.180
II-M-2	610	0.80	1.57	79.41	6.0	47.40	0.122
II-M-F	590	0.84	1.47	76.18	5.0	18.40	0.154
II-M-3	710	1.20	1.83	82.88	5.0	23.85	0.199
II-A-1	160	0.27	0.35	21.84	3.0	11.90	0.379
II-A-2	210	0.50	0.62	28.04	1.5	20.05	0.322
II-A-3	240	0.48	0.48	33.50	2.9	17.05	0.445
II-S-4	350	1.05	0.77	44.67	6.8	46.20	0.209
II-S-5	270	0.96	0.64	28.78	8.4	76.05	0.155
III-S-1	540	2.49	1.76	58.56	4.4	23.10	0.117
III-S-3	860	3.62	2.63	86.36	5.0	34.75	0.161
III-MA-1	430	0.86	1.15	430.00	6.0	42.65	0.298
III-MA-3	270	0.55	0.79	270.00	12.5	34.85	0.567
III-MA-S	152	0.27	0.20	27.80	1.0	29.85	0.598
III-G-1	220	0.89	0.57	27.05	9.3	31.00	0.199
III-G-3	430	1.17	0.96	80.65	8.2	6.35	0.167
III-G-OD	122	1.03	0.38	16.12	2.0	23.70	0.342
III-G-4	2180	4.07	5.74	298.28	6.0	17.60	0.094
III-S-5	1450	4.05	4.12	112.16	4.8	22.85	0.091

were tested by a *t* test for mean comparison using 95% as confidence level. For each selected metric and multimetric index, mean and standard error were computed using software STATGRAPHICS (version 7.0).

Results

Environmental factors

The physical and chemical parameters of the water are presented in Table 2. Oxygen saturation was high with all values higher than 50%, excepting for site I-S-5 (13%) where the total phosphorous was one of the highest values analysed (1930 μg l^{-1}). Accentuated conductivity values were observed in sites III-G-4 (2180 μS cm^{-1}) and III-S 5 (1450 μS cm^{-1}). Also in those sites the chloride was high (298.28 mg l^{-1} and 112.16 mg l^{-1}), indicating the effects of urban pollution due to water supply treatment. The highest ammonium value was detected at site I-MA-4 (1.45 mg l^{-1}), located 3 km downstream from a water treatment plant, presenting also accentuated values of nitrate (11.06 mg l^{-1}), total phosphorous (1910 mg l^{-1}), and chlorophyll *a*

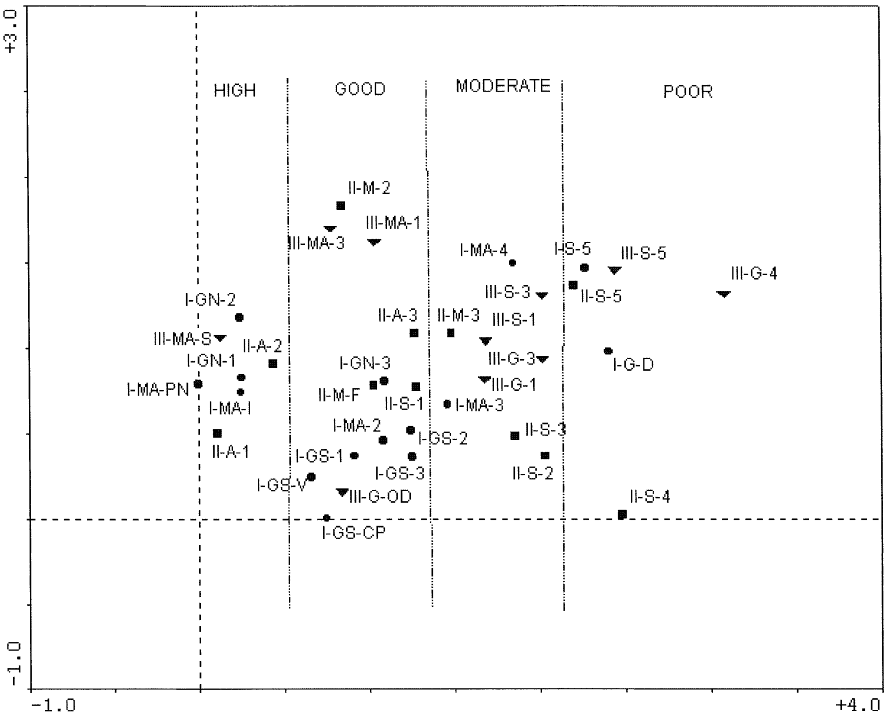

Figure 2. Ordination (DCA) of sampling sites. The vertical bars indicate the boundaries between quality classes after the post-validation. Stream type I (circles); stream type II (squares); stream type III (triangles).

(8.82 μg l^{-1}). The highest chlorophyll *a* value was observed at site III-S-3 (15.93 μg l^{-1}), which also presented the highest observed value of total phosphate (3990 mg l^{-1}). Concerning BOD5, the highest value was obtained at site II-S-2 (14.6 mg l^{-1}), which also presented accentuated values of nitrate (23.13 mg l^{-1}), total phosphate (990 mg l^{-1}), and chlorophyll *a* (8.54 μg l^{-1}). At site III-MA-3, BOD5 was high (12.5 mg l^{-1}), showing also a high chloride value (270 mg l^{-1}).

Macroinvertebrates

The DCA was based on macroinvertebrate composition and their abundances (individuals by m^2) at the 36 studied sites (Fig. 2). Pearson correlations between environmental parameters of water and first and second DCA axes are presented in Table 3. The results show that organic pollution gradient is associated with first axis. Some influence of the morphological degradation was also detected in the first axis by two variables related to riparian vegetation (shore line covered with woody riparian vegetation and average width of woody riparian vegetation: Table 3. Kmeans classification was obtained using the site coordinates in the first DCA axis (post-classification). Pseudo-F-statistic value detected 12 as the most suitable number of groups (Table 4). With such a high number of groups, it is impossible to develop an assessment methodology based on five quality classes (high, good, moderate, poor and bad). To circumvent this problem, these were converted into a lower number. The ideal number would be five. However, analysing the results of Kmeans for classifications with lower number of groups, four seems to be more consistent with 12 groups' classification boundaries (Table 4). In addition, all BMWP′ values (Appendix 1) are higher than 60 (the boundary between moderate and poor quality), which suggest that the organic pollution gradient, from high status to bad status, was not totally covered, missing the bad quality class. Boundaries of the new classification (post-classification) are represented in Figure 2 by vertical bars.

Observing Figure 2, the three stream types are present all along the same pollution gradient, with sites included in the four quality classes. In addition, sites classified as high quality class have their communities significantly correlated for $P < 0.01$ (Table 5), which suggests that the three system A types belong only to one stream type.

Table 3. Correlation between the environmental parameters and the DCA axis. The parameters without any significant correlation with the first and second axes were excluded from the table. [(*) $p < 0.05$; (**) $p < 0.01$]

		Axis 1	Axis 2
Morphology	Shoreline covered with woody riparian vegetation;	−0.45**	–
	average width of woody riparian vegetation	−0.33*	–
Mineral substrates (% of coverage)	Megalithal (>40 cm);	0.68**	–
	Mesolithal (>6 cm to <20 cm)	−0.33*	–
	Microlithal (>2 cm to <6 cm)	−0.47**	–
	Akal (>0.2 cm to 2 cm)	0.54**	–
	Psammal/psammopelal (>6 μm to <2 mm)	0.81**	–
Biotic microhabitats	Algae;	0.44**	–
	Submerged macrophytes	0.55**	–
	Emergent macrophytes	0.59**	–
Physical and chemical parameters	pH	0.58**	–
	Conductivity	0.70**	0.40*
	Alkalinity	0.75**	0.38*
	Total hardness	0.69**	0.42**
	Nitrite	0.33*	–
	BOD5	0.42**	–
	Chloride	–	0.51**
	Dissolved oxygen content	−0.36**	–
	Oxygen saturation	−0.34*	–
	Chlorophyll	0.67**	–
	Maximum current velocity	−0.55**	0.40*
	Reduction phenomena	0.61**	–

Table 4. Comparison among K Means classifications (4, 5 and 12 groups). Solid lines represent the adopted classification.

Pseudo-F-statistic	136.8	156.6	446.3
Number of K Means groups	4Gr	5Gr	12Gr
I-MA-PN	1	1	1
II-A-1	1	1	1
III-MA-S	1	1	1
I-GN-2	1	1	1
I-MA-I	1	1	1
I-GN-1	1	1	1
II-A-2	1	1	2
I-GS-V	2	2	3
I-GS-CP	2	2	3
III-MA-3	2	2	3
III-G-OD	2	2	4
II-M-2	2	2	4
I-GS-1	2	2	4
II-M-F	2	3	5
III-MA-1	2	3	5
I-MA-2	2	3	5
I-GN-3	2	3	5
I-GS-2	2	3	6
I-GS-3	2	3	6
II-A-3	2	3	6
II-S-1	2	3	6
I-MA-3	3	3	7
II-M-3	3	3	7
III-G-1	3	4	8
III-S-1	3	4	8
I-MA-4	3	4	9
II-S-3	3	4	9
III-G-3	3	4	9
III-S-3	3	4	9
II-S-2	3	4	9
II-S-5	4	5	10
I-S-5	4	5	10
I-G-D	4	5	11
III-S-5	4	5	11
II-S-4	4	5	11
III-G-4	4	5	12

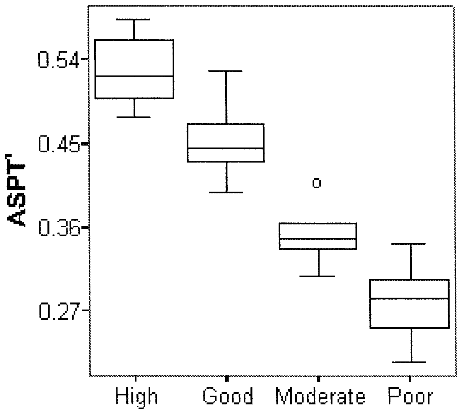

Figure 3. Box Cox graphic for the selected tolerance metric.

Metrics and indices

Pearson correlation and linear regressions between the pollution gradient, defined by the first axis (independent variable for linear regression), and the studied metrics and indices (dependent variable for linear regression) were performed in order to evaluate how metrics and indices explain the gradient detected by the first DCA axis (Table 6). Despite the relatively high number of significant correlations, only for a few metrics is the R^2 higher than 0.5 (Table 6). For these selected metrics, Box Cox graphic analyses were done and are presented in Figs 3–5. For nearly all the different type of metrics (tolerance and richness), it

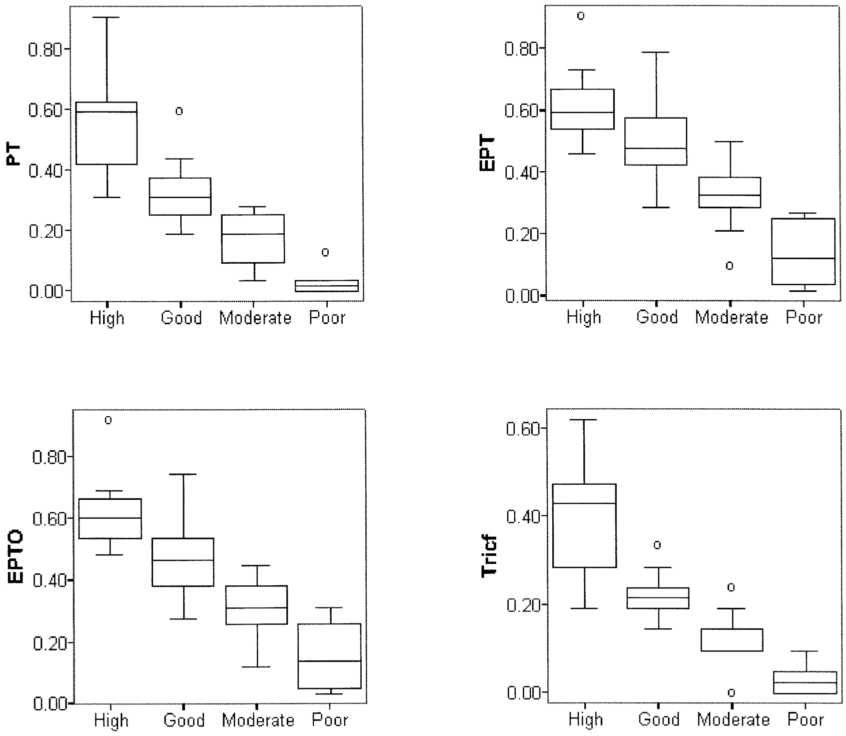

Figure 4. Box Cox graphics for selected richness metrics.

Table 5. Significant Pearson correlation coefficients ($p < 0.01$) among high quality sites (post-classification)

Sites	I-GN-1	**I-GN-2**	**I-MA-1**	**I-MA-PN**	**II-A-1**	**II-A-2**	**III-MA-S**
I-GN-1	1						
I-GN-2	0.694	1					
I-MA-1	0.610	0.528	1				
I-MA-PN	0.308	0.291	0.384	1			
II-A-1	0.442	0.316	0.579	0.635	1		
II-A-2	0.446	0.433	0.589	0.474	0.527	1	
III-MA-S	0.262	0.293	0.209	0.664	0.470	0.527	1

Table 6. Values of Pearson correlation coefficients (r) and R^2 linear regression between first DCA axis (pollution gradient) and metric scores ($^{*}p < 0.05$; $^{**}p < 0$). R^2 higher than 0.5 are in bold

	Metrics	r		R^2
Tolerance	DSI	0.15		0.02
	SI	0.44	**	0.19
	BBI	0.62	**	0.38
	IBE	0.38	*	0.15
	MAS	0.44	**	0.19
	BMWP	0.61	**	0.37
	BMWP′	0.68	**	0.47
	ASPT	0.78	**	**0.62**
	ASPT′	0.93	**	**0.87**
Richness	Ephemeroptera	0.51	**	0.26
(number of *Taxa*)	Plecoptera	0.68	**	0.47
	Trichoptera	0.69	**	0.48
	Odonata	0.33	*	0.11
	Tricf	0.75	**	**0.57**
	Gastropoda	0.20		0.04
	Oligochaeta	0.63	**	0.39
	Hirudinea	0.15		0.02
	Crustacea	0.12		0.01
	Heteroptera	0.34	*	0.11
	Coleoptera	0.16		0.02
	Diptera	0.12		0.01
	EPT	0.72	**	**0.52**
	EPTO	0.73	**	**0.53**
	PT	0.76	**	**0.57**
	EPT/Chironomidae	0.57	**	0.33
	Number of *taxa*	0.33	*	0.11
Composition	Plecoptera	0.81	**	**0.66**
(%)	Gastropoda	0.08		0.01
	Bivalvia	0.04		0.00
	Hirudinea	0.13		0.02
	Crustacea	0.08		0.01
	Odonata	0.60	**	0.36
	Heteroptera	0.15		0.02
	Coleoptera	0.55	**	0.30
	Diptera	0.42	*	0.17
	Lumbricidae	0.03		0.00
	Tubificidae	0.46	**	0.21
	Elmidae	0.51	**	0.26
	Chironomidae	0.48	**	0.24
	Trichoptera	0.52	**	0.27
	Ephemeroptera	0.52	**	0.27
	Oligochaeta	0.68	**	0.47
	Tanytarsini	0.04		0.00
	Hydropsychidae	0.13		0.02
	Hydropsychidae/Trichoptera	0.20		0.04
	Heptageniidae/Ephemeroptera	0.50	**	0.25
	Caenidae/Ephemeroptera	0.32		0.10
	GOLD	0.78	**	**0.61**
	EPT %	0.68	**	0.46
	Dominante taxa (fam.)	0.51	**	0.26
	Dominante taxa (orders)	0.52	**	0.27
Diversity	Simpson-Index	0.34	*	0.12
	Shannon–Wiener-Index	0.51	**	0.26
	Evenness	0.45	**	0.20
Trophic structure	Scrapers	0.46	**	0.21
(%)	Collectors	0.68	**	0.46
	Shredders	0.36	*	0.13
	Predators	0.47	**	0.22
	Shreders/Colectors	0.38	*	0.14
	Scrapers/Colectors	0.42	*	0.17
	Shredders/Total	0.37	*	0.14
	Collectors/Total	0.61	**	0.37
	Scrapers/Total	0.48	**	0.23
	TsP	0.48	**	0.23
	P/TsP	0.44	**	0.19

was possible to find at least one metric with power to discriminate the four quality classes (Table 7). The only exception occurs within the composition metrics, where the two selected metrics are unable to discriminate high and good status (GOLD) or moderate and poor status (Plecoptera%) (Fig. 5). GOLD was considered the best composition metric, because a decreasing linear tendency is observed along the pollution gradient.

Three multimetric indices were created by a combination of the most suitable metrics: Tolerance, Richness and Composition metrics (Table 8). Box Cox exploratory graphics for those indices, presented in Fig. 6, show that nearly all the multimetric indices generated are able to discriminate between the four quality classes (Table 7). The value of the percentile 25 of the poor status class is always higher than zero for all the multimetric indices. This fact allows the

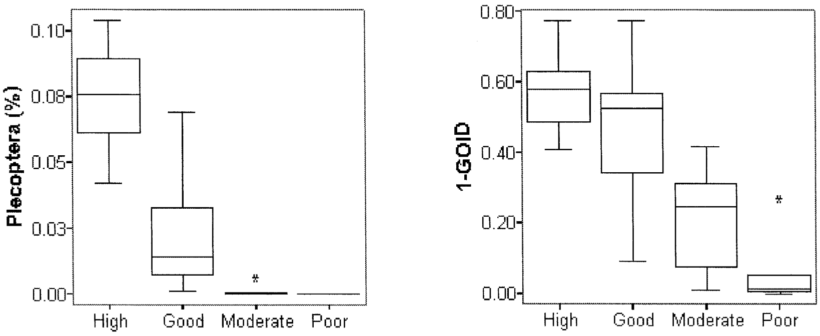

Figure 5. Box Cox graphics for selected composition metrics.

Table 7. Boundaries between quality classes adopted for selected metrics and multimetric index scores.

		Boundaries High/Good	Good/ Moderate	Moderate/Poor	Poor/Bad
Metrics					
Tolerance	ASPT′	0.50	0.43	0.34	0.25
Richness	EPT	-	0.43	0.3	0.04
	EPTO	0.54	0.38	0.25	0.05
	Tricf	0.29	0.19	0.10	0
Composition	% Plecoptera	0.06	0.01	–	–
	1-GOLD	–	0.34	0.08	0.01
Multimetric indices					
	IM7	0.54	0.40	0.27	0.11
	IM8	-	0.40	0.27	0.10
	IM9	0.48	0.34	0.22	0.10

establishment of the boundary between the poor and the bad status. A better discrimination among the quality classes was observed for IM9, Box Plots.

The test t, of mean comparison between consecutive quality classes (post-classification and multimetric classification) for a confidence interval of 95%, shows significant differences between quality classes (Table 9).

The multimetric classification (IM9) of 7 sites was underestimated in relation to the post-classification (Table 10). More differences are observed with the pre-classification (over and under estimations). This fact is a clear consequence of the absence of historical data to support the site selection (pre-classification). The multimetric classification of IM9 for the total sample and the transport habitat community was very similar (Table 11), contrasting with the almost consist ent underestimation observed in the deposition habitat community. A few differences on multimetric classifications were observed between the total sample and the coarse fraction, although the observed five overestimated (Table 11).

Discussion

Stream types

The Water Framework Directive requires ecological quality monitoring on the basis of stream types which correspond to groups of streams with similar characteristics. Under quite pristine conditions, different communities are expected for each stream type (reference conditions). Two possible methodologies may be used to establish the stream types: system A, based on geology, altitude and drainage area, with *a priori*

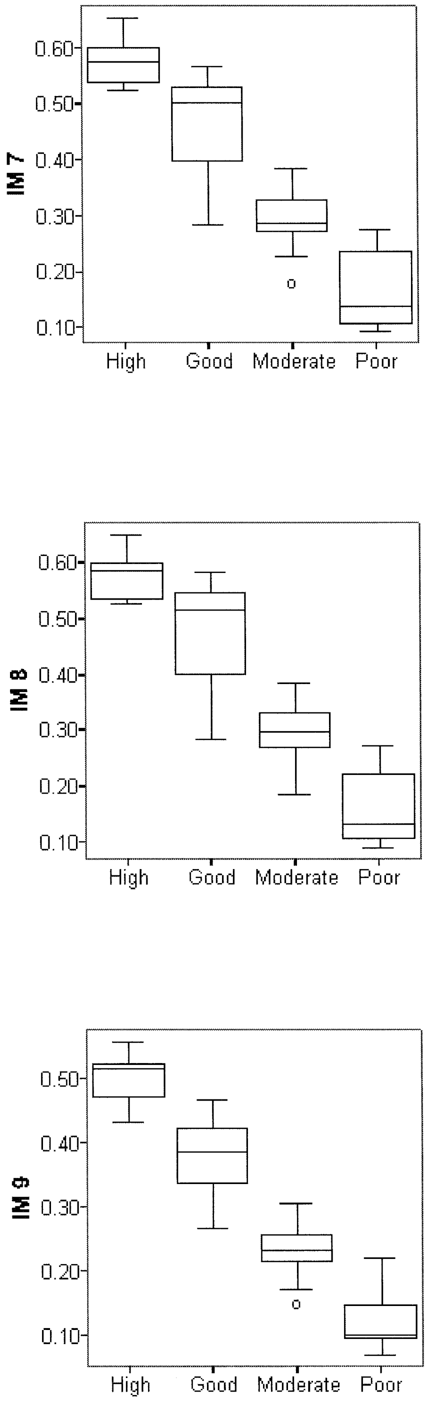

Figure 6. Box Cox graphics for selected multimetric indices.

Table 8. Components of multimetric indices.

	Metric category		
	Tolerance	Richness	Composition
IM7	ASPT′	EPTO	GOLD
IM8	ASPT′	EPT	GOLD
IM9	ASPT′	TRICF	GOLD

precise boundaries for those attributes; and system B, based on more abiotic variables, but treated as continuous variables.

In this study, site selection was done according to system A. However, DCA results did not split the stream types along the organic pollution gradient. For high ecological status (under low pollution effect), Pearson correlation ($P < 0.01$) indicated non significant differences among high-status-site communities (Table 5). According to these results, the three initial stream types probably belong to only one type. This confirms the most recent studies for different European regions (Charvet et al., 2000; Alves et al., 2002), suggesting system B.

Assessment

Mediterranean streams are subject to accentuated hydrological variations, influencing the colonisation processes, mortality and recovery of macroinvertebrate communities (Jackson & Fisher, 1986). However, similar patterns of variation of communities tend to be repeated during sequent years (Resh et al., 1990; Boulton et al., 1992; Stanley et al., 1994; Gasith & Resh, 1999), despite some significant differences in densities. Probably for this reason, composition metrics discriminated quality classes with a lower efficiency than the others (tolerance and richness). Richness (number of *taxa*) shows less variability than densities. Concerning the two selected composition metrics (GOLD – percentage of Gasteropoda, Oligochaeta and Diptera and %PLEC – percentage of Plecoptera), GOLD has some advantages when compared with %PLEC. Firstly, it is based on *taxa* which stay in stream for a long time during the year, in opposition to Plecoptera, mainly present up to early spring in southern Portugal streams (Morais, 1995). Secondly, the variation pattern with pollution is more gradual, allowing the establishment of quality classes with similar ranges of variation.

Table 9. Values of *t* Test of Equality of Means (95% confidence interval) between consecutive quality classes of post-classification and multimetric classification. (**$p < 0.01$; ***$p < 0.001$).

		Boundaries					
		High/Good		Good/ Moderate		Moderate/ Poor	
Post-classification	**IM7**	3.938 ***	19	5.557 ***	20	3.363 **	10
	IM8	3.460 **	19	5.458 ***	21	3.691 **	10
	IM9	5.198 ***	14	6.734 ***	19	3.854 **	10
Multimetric classification	**IM7**	4.079 **	14	9.303 ***	20	6.620 ***	15
	IM8	–	–	10.181 ***	27	6.007 ***	16
	IM9	8.359 ***	13	8.708 ***	21	6.104 ***	17

Concerning BMWP′ (Alba Tercedor & Sanchez Ortega, 1988), although the significant correlation with the gradient of organic pollution (Table 6), it was rejected due to the low linear regression R^2 (0.36) between the first axis of the DCA (independent variable) and BMWP′ scores (dependent variable). These results did not confirm the good ones obtained in other studies (Zamora-Muñoz & Alba-Tercedor, 1996). In contrast, ASPT′, which represents a mean score per family, was extremely efficient in discriminating all quality classes

Concerning the EPTO richness metrics, it was considered better than the EPT. The addition of Odonata *taxa* to metric can compensate for the absence of Plecoptera when more lentic habitats are present. Under these situations, Odonata tends to increase at non-impaired sites. In each case, TRICF (number of Trichoptera families) was considered the best richness metric, despite of the existence of outliers in the distribution of good and moderate ecological status.

All three multimetric indices developed in this study (Fig. 6, Table 8) are able to discriminate good and moderate ecological status (Table 7). IM9 (ASPT′ + TRICF + (1-GOLD)) had the highest R^2 and is consequently the best index to discriminate all the quality classes. For any consecutive quality class, the percentile 25 of the upper class is never superimposed with the percentile 75 of the next class.

It is possible to apply IM9 to siliceous basins of the ecoregion 1, because the metrics involved are not dependent only on taxa collected in this study. The extension of this index to other ecoregions needs a correction to the expression used to convert the metric TRICF (number of Trichoptera families) to a range from 0 to 1, converted to the number of Trichoptera families in the new ecoregion.

The application of IM9 to the coarse fraction, transport habitat communities and of deposition habitat communities (Table 10) is quite similar. Differences in obtained quality classes (overestimation and underestimation) occurred only between adjacent classes. Changes between good and moderate ecological status only occurred in four cases. A general pattern of overestimation was observed in the deposition habitat community. This is a direct influence of the generally higher densities of Oligochaeta and Chironomidae in the deposition areas, increasing the GOLD scores and, consequently, decreasing the IM9 scores. The slight overestimation observed in the coarse fraction can result from Naididae, generally with higher densities in the fine fraction than in the coarse fraction.

The multimetric index developed in this study (IM9) was only applied in spring. More studies throughout the year are needed to extend its use to other hydrological conditions (Stanley et al., 1997; Rabeni & Wallace, 1998). The application of IM9 is advisable for Mediterranean streams with high hydrological variability because: it is based on three pure metrics (richness, tolerance and composition); only the family level identification is required (important when local identification keys are absent); and it is not redundant, because this multimetric index is composed of metrics with different sensitivities. ASPT′ and TRICF are more sensitive to intolerant taxa while GOLD is more sensitive to more tolerant taxa. Despite the similar results obtained in this study between total community, course fraction and transport habitat community, it is advisable to use only total community to assess the water quality in future monitoring programmes. Possible future reduction in assessment time consumption, both for sampling procedure (to sample only one of the habitats), and for sorting effort (to sort and analyse only the coarse fraction), can be imple-

Table 10. Comparison of site quality classes obtain by pre-classification, post-classification and multimetric classification. 1 – bad status; 2 – poor status; 3 – moderate status; 4 – good status; 5 – high status.

Site	Pre-classification	Post-classification	Multimetric index classification
I-GN-1	5	5	5
I-GN-2	4	5	5
I-GN-3	3	4	4
I-MA-I	5	5	4
I-MA-2	4	4	4
I-MA-3	3	3	3
I-MA-PN	5	5	5
I-GS-1	5	4	4
I-GS-2	4	4	4
I-GS-3	3	4	4
I-GS-V	5	4	4
I-GS-CP	5	4	4
I-MA-4	2	3	3
I-G-D	2	2	2
I-S-5	1	2	1
II-S-1	5	4	4
II-S-2	4	3	2
II-S-3	3	3	2
II-M-2	4	4	3
II-M-F	4	4	4
II-M-3	3	3	3
II-A-1	5	5	5
II-A-2	4	5	5
II-A-3	3	4	3
II-S-4	2	2	2
II-S-5	1	2	1
III-S-1	5	3	3
III-S-3	3	3	2
III-MA-1	5	4	3
III-MA-3	3	4	3
III-MA-S	5	5	4
III-G-1	5	3	3
III-G-3	3	3	3
III-G-OD	5	4	4
III-G-4	2	2	1
III-S-5	1	2	3

Table 11. Multimetric classification (IM9) for total fraction, coarse fraction, transport habitat and depositional habitat. 1 – bad status; 2 – poor status; 3 – moderate status; 4 – good status; 5 – high status.

Site	Total fraction	Coarse fraction	Transport habitat	Depositional habitat
I-GN-1	5	5	5	4
I-GN-2	5	5	4	5
I-GN-3	4	4	4	3
I-MA-I	4	5	5	4
I-MA-2	4	4	4	4
I-MA-3	3	3	3	3
I-MA-PN	5	5	5	4
I-GS-1	4	5	4	3
I-GS-2	4	4	4	4
I-GS-3	4	4	3	5
I-GS-V	4	4	4	4
I-GS-CP	4	4	4	4
I-MA-4	3	3	3	1
I-G-D	2	2	2	2
I-S-5	1	1	1	1
II-S-1	4	4	4	4
II-S-2	2	2	2	2
II-S-3	2	2	3	2
II-M-2	3	2	4	3
II-M-F	4	4	3	4
II-M-3	3	4		3
II-A-1	5	3	5	4
II-A-2	5	5	5	4
II-A-3	3	5	3	5
II-S-4	2	3		2
II-S-5	1	2		2
III-S-1	3	3	3	2
III-S-3	2	2	2	2
III-MA-1	3	4	3	3
III-MA-3	3	3	3	3
III-MA-S	4	4	4	4
III-G-1	3	3	3	3
III-G-3	3	2	2	3
III-G-OD	4	4	4	5
III-G-4	1	2		2
III-S-5	3	3	3	2

mented only after testing the assessment efficiency for a larger number of collecting sites.

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Appendix 1. Scores of tolerance metrics

Site	DSI	SI	BBI	IBE	MAS	BMWP	BMWP′	ASPT	ASPT′
I-GN-1	0.67	2.06	10.00	14.00	3.00	254	280	6.35	5.60
I-GN-2	0.59	2.03	10.00	−1.00	2.75	246	274	6.65	5.96
I-GN-3	0.86	1.90	10.00	12.60	2.82	205	213	6.03	5.20
I-MA-I	0.25	1.99	10.00	14.00	3.00	232	255	6.11	5.31
I-MA-2	0.54	1.94	9.00	10.00	2.83	149	161	5.96	4.88
I-MA-3	0.46	2.13	10.00	9.00	2.80	130	135	5.65	4.66
I-MA-PN	0.53	1.68	10.00	10.00	2.75	154	150	7.00	6.25
I-GS-1	0.44	2.11	10.00	11.00	3.00	168	162	6.22	5.23
I-GS-2	0.52	2.11	10.00	10.00	2.82	159	154	6.12	4.97
I-GS-3	0.27	2.13	10.00	10.00	2.82	135	133	6.14	4.75
I-GS-V	0.60	2.11	10.00	9.40	2.78	141	138	6.71	5.75
I-GS-CP	0.33	2.18	10.00	10.00	2.78	144	137	6.26	5.71
I-MA-4	0.62	1.69	9.00	8.00	2.00	78	98	4.59	3.77
I-G-D	0.45	2.65	8.00	8.00	2.20	80	75	4.44	3.26
I-S-5	0.02	2.46	6.00	3.00	1.00	38	44	4.22	2.93
II-S-1	0.42	2.03	10.00	12.40	3.00	187	186	5.84	4.89
II-S-2	0.01	2.10	6.00	3.00	2.00	39	137	4.33	4.28
II-S-3	0.01	1.98	10.00	10.00	2.60	140	140	5.38	4.12
II-M-2	0.14	2.54	9.00	11.00	2.67	137	131	5.27	5.04
II-M-F	0.81	2.18	10.00	9.00	2.43	138	101	6.27	4.59
II-M-3	0.28	2.12	9.00	9.00	2.00	114	120	5.43	4.29
II-A-1	0.31	1.70	9.00	10.40	2.67	106	185	5.30	6.17
II-A-2	0.32	2.13	10.00	12.00	3.00	168	205	6.46	5.39
II-A-3	0.69	2.13	10.00	11.00	2.43	199	178	6.42	4.68
II-S-4	0.42	2.72	10.00	9.00	3.00	170	139	5.86	4.09
II-S-5	1.81	2.53	8.00	10.40	2.43	122	60	5.08	3.53
III-S-1	0.19	2.14	8.00	9.40	2.20	93	93	4.89	4.04
III-S-3	0.23	2.02	10.00	8.00	2.00	86	91	5.06	3.96
III-MA-1	0.15	1.98	10.00	11.00	2.00	130	135	5.91	5.00
III-MA-3	1.49	2.75	10.00	8.00	2.00	129	131	5.86	5.24
III-MA-S	0.65	1.86	10.00	11.60	3.00	122	143	6.42	5.72
III-G-1	0.31	2.23	9.00	8.40	2.00	118	114	5.36	4.22
III-G-3	0.53	1.92	9.00	9.00	2.33	134	128	5.36	4.13
III-G-OD	0.67	2.25	10.00	8.40	2.75	112	107	6.22	5.35
III-G-4	0.01	2.73	7.00	4.00	3.00	54	82	4.50	3.57
III-S-5	0.56	1.81	8.00	9.00	2.20	88	97	4.63	3.73

Appendix 2. Scores of richness metrics

Site	Ephem.	Plecopt.	Trich.	Odonata	Tricf	Gastrop.	Oligo.	Hirud.	Crustac.
I-GN-1	18	5	24	6	13	6	5	2	1
I-GN-2	12	3	16	6	11	4	2	1	0
I-GN-3	17	3	11	5	7	10	4	2	0
I-MA-I	17	5	16	2	9	5	3	1	0
I-MA-2	20	2	8	1	4	3	3	0	0
I-MA-3	18	1	7	0	4	5	4	1	0
I-MA-PN	14	6	4	4	4	1	0	0	0
I-GS-1	18	3	8	1	6	3	3	1	0
I-GS-2	24	4	8	0	4	5	3	1	1
I-GS-3	20	1	9	1	4	1	2	0	0
I-GS-V	13	3	8	1	5	1	3	0	1
I-GS-CP	14	6	6	1	3	1	1	2	0
I-MA-4	4	1	0	2	0	5	4	2	0
I-G-D	9	0	1	2	1	6	6	1	0
I-S-5	3	0	0	1	0	2	7	0	0
II-S-1	22	7	12	2	5	5	5	2	3
II-S-2	9	4	4	1	3	4	5	0	1
II-S-3	13	2	5	1	3	11	7	3	2
II-M-2	15	1	6	2	4	2	3	0	2
II-M-F	9	1	7	0	5	4	2	1	1
II-M-3	13	1	2	2	2	2	5	2	2
II-A-1	12	7	12	4	8	3	3	1	0
II-A-2	15	3	14	1	9	5	5	0	0
II-A-3	13	2	7	5	5	10	4	1	0
II-S-4	13	0	1	4	1	13	4	4	1
II-S-5	2	0	0	0	0	0	4	0	0
III-S-1	13	0	2	0	2	4	4	2	3
III-S-3	7	2	2	0	2	3	7	2	1
III-MA-1	7	3	6	0	4	2	3	2	2
III-MA-3	7	1	7	2	5	2	2	0	3
III-MA-S	15	3	7	4	4	1	2	0	1
III-G-1	13	0	6	3	3	2	4	1	0
III-G-3	13	0	9	1	5	4	6	1	1
III-G-OD	16	2	4	0	3	1	2	1	0
III-G-4	1	0	0	2	0	1	4	0	0
III-S-5	9	0	4	2	2	5	5	0	3

Continued on p. 208

Appendix 2.. Continued

Site	Heter.	Coleo.	Dipt.	EPT	EPTO	PT	EPT/Chiro	No. taxa
I-GN-1	1	16	22	47	53	29	279.97	86
I-GN-2	2	12	23	31	37	19	194.92	66
I-GN-3	1	14	23	31	36	14	194.95	72
I-MA-I	3	14	21	38	40	21	110.25	71
I-MA-2	2	14	16	30	31	10	333.46	56
I-MA-3	0	8	12	26	26	8	200.06	46
I-MA-PN	1	13	8	24	28	10	319.20	37
I-GS-1	0	12	17	29	30	11	256.10	51
I-GS-2	1	6	15	36	36	12	163.53	54
I-GS-3	2	9	17	30	31	10	116.72	51
I-GS-V	1	8	10	24	25	11	108.95	37
I-GS-CP	1	11	10	26	27	12	72.22	41
I-MA-4	2	6	16	5	7	1	72.17	36
I-G-D	2	10	15	10	12	1	24.17	44
I-S-5	0	4	11	3	4	0	8.18	24
II-S-1	1	10	19	41	43	19	171.88	67
II-S-2	3	18	22	17	18	8	19.05	57
II-S-3	2	12	18	20	21	7	33.28	62
II-M-2	0	6	11	22	24	7	92.21	38
II-M-F	1	10	9	17	17	8	639.82	38
II-M-3	1	9	17	16	18	3	47.77	46
II-A-1	0	13	14	31	35	19	132.60	55
II-A-2	0	17	15	32	33	17	443.61	63
II-A-3	1	15	22	22	27	9	118.09	64
II-S-4	2	23	25	14	18	1	72.15	70
II-S-5	0	5	14	2	2	0	2.08	21
III-S-1	0	4	10	15	15	2	44.02	33
III-S-3	2	5	13	11	11	4	48.38	37
III-MA-1	0	5	16	16	16	9	85.25	32
III-MA-3	0	7	12	15	17	8	145.83	36
III-MA-S	0	8	11	25	29	10	493.75	36
III-G-1	1	5	13	19	22	6	110.64	37
III-G-3	2	7	13	22	23	9	223.51	47
III-G-OD	0	7	10	22	22	6	143.81	29
III-G-4	2	11	17	1	3	0	2.42	32
III-S-5	2	9	15	13	15	4	32.10	47

Appendix 3. Scores of composition metrics

Site	Plecop.	Gastro.	Bivalv.	Oligo.	Hirud.	Crustac.	Odonata (%)	Hetero..	Coleopte.
I-GN-1	0.104	9.784	0.031	1.909	0.021	0.010	1.961	0.073	2.584
I-GN-2	0.086	7.652	0.079	1.798	0.009	0.000	2.654	0.123	2.795
I-GN-3	0.007	56.329	0.008	0.620	0.136	0.000	0.195	0.008	1.010
I-MA-I	0.056	4.607	0.050	0.932	0.050	0.000	1.309	0.076	2.669
I-MA-2	0.016	18.043	0.000	3.361	0.000	0.000	0.049	0.099	7.662
I-MA-3	0.001	16.427	0.000	0.218	0.073	0.000	0.000	0.000	3.155
I-MA-PN	0.093	0.301	0.000	0.000	0.000	0.000	2.105	0.301	29.774
I-GS-1	0.039	6.300	0.000	23.126	0.080	0.000	0.080	0.000	7.416
I-GS-2	0.006	2.128	0.000	14.590	0.478	0.043	0.000	0.043	5.124
I-GS-3	0.009	0.936	0.000	6.383	0.000	0.000	0.170	0.170	12.340
I-GS-V	0.032	10.145	0.000	1.884	0.000	0.580	0.145	0.145	7.101
I-GS-CP	0.058	1.895	0.000	0.947	0.421	0.000	0.105	0.105	4.842
I-MA-4	0.001	1.446	0.000	41.867	0.783	0.000	0.181	0.120	1.325
I-G-D	0.000	12.700	0.008	29.803	0.226	0.000	0.016	0.073	0.598
I-S-5	0.000	0.348	0.000	24.882	0.000	0.000	0.011	0.000	0.056
II-S-1	0.009	12.298	0.000	5.504	0.242	0.468	0.081	0.016	2.469
II-S-2	0.000	0.075	0.000	3.647	0.000	0.004	0.257	0.072	0.472
II-S-3	0.001	7.327	0.011	8.259	0.191	0.045	0.449	0.022	9.922
II-M-2	0.029	31.462	0.000	4.094	0.000	7.953	1.170	0.000	8.070
II-M-F	0.001	7.367	0.000	0.483	0.121	0.121	0.000	0.121	5.556
II-M-3	0.006	0.440	0.000	3.077	1.495	0.396	0.176	0.044	0.879
II-A-1	0.076	3.744	0.000	0.333	0.250	0.000	1.165	0.000	23.211
II-A-2	0.042	5.375	0.000	1.344	0.000	0.000	0.071	0.000	6.223
II-A-3	0.00	8.33	0.00	2.11	0.04	0.00	0.52	0.04	3.11
II-S-4	0.000	7.506	0.000	67.043	0.527	0.002	0.122	0.046	3.889
II-S-5	0.000	0.000	0.000	1.577	0.000	0.000	0.000	0.000	0.643
III-S-1	0.000	1.575	0.000	27.487	1.718	1.575	0.000	0.000	5.154
III-S-3	0.000	12.393	0.000	31.622	0.162	0.003	0.000	0.362	0.100
III-MA-1	0.033	5.209	0.000	2.681	0.868	21.859	0.000	0.000	0.536
III-MA-3	0.012	70.961	0.000	0.693	0.000	0.455	0.065	0.000	0.152
III-MA-S	0.067	5.606	0.000	3.074	0.000	1.266	3.797	0.000	23.870
III-G-1	0.000	0.850	0.061	3.944	0.121	0.000	0.303	0.364	8.799
III-G-3	0.000	42.867	0.114	31.583	0.043	0.057	0.029	0.613	1.398
III-G-OD	0.069	0.142	0.000	1.416	0.425	0.000	0.000	0.000	5.949
III-G-4	0.000	0.175	0.000	43.396	0.000	0.000	0.140	0.105	0.943
III-S-5	0.000	7.614	0.066	18.838	0.000	1.401	0.028	0.085	0.931

Continued on p. 210

Appendix 3. Continued

Site	Dipt..	Lumbric.	Tubificid.	Elmid.	Chiro.	Tricho.	Ephem.	Tanytar.s	Hydrops.ae (%)
I-GN-1	34.696	0.000	0.011	0.022	0.168	0.119	26.250	0.083	0.016
I-GN-2	46.390	0.001	0.000	0.025	0.159	0.019	27.929	0.066	0.000
I-GN-3	20.808	0.002	0.000	0.009	0.159	0.025	17.608	0.022	0.003
I-MA-I	53.323	0.002	0.004	0.010	0.345	0.136	16.994	0.262	0.006
I-MA-2	14.829	0.010	0.001	0.020	0.090	0.239	30.400	0.015	0.021
I-MA-3	58.439	0.000	0.002	0.029	0.130	0.005	21.093	0.080	0.003
I-MA-PN	22.256	0.000	0.000	0.232	0.075	0.035	32.481	0.003	0.014
I-GS-1	18.022	0.002	0.001	0.065	0.113	0.036	37.480	0.014	0.007
I-GS-2	30.178	0.000	0.008	0.038	0.220	0.072	39.644	0.038	0.014
I-GS-3	37.277	0.000	0.002	0.109	0.257	0.068	34.979	0.010	0.019
I-GS-V	30.870	0.006	0.001	0.045	0.220	0.168	29.130	0.007	0.000
I-GS-CP	36.737	0.000	0.000	0.034	0.360	0.131	36.105	0.005	0.000
I-MA-4	16.566	0.011	0.405	0.000	0.069	0.000	37.651	0.002	0.000
I-G-D	55.663	0.000	0.018	0.000	0.414	0.000	0.905	0.032	0.000
I-S-5	74.601	0.004	0.102	0.000	0.367	0.000	0.101	0.026	0.000
II-S-1	29.277	0.007	0.036	0.018	0.239	0.081	40.607	0.030	0.046
II-S-2	95.144	0.000	0.028	0.001	0.892	0.001	0.250	0.104	0.000
II-S-3	68.086	0.002	0.075	0.092	0.601	0.005	5.147	0.049	0.003
II-M-2	26.667	0.013	0.021	0.000	0.239	0.036	14.035	0.048	0.015
II-M-F	44.686	0.001	0.004	0.029	0.027	0.169	24.517	0.007	0.159
II-M-3	65.011	0.011	0.006	0.003	0.335	0.026	25.275	0.069	0.022
II-A-1	37.022	0.000	0.002	0.161	0.234	0.101	16.556	0.030	0.001
II-A-2	25.813	0.006	0.001	0.049	0.072	0.115	45.332	0.018	0.084
II-A-3	57.44	0.02	0.00	0.01	0.19	0.07	21.15	0.10	0.06
II-S-4	19.932	0.001	0.000	0.034	0.194	0.000	0.931	0.030	0.000
II-S-5	97.605	0.002	0.005	0.001	0.962	0.000	0.175	0.064	0.000
III-S-1	44.166	0.037	0.236	0.048	0.341	0.073	10.952	0.079	0.072
III-S-3	54.155	0.001	0.023	0.000	0.227	0.003	0.877	0.022	0.003
III-MA-1	57.482	0.000	0.025	0.002	0.188	0.038	4.316	0.022	0.030
III-MA-3	18.948	0.003	0.000	0.001	0.103	0.037	3.898	0.005	0.010
III-MA-S	33.092	0.002	0.000	0.222	0.051	0.042	18.445	0.000	0.013
III-G-1	53.277	0.003	0.036	0.078	0.172	0.078	24.454	0.094	0.062
III-G-3	17.960	0.000	0.007	0.001	0.098	0.018	3.481	0.033	0.016
III-G-OD	20.963	0.003	0.000	0.055	0.153	0.027	61.473	0.010	0.001
III-G-4	55.136	0.000	0.433	0.000	0.413	0.000	0.105	0.000	0.000
III-S-5	46.710	0.000	0.012	0.000	0.405	0.013	23.021	0.071	0.008

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Appendix 3. Continued

Site	Hydrops./ Tricho.	Hept./ Ephem.	%Cae./ Ephem.	GOLD	EPT %	Dominante taxa (fam.)	Dominante taxa (orders)
I-GN-1	0.132	0.026	0.215	0.464	48.579	0.178	0.347
I-GN-2	0.023	0.004	0.028	0.558	38.438	0.285	0.464
I-GN-3	0.122	0.004	0.056	0.778	20.834	0.406	0.563
I-MA-I	0.045	0.311	0.058	0.589	36.178	0.345	0.533
I-MA-2	0.089	0.000	0.099	0.362	55.957	0.195	0.304
I-MA-3	0.486	0.003	0.007	0.751	21.689	0.450	0.584
I-MA-PN	0.391	0.051	0.032	0.226	45.263	0.232	0.325
I-GS-1	0.200	0.070	0.253	0.474	44.976	0.229	0.375
I-GS-2	0.194	0.011	0.072	0.469	47.416	0.306	0.396
I-GS-3	0.275	0.029	0.112	0.446	42.723	0.257	0.373
I-GS-V	0.000	0.045	0.020	0.429	49.130	0.220	0.309
I-GS-CP	0.000	0.344	0.041	0.396	54.947	0.360	0.367
I-MA-4	0.000	0.000	0.000	0.599	37.711	0.405	0.419
I-G-D	0.000	0.000	0.107	0.982	0.913	0.414	0.557
I-S-5	0.000	0.000	0.000	0.998	0.101	0.377	0.746
II-S-1	0.567	0.005	0.044	0.471	49.613	0.239	0.406
II-S-2	0.143	0.000	0.129	0.989	0.329	0.892	0.951
II-S-3	0.610	0.000	0.052	0.837	5.686	0.601	0.681
II-M-2	0.419	0.033	0.125	0.622	20.585	0.277	0.315
II-M-F	0.943	0.000	0.123	0.525	41.546	0.411	0.447
II-M-3	0.817	0.000	0.068	0.685	28.484	0.335	0.650
II-A-1	0.008	0.367	0.050	0.411	34.276	0.234	0.370
II-A-2	0.730	0.064	0.039	0.325	61.103	0.374	0.453
II-A-3	0.88	0.00	0.08	0.68	28.41	0.21	0.57
II-S-4	1.000	0.000	0.052	0.945	0.933	0.670	0.670
II-S-5	0.000	0.000	0.667	0.992	0.175	0.962	0.976
III-S-1	0.980	0.000	0.379	0.732	18.253	0.341	0.442
III-S-3	0.990	0.000	0.011	0.982	1.20219	0.298	0.542
III-MA-1	0.797	0.000	0.053	0.654	11.3636	0.360	0.575
III-MA-3	0.278	0.006	0.011	0.906	8.72672	0.708	0.710
III-MA-S	0.304	0.078	0.010	0.418	29.2948	0.222	0.331
III-G-1	0.791	0.000	0.541	0.581	32.2816	0.336	0.533
III-G-3	0.883	0.000	0.664	0.924	5.3067	0.419	0.429
III-G-OD	0.053	0.180	0.263	0.225	71.1048	0.214	0.615
III-G-4	0.000	0.000	0.001	0.987	0.10482	0.433	0.551
III-S-5	0.652	0.000	0.780	0.732	24.3185	0.405	0.467

Appendix 4. Diversity indices

Site	Indice Simpson	Indice Shannon–Wiener	Evenness
I-GN-1	0.92	3.09	0.66
I-GN-2	0.87	2.54	0.57
I-GN-3	0.80	2.34	0.52
I-MA-I	0.88	2.84	0.63
I-MA-2	0.92	3.07	0.72
I-MA-3	0.74	1.84	0.46
I-MA-PN	0.91	2.92	0.74
I-GS-1	0.91	3.06	0.73
I-GS-2	0.92	3.01	0.71
I-GS-3	0.92	3.05	0.74
I-GS-V	0.92	2.98	0.77
I-GS-CP	0.88	2.79	0.70
I-MA-4	0.80	2.05	0.55
I-G-D	0.86	2.32	0.58
I-S-5	0.76	1.74	0.52
II-S-1	0.94	3.19	0.71
II-S-2	0.28	0.75	0.23
II-S-3	0.57	1.39	0.33
II-M-2	0.70	1.96	0.45
II-M-F	0.89	2.86	0.74
II-M-3	0.79	2.25	0.59
II-A-1	0.86	2.58	0.64
II-A-2	0.95	3.36	0.79
II-A-3	0.91	3.08	0.71
II-S-4	0.89	2.72	0.62
II-S-5	0.62	1.65	0.37
III-S-1	0.90	2.75	0.73
III-S-3	0.81	1.96	0.52
III-MA-1	0.81	2.22	0.58
III-MA-3	0.49	1.33	0.35
III-MA-S	0.92	3.09	0.78
III-G-1	0.85	2.56	0.66
III-G-3	0.73	1.85	0.45
III-G-OD	0.93	3.01	0.80
III-G-4	0.78	1.96	0.54
III-S-5	0.88	2.50	0.62

Appendix 5. Scores of multimetric indices

Site	IM7	IM8	IM9
I-GN-1	0.654	0.650	0.555
I-GN-2	0.543	0.529	0.505
I-GN-3	0.436	0.428	0.341
I-MA-I	0.527	0.540	0.440
I-MA-2	0.534	0.549	0.420
I-MA-3	0.368	0.385	0.282
I-MA-PN	0.614	0.606	0.516
I-GS-1	0.504	0.518	0.427
I-GS-2	0.531	0.555	0.387
I-GS-3	0.502	0.516	0.387
I-GS-V	0.510	0.520	0.446
I-GS-CP	0.531	0.542	0.423
I-MA-4	0.277	0.268	0.236
I-G-D	0.159	0.154	0.106
I-S-5	0.095	0.091	0.072
II-S-1	0.568	0.583	0.400
II-S-2	0.229	0.234	0.173
II-S-3	0.291	0.298	0.218
II-M-2	0.413	0.417	0.339
II-M-F	0.389	0.400	0.371
II-M-3	0.330	0.329	0.258
II-A-1	0.589	0.586	0.515
II-A-2	0.577	0.593	0.531
II-A-3	0.399	0.385	0.323
II-S-4	0.236	0.223	0.149
II-S-5	0.108	0.109	0.096
III-S-1	0.288	0.298	0.234
III-S-3	0.179	0.186	0.147
III-MA-1	0.356	0.366	0.327
III-MA-3	0.286	0.285	0.268
III-MA-S	0.536	0.529	0.432
III-G-1	0.386	0.381	0.307
III-G-3	0.273	0.282	0.221
III-G-OD	0.546	0.560	0.467
III-G-4	0.117	0.106	0.099
III-S-5	0.277	0.274	0.222

Appendix 6. Mean and standard error of selected metrics and multimetric indices for each quality class

		Quality classes							
		High		Good		Moderate		Poor	
		Mean	Std. error	Mean	Std. error	Mean	Std. error	Mean	Std. error
Richeness	PT	17.86	2.50	10.43	0.88	4.36	1.02	1.50	0.87
	EPT	32.57	2.98	25.79	2.02	14.18	2.41	9.50	2.96
	EPTO	36.43	3.19	27.29	2.12	15.18	2.45	12.0	3.24
	TRICF	8.29	1.27	4.57	0.29	2.18	0.50	1	0.41
Composition	GOLD	0.57	0.05	0.47	0.05	0.18	0.05	0.09	0.06
	% Plecoptera	7.49	0.81	2.32	0.57	0.07	0.05	0	0
Tolerance	ASPT′	5.77	0.14	5.02	0.09	3.99	0.14	3.66	0.17
Multimetric index	IM7	0.58	0.02	0.47	0.02	0.26	0.03	0.20	0.04
	IM8	0.58	0.02	0.47	0.02	0.26	0.03	0.19	0.04
	IM9	0.50	0.02	0.39	0.02	0.20	0.02	0.14	0.03