



Assessment of temporary streams: the robustness of metric and multimetric indices under different hydrological conditions

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Abstract

The generalization of ecological results from temporary streams needs the study of those ecosystems across a broad scale of running waters, covering the geographical ecological constraints involved (e.g., precipitation, temperature and geology). For the particular situation of Mediterranean streams in Southern Europe, high changes in water level, with unpredicted flood events, represent an important factor determining the structure and function of Mediterranean streams. This variability, inducing accentuated changes in the macroinvertebrate community, tends to influence the assessment methodologies. A set of metrics commonly used in Europe to assess organic degradation (Shannon–Wiener index, evenness, richness, BBI, IBE, BMWP', ASPT', DSFI, EPT, number of Trichoptera families, percentage of Gasteropoda, Oligochaeta and Diptera) and a multimetric index (IM9) developed to assess organic degradation in southern siliceous Portuguese basins were tested to evaluate quality at two contrasting sites (one unimpaired and another impaired by an identified point source of pollution). The multimetric index (IM9) composed by: ASPT' (average score per taxon); GOLD (one minus percentage of Gasteropoda, Oligochaeta and Diptera); and TRICF (number of Trichoptera families), was the most suitable assessment methodology. IM9 presented a quite stable temporal pattern from February in late winter until June in early summer, even under the effect of episodic floods. A stepwise regression showed that most of tested metrics were significantly related to environmental variables (soluble reactive phosphorous, dissolved inorganic nitrogen and dissolved oxygen). Only richness, IBE and BMWP' were not significantly influenced by environmental variables. Future research must be done covering the complete gradient of organic degradation, including the extension of multimetric assessment methodologies to temporary streams located in other regions under different geological and climatic conditions.

Introduction

The study of temporary Mediterranean streams in Southern Europe was initiated in the last two decades to evaluate the value of these systems for conservation and landscape management (Puig et al., 1991; Sabater et al., 1995; Gasith & Resh, 1999; Bonada et al., 2000; Prat & Munne, 2000). Research was focused mainly on community composition and on physical and chemical processes. In Mediterranean streams, the assessment using biotic indices started in the permanent streams (e.g., Cortes et al., 1986; Cortes & Monzon, 1991). Due to methodological difficulties, only recently were these studies extended to

temporary streams (Graça & Coimbra, 1998; Prenda & Gallardo-Mayenco, 1996; Coimbra et al., 1996; Rueda et al., 2002), defined as those that flow only seasonally (Boulton & Suter, 1986; Lake et al., 1986).

In southern Portugal, under Mediterranean climatic conditions, the annual precipitation distribution determines that many streams have developed spatial and temporal discontinuities of flow regime with superficial flow interruption during the summer dry period, in contrast to high discharges, during floods, observed from late autumn to early spring. The superficial summer flow interruption represents a predicted natural constraint, leading to stagnant pools or even

to drying out completely. In this period, macroinvertebrate abundances and richness tend to decrease (Richards & Minshall, 1992; Stanley et al., 1994; Gasith & Resh, 1999). The relatively harsher environmental conditions experienced in temporary streams have been used to explain this reduced species abundance and richness (Boulton & Suter, 1986). During the flow period, precipitation may originate floods which cause disturbances, varying from small movements of substrate, to large changes in the system structure and in the morphology of the stream channel, such as detritus exportation, sediment deposition and the dislodging of biological communities (Boulton & Suter, 1986; Resh et al., 1988; Townsend, 1989; Stanley et al., 1994; Lake et al., 1998; Meyer & Meyer, 2000). The effect of these hydrological events is one of the principal environmental factors influencing the structure and function of macroinvertebrate communities in Mediterranean streams (Langton & Casas, 1998; Prenda & Gallardo-Mayenco, 1996; Prenda & Gallardo-Mayenco, 1999; Puig et al., 1991; Ubero-Pascal et al., 2000). Another characteristic of these systems is the rapid capacity to recover following these natural disturbances (Boulton et al., 1988; Closs & Lake, 1994; Doeg et al., 1989; Grimm & Fisher, 1989).

Flow conditions are normally associated with taxa more sensitive to organic contamination (Coimbra et al., 1996; Prenda & Gallardo-Mayenco, 1996; Prenda & Gallardo-Mayenco, 1999; Ubero-Pascal et al., 2000). In contrast, under lentic conditions, these taxa tend to decrease and tolerant taxa become dominant in the community (Coimbra et al., 1996). As a result of this dynamic (lentic *versus* lotic), an annual temporal pattern of macroinvertebrate community composition is observed. More sensitive taxa, usually occurring with higher densities during the flow period, are substituted by more tolerant taxa in the summer lentic period. Hydrological variability in temporary streams, and the subsequent biological adaptation strategies to survive, are the main problems concerning biological assessment methodologies, because distortions and mistakes can be introduced into ecosystem quality evaluations. Natural unpredicted (due to episodic floods) and predicted (superficial flow interruption included in annual hydrological cycle) constraints on macroinvertebrate community structure (Boulton & Lake, 1992; Peterson & Boulton, 1999; Prenda & Gallardo-Mayenco, 1999; Delluchi, 2002) should be covered by any assessment methodology. It must be sufficiently robust to integrate this natural

variability, in order to be applicable over any temporal period (not dependent on a single hydrological condition in time). Studies concerning the development of assessment methodologies of temporary streams, covering different hydrological conditions, are scarce (Zamora Muñoz et al., 1995; Coimbra et al., 1996; Graça & Coimbra, 1998) and multimetric approaches are quite absent (Pinto et al., 2004).

The objective of this study was to understand the influence of hydrological conditions on assessment methodologies based on macroinvertebrate communities. A set of tolerance and diversity metrics commonly used in European permanent streams (AQEM consortium, 2002; Hering et al., 2004), and a multimetric index recently developed for southern Portugal (Pinto et al. 2003), were tested from late winter to autumn, at two sites with contrasting ecological status (impaired and non-impaired). The influence of environmental variables on the metrics and the multimetric index was investigated and the stability of metric scores, observed over the whole sampling period (covering different hydrological situations), was used as a criterion to evaluate the robustness of the assessment of temporary streams.

Study area

Grândola is a second-order Mediterranean stream, located 10 Km south of Grândola village (38° 18' N, 8° 56' W), in south-western Portugal (Fig. 1). It receives the effluent from the wastewater treatment plant (WWTP) of Grândola village, characterised as a secondary treatment by an activated sludge process for a load of 6750 inhabitants equivalent. The catchment area is 265 km² and the stream length is about 40 km. Elevation ranges from 270 m at the headwaters to 16 m at its confluence with the Sado river. The catchment area is composed of siliceous and metamorphic rocks with shallow overlying soils. Maximum mean monthly temperature is 34 °C (July), minimum is 9 °C (January) and mean annual precipitation is about 678 mm, ranging from 376 to 1139 mm, irregularly distributed throughout the year and among different years. Most rainfall occurs seasonally, from late autumn to early spring. Heavy storms may cause the stream to flood. Flash floods during spring recede much faster than those in winter, and discharge can return to baseflow in a few days. This precipitation regime results in an irregular stream hydrology with lowest discharge usually recorded during summer, when precipitation

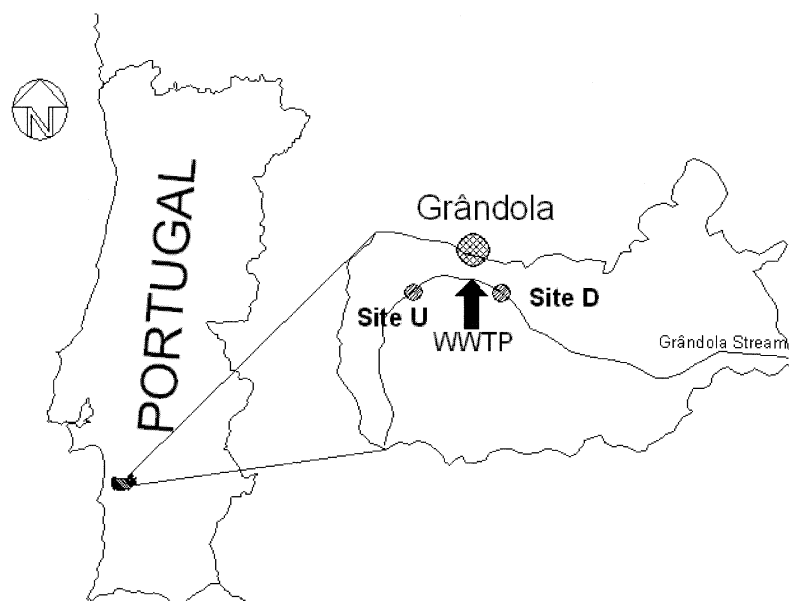


Figure 1. Map of the study area in Portugal showing the location of Waste Water Treatment Plan of Grândola village and the two study sites: upstream, non-impaired site (U); downstream impaired site (D).

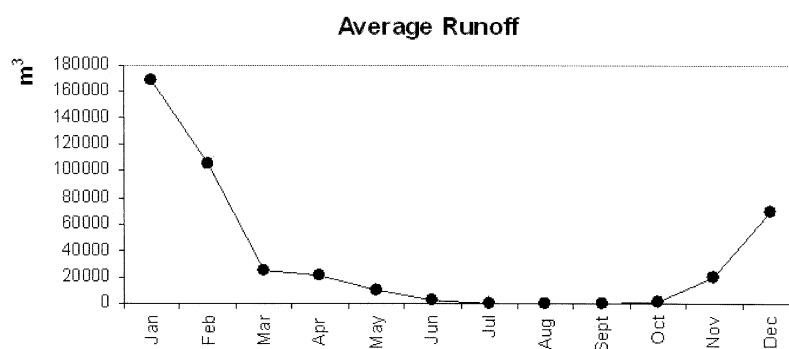


Figure 2. Mean monthly runoff values in Grândola watershed. Values recorded by Portuguese National Institute of Water (INAG)

drops to zero (Fig. 2). Like many small streams in the region, Grândola stream is temporary; the channel becomes fragmented into a series of isolated pools during summer each year. Most pools are small and shallow (<50 m² in area and <50 cm deep) and persist in solid rock basins, having little or no contact with subsurface flow.

Two sampling sites were established: one slightly impaired, presenting good quality (site U) (Pinto et al., 2004), is located 10 km upstream from the entrance of WWTP effluent; the other one (site D), located 3 km downstream of the WWTP, is under the effect of its effluent discharge. Each site covers a reach of 100 m with an average surface stream width of about 3 m. The physiographic features of two selected sites are presented in Table 1. The vegetation on facing

slopes is dominated by *Quercus suber* L. at both sites. Riparian vegetation is dense and mainly comprises *Quercus faginea* Lam., *Fraxinus angustifolia* Vahl and *Erica lusitanica* Rudolphi at the non-impaired site and *Alnus glutinosa* (L.), *Salix atrocinerea* Brot., *Fraxinus angustifolia* and *Populus nigra* L. at the impaired site. The algae community is dominated by diatoms during spring. However, during low-flow summer conditions, filamentous green algae mats can be very abundant, mainly at the impaired site. The streambed substrate is dominated by bedrock, boulders and cobbles. During summer, the upper site does not present superficial flow from July to the first rainfall in autumn, while at the downstream site, the baseflow is residual in summer and maintained only by WWTP effluent. In the sense of Barbour et al. (1996), this site is impaired

Table 1. Physiographic features of the two study sites on Grândola stream

	Non-impaired site	Impaired site
Dominant substrata	Bedrock and cobbles	Bedrock
Riparian vegetation	<i>Quercus faginea</i> , <i>Fraxinus angustifolia</i> and <i>Erica lusitanica</i>	<i>Alnus glutinosa</i> , <i>Salix atrocinerea</i> , <i>Fraxinus angustifolia</i> and <i>Populus nigra</i>
Vegetation on facing slopes	<i>Quercus suber</i>	<i>Quercus suber</i>
Algae community	Diatoms in spring; filamentous green algal mats during summer	Diatoms in spring; filamentous green algal mats during summer
Reach slope (m/m)	4.8×10^{-5}	1.0×10^{-4}

because the effluent makes up at least 25% of the total receiving stream flow under low flow conditions.

During the study period, a flood in which discharge increased from 52 to 1515 l s⁻¹ at its peak, occurred one day before the second sampling date (10 April 2002).

Methodology

Sampling was undertaken from February 2002, in late winter, to October 2002, in autumn, covering the summer period with its non-superficial flow.

Water temperature (°C), conductivity (µS cm⁻¹) dissolved oxygen (mg l⁻¹) and pH were measured *in situ* with specific probes (WTW thermo-conductimeter, WTW oxymeter and WTW pH meter, respectively). Water velocity was measured using a current meter at middle depth in one transect for both reach sites, measuring from right to left bank at each 0.20 m distance. Discharge was calculated integrating depth, distance to the right bank and water velocity for each transect. Water samples were collected and transported to the laboratory, preserved on ice. In the laboratory, samples were filtered with glass micro-fibre filters (Whatman GFF, 0.7 µm – pore size) and processed for a minimum of two hours, according to APHA (1989). Soluble reactive phosphorus (SRP) was analysed using the stannous chloride method, ammonium was determined using the phenate method, nitrate was analysed using the ultraviolet spectrophotometric screening method, and nitrites were analysed according to the colorimetric method. Dissolved in-

organic nitrogen (DIN) was obtained by the sum of ammonium, nitrate and nitrite.

At each reach site, 6 surber samples (25 cm square side with a mesh size of 0.5 mm) were taken to evaluate the benthic macroinvertebrate communities. Samples were fixed in the field with a 40% formalin solution. At the laboratory, samples were washed on metallic sieves (0.5 mm mesh size) and completely sorted only with the naked eye. Macroinvertebrates were preserved in a 70% alcohol solution and identified, whenever possible, down to the species level.

Prior to any data analysis, a taxonomic adjustment (see also Schmidt-Kloiber & Nijboer (2004)) was done according to abundances of each taxonomical level and their ecological information (AQEM consortium, 2002). The objective of this procedure is to avoid the inclusion of taxa at different taxonomical levels (AQEM consortium, 2002). To prevent distortions, caused by the most abundant taxa, multivariate analyses were carried out on transformed data: $x' = \log(x + 1)$ for macroinvertebrate abundance. Temporal and spatial gradients of macroinvertebrate communities were evaluated using multivariate techniques. In the first step, a detrended correspondence analysis (DCA) (ter Braak & Smilauer, 1998) was performed to detect if the data had an unimodal or linear structure according to the gradient length of the first axis. In the second step, a principal component analysis (PCA) was performed because the gradient length was shorter than 3 in the DCA analysis (ter Braak & Smilauer, 1998). An indirect analysis was chosen because the objective was to have an ordination based only on macroinvertebrate community structure iden-

Table 2. Definitions of studied metrics

Metric or index	Definition	Symbol	Reference
Shannon–Wiener index	$\sum_{i=1}^s \left(\frac{n_i}{A} \right) \ln \left(\frac{n_i}{A} \right)$	H'	Shannon & Weaver, 1949
Evenness	$\frac{H'}{s}$	E	Shannon & Weaver, 1949
Richness	Number of <i>taxa</i>	S	
Belgium Biotic Index	Combination of richness a tolerance of selected taxa	BBI	De Paw & Vanhooren, 1983
Danish Stream Fauna Index	Combination of richness a tolerance of selected taxa	DSFI	Skriver et al., 2001
Indice Biotico Estesio	Combination of richness a tolerance of selected taxa	IBE	Ghetti, 1997
Biological Monitoring Working Party (Spanish version)	Sum of selected tolerance score taxa	BMWP'	Alba Tercedor & Sanchez Ortega, 1988
Average Score per Taxa (Spanish version)	BMWP' divided by the existent selected taxa	ASPT'	Alba Tercedor & Sanchez Ortega, 1988
Ephemeroptera Plecoptera and Trichoptera taxa	Number of Ephemeroptera, Plecoptera and Trichoptera	EPT	Barbour et al., 1999
Trichoptera Families	Number of Trichoptera Families	TRICF	Pinto et al., 2003
Gasteropoda Oligochaeta and Diptera	Symmetric of Gasteropoda Oligochaeta and Diptera taxa	GOLD	Pinto et al., 2003
Multimetric index 9	Multimetric index Combining ASPT', TRICF and GOLD	IM9	Pinto et al., 2003

Table 3. Class boundaries used as scoring criteria for each studied metric.

Boundary	High/ Good	Good/ Moderate	Moderate/ Poor	Poor/ Bad
BMWP'	100	60	30	15
ASPT'	0.50	0.43	0.34	0.25
BBI	8	6	4	2
IBE	8	6	4	2
EPT	–	0.43	0.3	0.04
TRICF	0.29	0.19	0.10	0
GOLD	–	0.34	0.08	0.01
IM9	0.48	0.34	0.22	0.10

tified at the two selected sites, under different impaired statuses of quality. In order to relate the measured environmental variables with macroinvertebrate community structure, defined in PCA components, Pearson correlations were performed (between site scores along the axes and environmental variable values).

A set of metrics and index to assess quality in lotic ecosystems (see Brabec et al., 2004; Buffagni et al.,

2004; Offenböck et al. 2004) were computed for both sites over the study period (Table 2). The objective of those calculations was to detect temporal patterns. All the selected metrics and index are common used to assess organic pollution. Shannon–Wiener diversity index (H') (Shannon & Weaver, 1949), taxa richness (S) and the Danish Stream Fauna Index (DSFI) (Skriver et al., 2001) have been also used to assess general degradation (AQEM consortium, 2002). For some metrics and index, boundaries between quality classes are already established. These boundaries are referred to in Table 3. The detection of metrics and indices with less inter-sample variability during the year (covering different hydrological situations), was done using Box-and-whiskers plots. This type of plot displays the statistics (i.e., median value, minimum, maximum, 25th, and 75th percentile) of a set of sample units. Another objective of these plots was to evaluate the robustness of assessment methodologies to discriminate between the two sampled sites contrasting quality status (non-impaired and impaired site). These plots were done covering two different periods, one from 10 February to 16 May (excluding

summer and autumn samples) and the other from 10 February to 14 October (over the whole study period). The first period concerns only situations with superficial flow (late winter and spring) where assessment methodologies are usually applied. The second one includes non-flow and small baseflow situations (water velocity $<45.5 \text{ l s}^{-1}$), in order to evaluate metrics and index efficiencies for a larger period of time. For both periods, the discrimination power of each metric or index was evaluated based on the degree of similarity (i.e., overlap of the interquartile and minimum and maximum ranges) or dissimilarity between plots.

Analyses of the environmental influence on metric and multimetric indices were undertaken using multiple regression. Stepwise regressions of environmental variables (independent variables) were performed to obtain a minimum of unexplained residual variance in terms of the smallest number of variables. Those independent variables which did not remove a significant proportion of the variation in metric and multimetric analysis were dropped from the regression model (Sokal & Rohlf, 1995). While the variables which best explained the variability of a given metric may not have been the only factors causing the observed variability, they did give an indication of the parameters which influence that metric.

Results

Environmental variables

During the study period an accentuated variation on discharge was observed (Fig. 3) and it is related to the hydrological annual cycle: a decrease from early spring to summer was observed followed by an increase in autumn. The occurrence of a flood during spring was responsible for a peak in discharge observed in samples taken on 11 April. In June the discharge was lower than 10 l s^{-1} , and in August dropped to zero, with no superficial flow at the non-impaired site. At the impaired site, the flow was residual, maintained only by WWTP effluent. Soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN) were higher at the impaired site than at the non-impaired site (Fig. 3), except in early April (11 April) under the effect of flooding, when DIN was similar at both sites, and SRP showed the most similar concentrations between the two sites. Increases in discharge usually resulted in elevated nitrogen and phosphorus (Marti & Sabater, 1996; Marti et al.,

1997). However, DIN was relatively high at the impaired site during late spring and summer, and SRP presented an increased pattern during the same period despite the low discharge. Nine days after the flood event (19 April), SRP and DIN dropped to very low values (0.0 mg l^{-1} and 0.5 mg l^{-1} , respectively). At the non-impaired site, SRP and DIN were generally very low (except for the flood situation), but did exhibit the same seasonal trend, with higher values recorded in late spring and summer. pH was higher at the non-impaired site than at the impaired site, but the variation patterns during the study period were similar for both sites, with a decrease in values obtained under the effect of flooding (Fig. 4). The same trend was observed for conductivity, with the lowest values recorded when an increase of discharge, resulting from flooding, was observed (Fig. 4). As for pH the variation pattern over the study period were similar for both sites, but higher values were recorded in impaired site. Concentrations of dissolved oxygen showed a decreased trend, related to the discharge decrease from spring to summer, with higher values recorded at the non-impaired site (Fig. 5). For both sites, the lowest values were recorded when the superficial flow ceased. The effect of flood on dissolved oxygen did not seem very clear. Temperatures were similar for both sites, with an increasing pattern related to a clear seasonal variation over the study period (Fig. 5).

Macroinvertebrate communities

The study reaches were chosen with respect to differences in the degree of organic pollution which would influence macroinvertebrate community structures. During the studied period richness was higher at the non-impaired site (Fig. 6), except during summer, when discharge was lowest: in June, under small baseflow, the richness became similar to the impaired site; in August, with an absence of flow, the richness dropped to values lower than at the impaired site. In contrast, at the impaired site, the highest richness was recorded during summer under a baseflow maintained only by the WWTP effluent (Fig. 6). A decreasing tendency was observed for both sites in samples collected one day after the flood occurred in spring (10 April). Abundances at the impaired site were always higher than at the non-impaired site (Fig. 6). The temporal pattern of variation was similar for both sites, with maximum values recorded during spring, and lower values recorded during summer (under lowest discharge) and winter (under highest discharge).

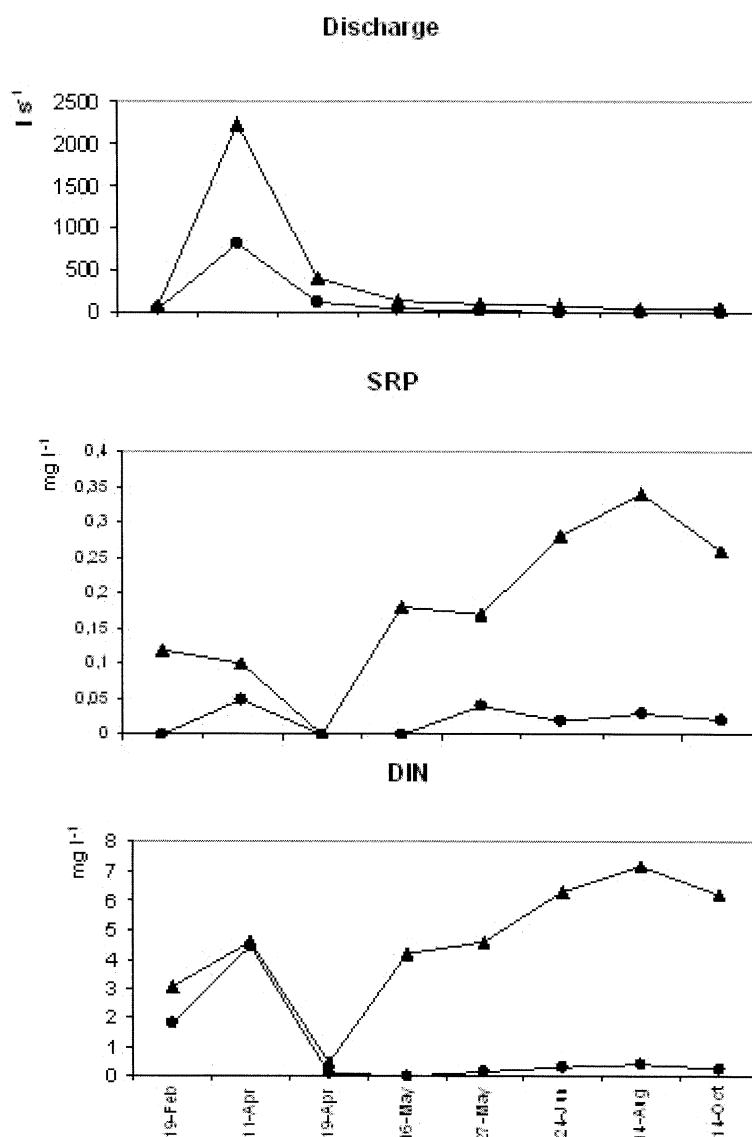


Figure 3. Temporal variation in discharge, dissolved inorganic nitrogen (DIN) and soluble reactive phosphorous (SRP) at the two sites over the study period (square: upstream non-impaired site; triangle: downstream impaired site).

However, minimum values were observed one day after the flood, reflecting the influence of accentuated discharges on macroinvertebrate dislodgement and mortality.

The results of PCA ordination showed that each principal component accounted for more than 20% of the variance (Table 4). The PCA ordination, performed to detect ecological gradients on macroinvertebrate communities, showed an opposition between the two sites along the first axis (Fig. 7). Along this axis, samples taken at the impaired site had positive scores, while those from the non-impaired site were

Table 4. Eigenvalues and percent of variance accounted for by the first two principal components in the PCA analysis

Component	Eigenvalues	% of variance	Cumulative % of variance
1st	0.317	31.7	31.7
2nd	0.218	21.8	53.5

negatives. Concerning community composition, more tolerant taxa, such as *Dero* sp., *Erpobdella* sp., Diptera

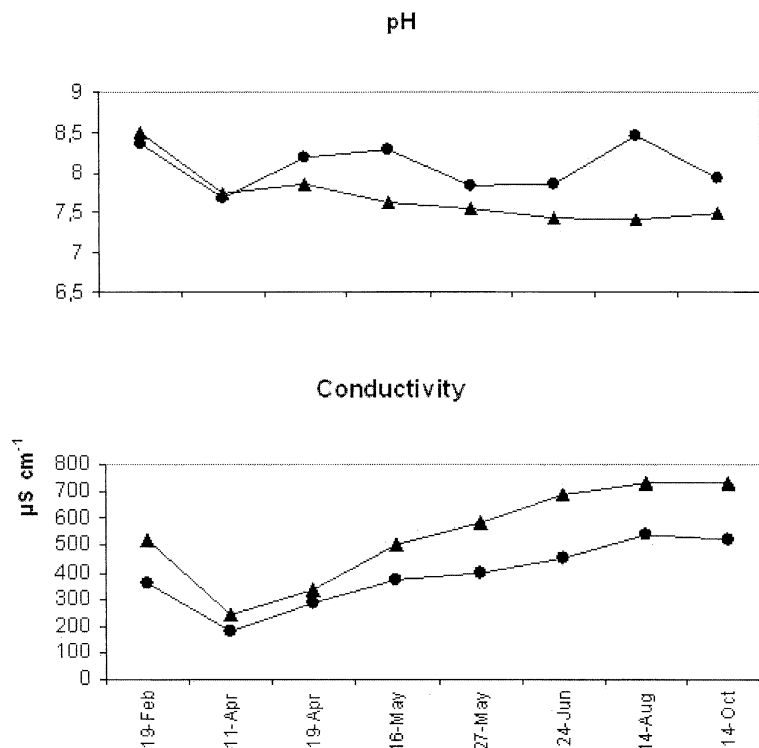


Figure 4. Temporal variation in water conductivity and pH at the two sites over the study period (square: upstream non-impaired site; triangle: downstream impaired site).

and Gasteropoda (*Physa acuta* Draparnaud), tended to be associated with samples taken at the impaired site [positive scores on the first principal component (Fig. 8)], while less tolerant ones, such as Ephemeroptera, Plecoptera and Trichoptera [negative scores on first principal component (Fig. 8)], were related to the upstream site (non-impaired). This fact indicated a relation between the first component and organic degradation that was confirmed by positive significant correlations obtained with soluble reactive phosphorous ($r = 0.68$, $p < 0.01$, $n = 16$), dissolved inorganic nitrogen ($r = 0.79$, $p < 0.01$, $n = 16$) and conductivity ($r = 0.67$, $p < 0.01$, $n = 16$), as well as the significant negative correlation obtained with dissolved oxygen ($r = -0.72$, $p < 0.01$, $n = 16$). A lower significant negative correlation was also detected between the first component and pH ($r = -0.59$, $p < 0.05$, $n = 16$) (Table 5). The temporal pattern of variability for macroinvertebrate communities, at both sites, seems to be associated with the second principal component. Samples taken under higher discharges (from 10 February to 27 May), with negative scores on the second component, are in opposition to samples taken during summer (24 June and 14 Au-

gust). Concerning community composition, for both sites, samples taken under higher discharges were related to taxa associated with lotic habitats (*Baetis* sp., Plecoptera and Trichoptera at non-impaired site and Simuliidae at impaired site). In contrast taxa associated with lentic habitats occurred mainly during summer (Odonata, Leptophlebiidae and *Atyaephra desmarestii* (Millet) at the non-impaired site and *Chironomus* sp. and *Dero* sp. at the impaired site). Autumn samples (14 October), for both sites, were taken just after the first rainfalls, corresponding to a discharge increase. For this reason, autumn samples on the second component are closer to samples taken during late winter and spring (19 February to 27 May). Despite the temporal pattern detected along the second principal component, neither discharge nor water velocity were correlated with this component ($p > 0.05$, $n = 16$). Probably the accentuated contrasting characteristics of water quality (dissolved oxygen, soluble reactive phosphorous, dissolved inorganic nitrogen and conductivity) masked the effect of hydrological conditions along the second principal component.

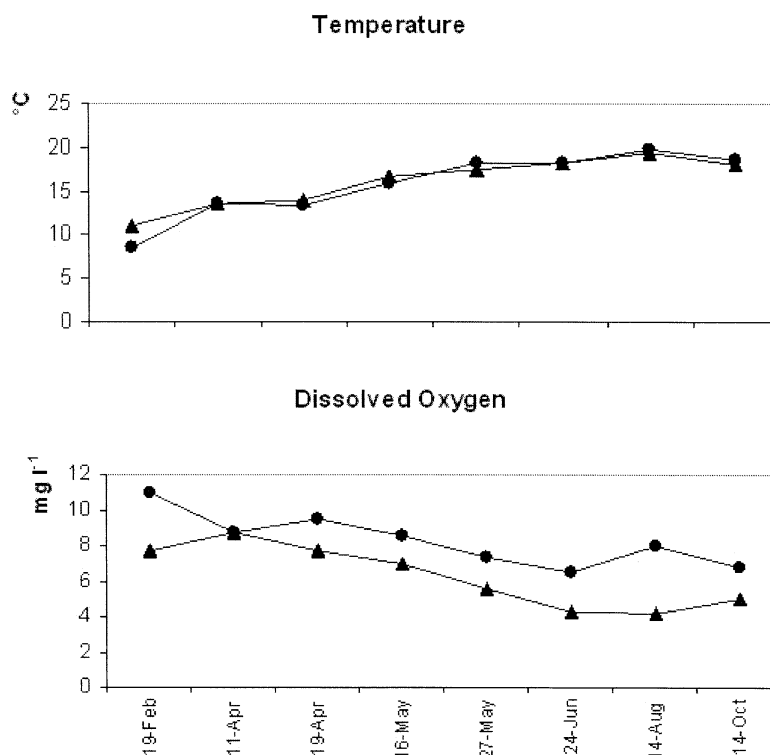


Figure 5. Temporal variation in water temperature and dissolved oxygen at the two sites over the study period (square: upstream non-impaired site; triangle: downstream impaired site).

Table 5. Pearson correlation coefficients between site scores on first two axis of PCA analysis and environmental variables. Significance levels of correlation coefficients: * = $p < 0.05$; ** = $p < 0.01$

	1st axis	2nd axis
Dissolved inorganic nitrogen	0.677** ($n = 16$)	0.289 ($n = 16$)
Soluble reactive phosphorous	0.787** ($n = 16$)	0.391 ($n = 16$)
Dissolved oxygen	-0.722** ($n = 16$)	-0.471 ($n = 16$)
pH	-0.586* ($n = 16$)	-0.176 ($n = 16$)
Temperature	0.235 ($n = 16$)	0.465 ($n = 16$)
Conductivity	0.666** ($n = 16$)	0.532* ($n = 16$)
Water velocity	-0.209 ($n = 16$)	-0.484 ($n = 16$)
Discharge	-0.043 ($n = 16$)	-0.185 ($n = 16$)

Assessment methodologies

When using benthic macroinvertebrate communities for bioassessment, temporal variation may influence judgement as to whether or not a site is degraded. For this reason, variability of tested metrics and indices were studied over the whole sampling period, for both sites (Figs 9, 10). Three different temporal patterns of variation were observed at the non-impaired site: the first one, concerning ASPT', BMWP', BBI, DSFI, IBE and TRICF, presented a quite stable trend from late winter (10 February) to spring (27 May), succeeded by a decrease in summer (24 June); the second one, including richness, Shannon–Wiener index and IM9, showed small fluctuations throughout the sampling period; the third one, represented by GOLD, showed small fluctuations only with an episodic decrease on 27 May. This episodic decrease is a consequence of an occasional peak of *Atyaephyra desmarestii*. Generally, at the impaired site, metrics and index scores had a more stable temporal pattern of variation, except BMWP' and TRICF. BMWP' showed a slightly decrease after the spring flood (11 April), and then it increased to summer (24 June). TRICF, also at the im-

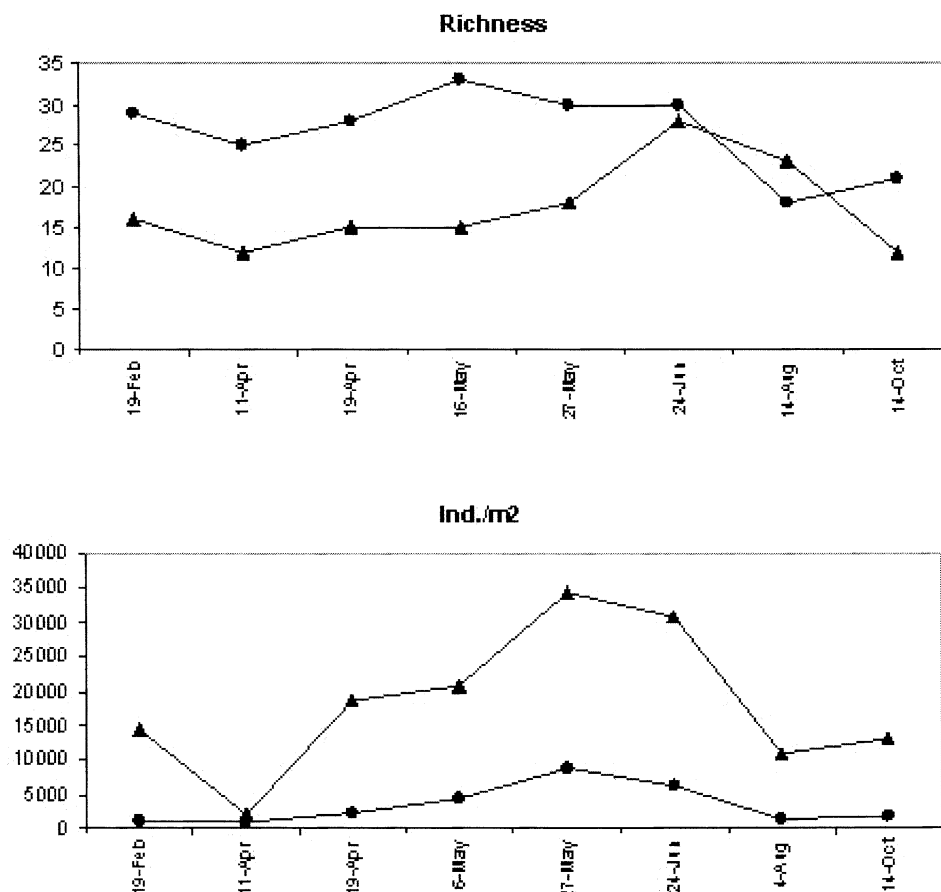


Figure 6. Temporal variation in richness and macroinvertebrates abundance (individuals m^{-2}) at the two sites over the study period (square: upstream non-impaired site; triangle: downstream impaired site).

paired site, presented an increase to spring, succeeded by a decrease to autumn (14 October) (Figs 9, 10). After the April flood, most of the metrics and the multimetric index showed a decrease. The only exception was the Shannon–Wiener index and evenness that, at the impaired site, increased just after the flood.

Quality classes, obtained by metrics and index whose boundaries between quality classes were established in the literature (Table 2), also showed some variability during the sampling period. IM9 and GOLD showed a more stable temporal pattern concerning the obtained classifications.

Box-and-whiskers plots comparing both sites, from 10 February to 27 May, showed almost no overlap between scores obtained for each metric or index (Figs 11, 12). When an overlap occurred (TRICF and ASPT'), it was always lower than 25% (Figs 11, 12). However, when the whole study period was analysed (10 February to 14 October), the Shannon–Wiener in-

dex, GOLD and IM9, did not show any score range of variation overlapping between non-impaired and impaired sites. Concerning the other metrics, most of them had overlaps higher than 25% (Figs 13, 14).

Stepwise regressions performed to evaluate the influence of environmental factors on metrics and indices scores (Table 6) showed that most of tested metrics were significantly related to environmental variables. However, richness (S), IBE and BMWP' were not significantly influenced by any regression model including environmental variables. By contrast, the Shannon–Wiener index and evenness were strongly (negatively) related to soluble reactive phosphorous, and DSFI could be influenced by a combination of dissolved oxygen, pH and discharge. In a similar way, ASPT' seems to be influenced by dissolved oxygen plus discharge and BBI was related to dissolved oxygen. In addition, TRICF and EPT were only weakly significant, respectively to dissolved inorganic nitro-

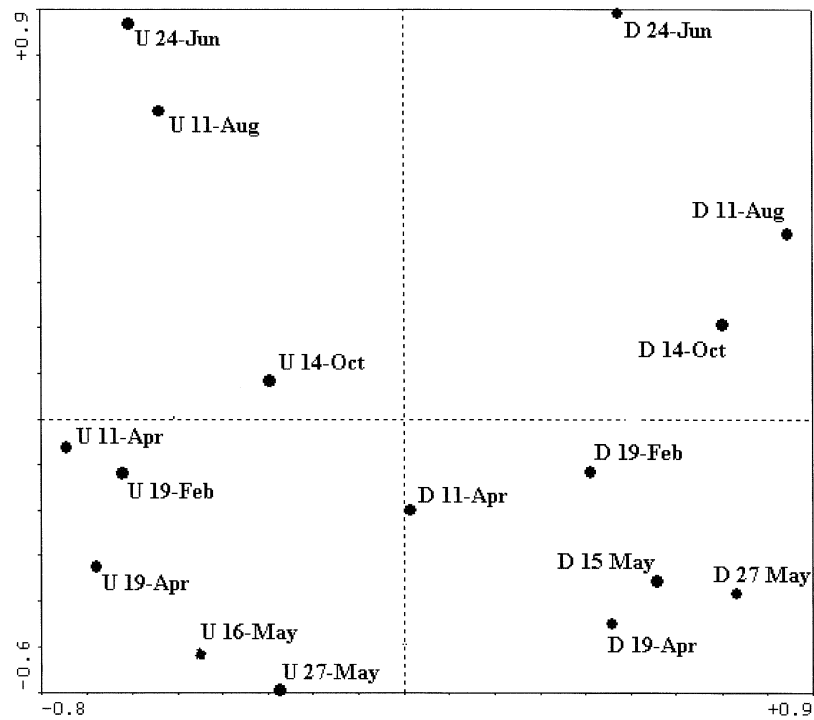


Figure 7. Two-dimensional PCA ordination plot of invertebrate communities in the two sites sampled during the study period.

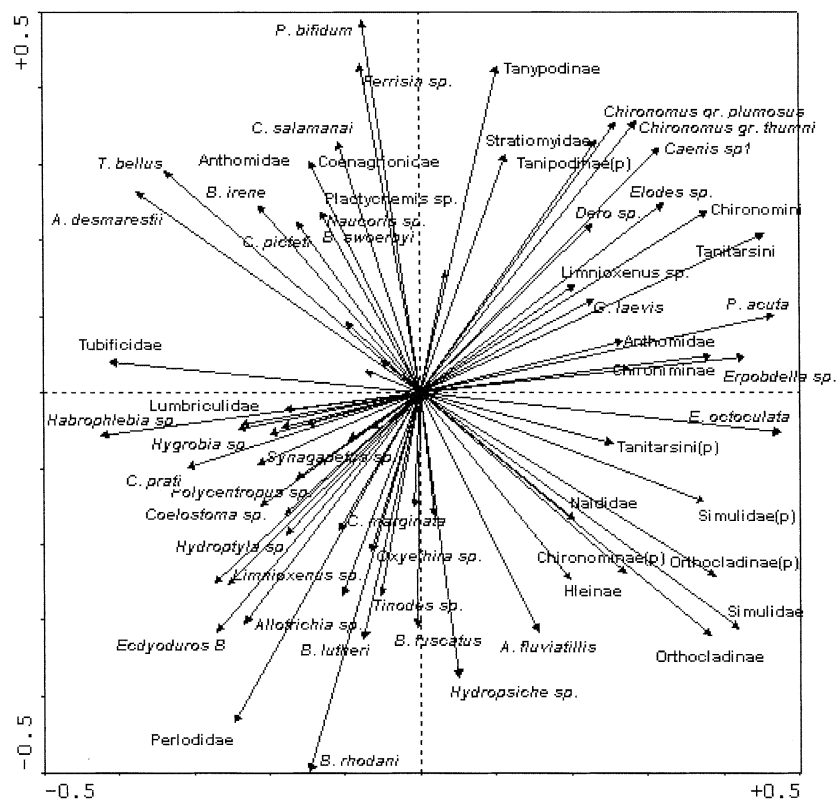


Figure 8. Two-dimensional PCA ordination plot of macroinvertebrate taxa.

Table 6. Summary of results of stepwise regression analyses of environmental variables that influence metric and multimetric indices. Variables are listed in order of their appearance in a stepwise model.

	Independent variable	Coefficient	r^2	F	p
H	SRP	-5.005	0.757	43.51	0.00001
S	No significant relations were found				
E	SRP	-1.456	0.777	48.88	0.00001
BBI	DO	0.498	0.431	10.60	0.006
IBE	No significant relations were found				
BMWP'	No significant relations were found				
ASPT'	DO	0.257	0.646	11.84	0.001
	Discharge	-0.005			
DSFI	DO	0.655	0.892	32.92	0.00001
	pH	-1.147			
	Discharge	-0.0007			
TRICF	DIN	-0.016	0.259	4.88	0.044
EPT	SRP	-1.189	0.277	5.36	0.036
GOLD	SRP	-1.932	0.481	12.98	0.002
IM9	SRP	-0.889	0.524	15.42	0.001

gen and soluble reactive phosphorus, while GOLD was strongly (negatively) related to soluble reactive phosphorus. Concerning the IM9 (multimetric index), the regression model including soluble reactive phosphorus as the sole independent variable had significant influence ($F = 15.42$, $p < 0.001$, $r^2 = 0.52$).

Discussion

The predominant pattern of physical variation in a temporary stream is the cycle of flood and flow cease. In Grândola stream, the strongest effect of a spring flood was a change in chemical and macroinvertebrate structure. The flood caused an acute increase in dissolved inorganic nitrogen at both study sites, and a decrease in pH and conductivity related to the dilution effect (Fisher & Minckley, 1978). An increase in dissolved inorganic nitrogen after floods was also reported in two mediterranean streams in Spain (Marti & Sabater, 1996). The lower values of conductivity present in rainfall diluted anion concentrations of the stream water, in chemical balance with the substrate. Macroinvertebrate abundance one day after the flood showed an enormous decrease at the impaired site. This result indicates that disturbances by floods not

only affect physical and chemical attributes but also biological structure. However, the post-flood increase in macroinvertebrate abundance was within the values recorded during the pre-flood period. Such resilience studies in temporary streams have been reported previously (e.g., Doeg et al., 1989; Grimm & Fisher, 1989; Lake & Schreiber, 1991). The main factor that may account for the apparent high resilience of the stream macroinvertebrate community was the rapid decrease in discharge from 1515 to 258 l s⁻¹. In fact the flood that occurred during the study period was of medium magnitude (peak discharge = 1515 l s⁻¹) compared with others reported in southern Portugal temporary streams (Bernardo & Alves, 1999). For this reason, the detected flood did not seem greatly to influence richness. Only a decreasing tendency was observed for both sites in samples collected one day after the flood. Floods of this magnitude occur more than once a year. These floods usually do not cause major physical changes in morphology, but they wash out benthic communities.

Following a flow decrease along spring-summer period, for both sites, only discharges lower than 17 and 94 l s⁻¹ at the non-impaired and impaired sites, respectively (27 May), seem to influence macroinvertebrate structure, with abundances decreasing to the lowest values, recorded in summer dry conditions (flow interruption at the non-impaired site, base-flow maintained only by WWTP effluent at the impaired site). These lowest values are related to adaptation strategies developed by invertebrates against predictable events over time. Delucchi (1989) refers to the fact that stream invertebrates may have survived the dry period as drought resistant forms in the substrate, or as terrestrial adults that emerged from the temporary stream before it dried. In contrast with the non-impaired site, at the impaired site, the highest richness was recorded in summer. Several factors may account for this pattern, among them the fact that invertebrates did not need to develop a life cycle to avoid the flow interruption conditions and that a relatively large diversity of food availability is present in summer, with a high density of filamentous green algae and diatoms.

Concerning environmental variables, major differences between sites were found for dissolved inorganic nitrogen and for soluble reactive phosphorus, with higher concentrations at the impaired site than at the non-impaired site, especially in spring and summer. These high concentrations came from the WWTP effluent and contributed to organic pollution degrad-

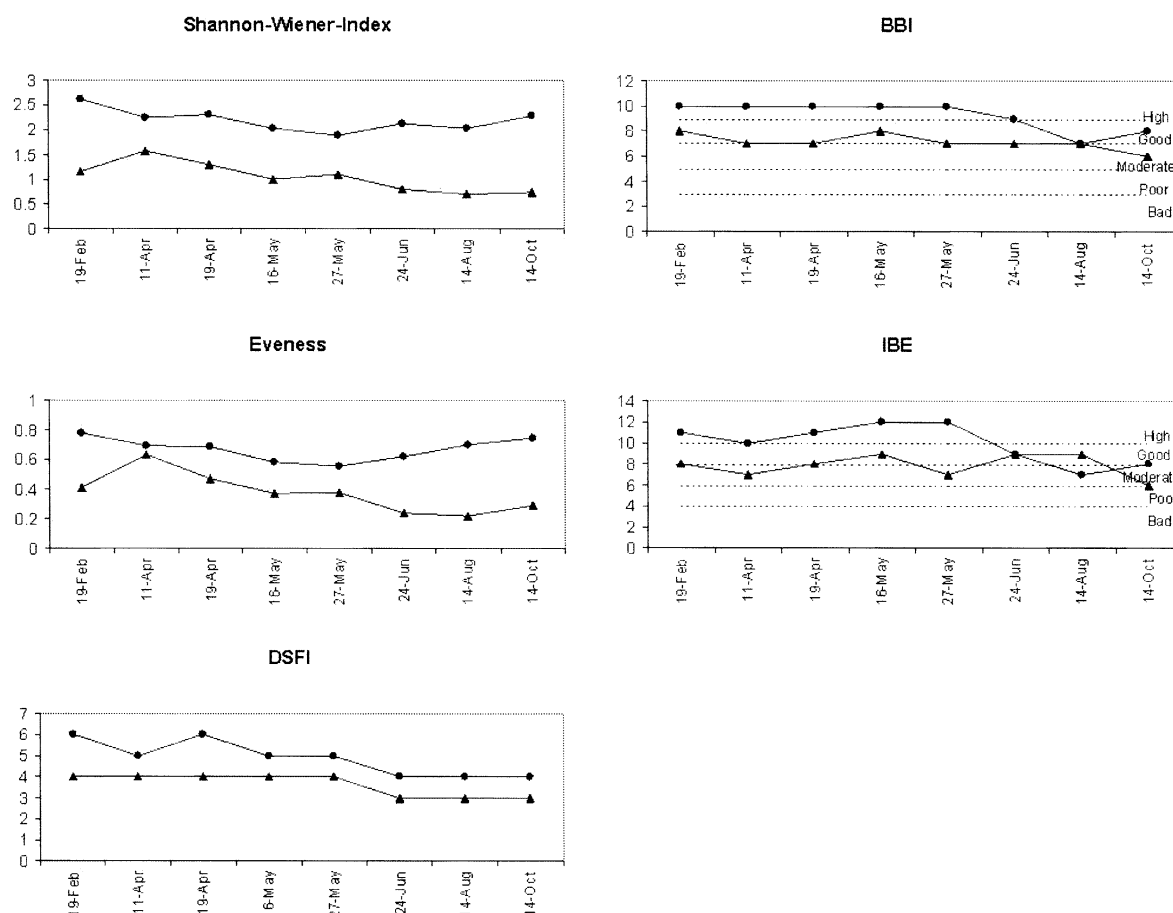


Figure 9. Temporal variation in metrics scores at the two sites over the study period (square: upstream non-impaired site; triangle: downstream impaired site). The horizontal dotted lines represent boundaries between quality classes.

ation at this site. In autumn, with the beginning of rainfall, dissolved inorganic nitrogen and soluble reactive phosphorus tended to be more diluted, presenting lower concentrations. Only under flood conditions did dissolved inorganic nitrogen and soluble reactive phosphorus becomes similar at the two sites: at the non-impaired site, an acute increase was observed in dissolved inorganic nitrogen, while at the impaired site, the values observed in late spring rose.

Organic pollution and temporal pattern are the main factors influencing the macroinvertebrate community structure of the studied sites. Along the first component in the PCA analysis, the two sites were clearly separated. Temporal variability was only expressed on the second component, denoting an opposition between samples taken under high and low discharge conditions, as occurred in other Mediterranean streams (Rossaro & Pietrangelo, 1993). These results indicated that most of data variance was ex-

plained by organic degradation (31% of all variance in first component). Concerning seasonal variation over the study period, the two communities followed the general temporal pattern observed in other mediterranean streams, presenting more sensitive taxa under high discharge, and more tolerant taxa under low discharge. The flood influenced macroinvertebrate community structure with a different magnitude at each site. A high decrease in abundance was observed at the impaired site (subjected to a higher discharge peak), and in the first principal component this sample appears clearly separated from the others recorded at the same site. Concerning the non-impaired site, only a slight flood disturbance was detected. The abundance decrease was low, and along the first principal component no evident separation was detected from the others taken at this site.

Generally, metrics and index followed the temporal pattern of macroinvertebrate community variation, al-

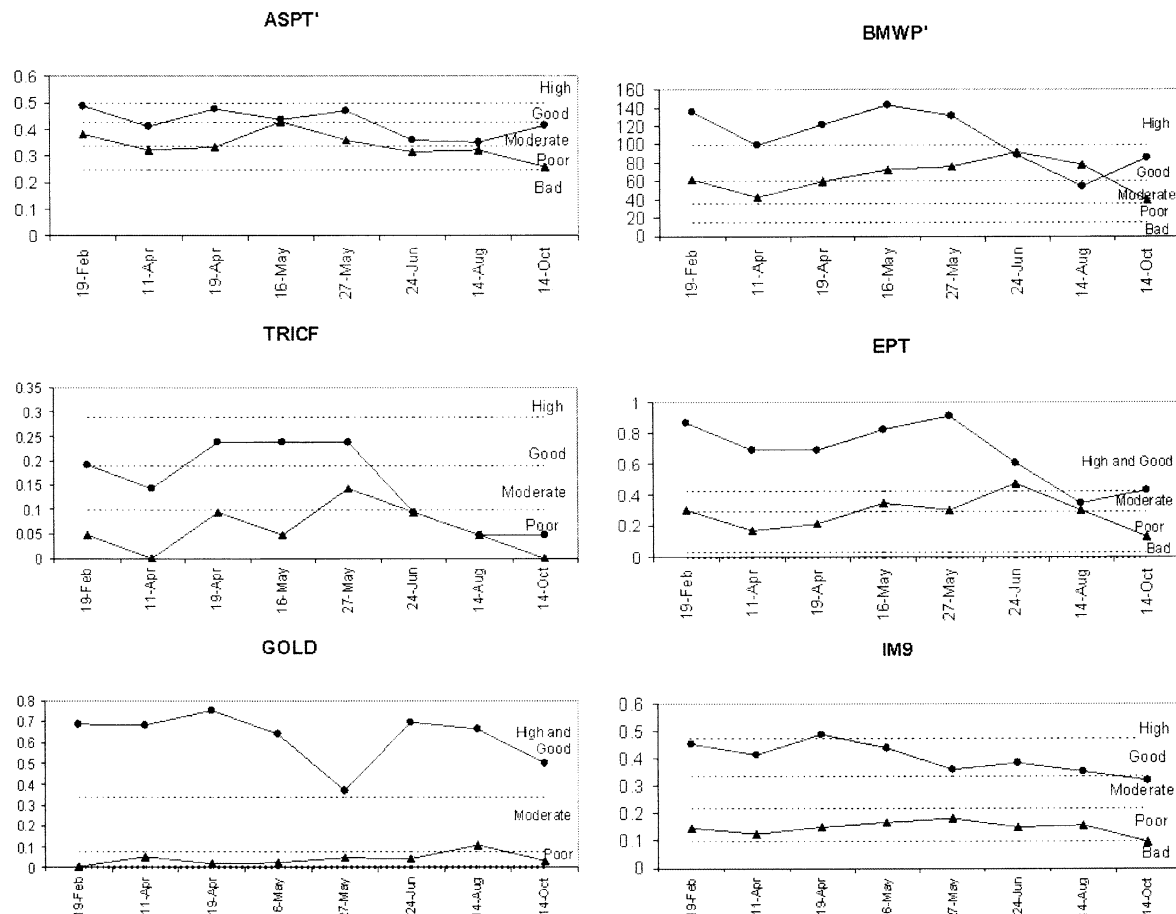


Figure 10. Temporal variation in metrics- and multimetric scores at the two sites over the study period (square: upstream non-impaired site; triangle: downstream impaired site). The horizontal dotted lines represent boundaries between quality classes.

though with differences in score magnitudes. In a different way, all metrics and the index were influenced by the flood. Most of them decreased after flood, reflecting the effect of organism dislodgement and mortality. The Shannon–Wiener diversity index and evenness had opposite patterns of variation at each site, increasing at the impaired site and decreasing at the non-impaired site. One day after the flood, the accentuated decrease in abundance at the non-impaired site reflected the increase of those metrics. However, this increase does not mean better quality; it is only a consequence of flood disturbance on macroinvertebrate community. Concerning GOLD, only a slightly increase was observed for both sites, which can result from a higher resistance of gasteropoda to being dislodged. (Stanley et al., 1994)

The seasonal effect of the discharge decreasing from winter to summer is more evident on non-

impaired metric scores (decreasing tendency), due to the substitution of sensitive taxa (spring) by tolerant taxa (summer). For this reason, the efficiency of metrics tends to decline under low discharge (Koetsier, 2002). The quite stable tendency observed at the impaired site is explained by the absence of sensitive taxa during the high discharge period (late winter to spring).

BMWP', commonly used in the Iberian Peninsula (Zamora Muñoz & Alba Tercedor, 1996; Cortes et al., 2002), showed accentuated fluctuations of high magnitude from 19 February to 14 October. Those fluctuations were very similar to the temporal pattern of richness variation, denoting dependence of this metric on the number of taxa. In fact, BMWP' being a sum of scores, it tends to be higher among communities with a higher number of taxa. ASPT', being an average score per taxa, is more stable, because it is independ-

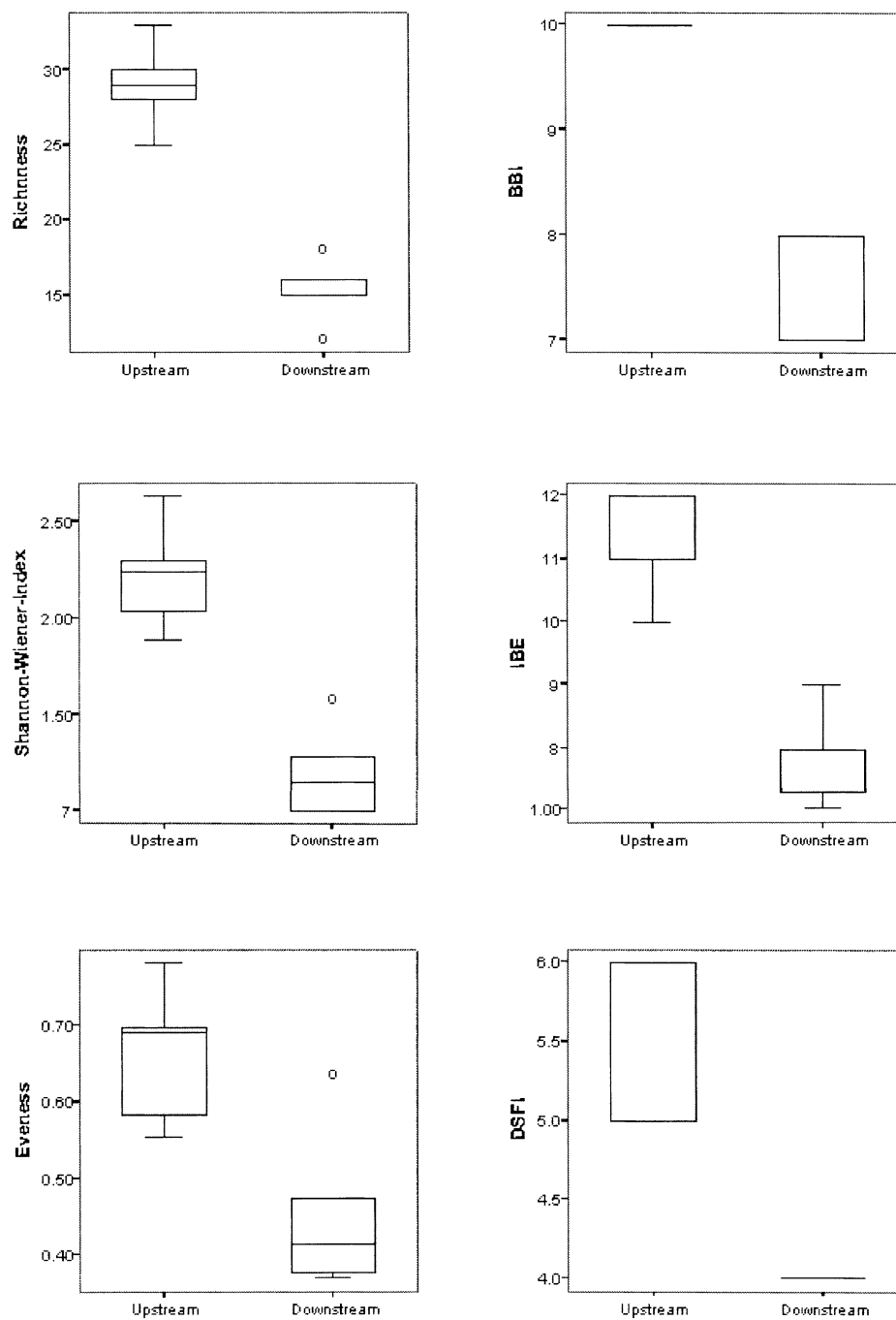


Figure 11. Discriminatory power of macroinvertebrate metric- for the two study sites from 10 February to 16 May (excluding summer and autumn samples). Range bars show maximum and minimum of non-outliers; boxes are interquartile ranges (25 percentile to 75 percentile); bars in boxes are medians; small open circles are outliers.

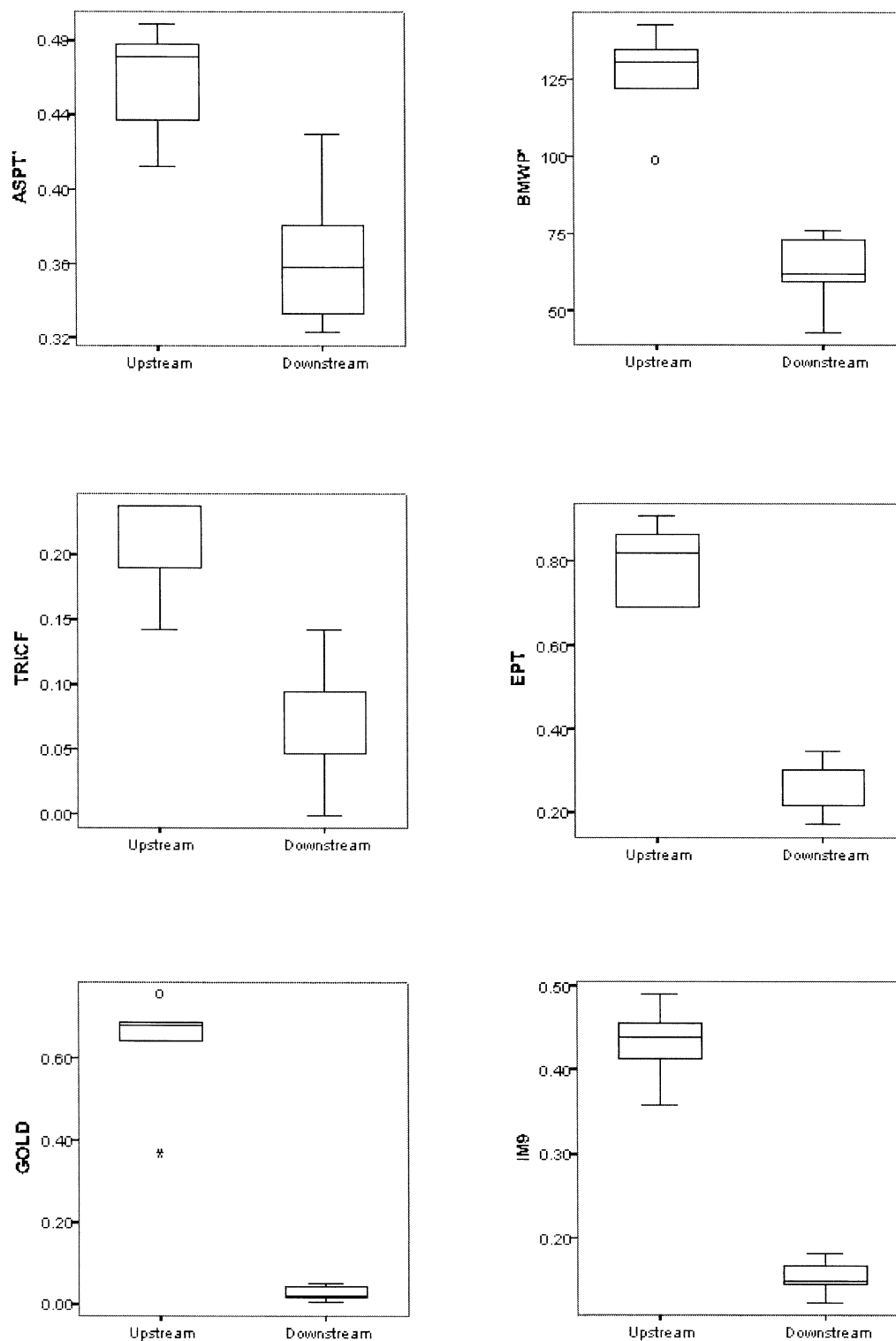


Figure 12. Discriminatory power of macroinvertebrate metric- and multimetric index for the two study sites from 10 February to 16 May (excluding summer and autumn samples). Range bars show maximum and minimum of non-outliers; boxes are interquartile ranges (25 percentile to 75 percentile); bars in boxes are medians; small open circles are outliers.

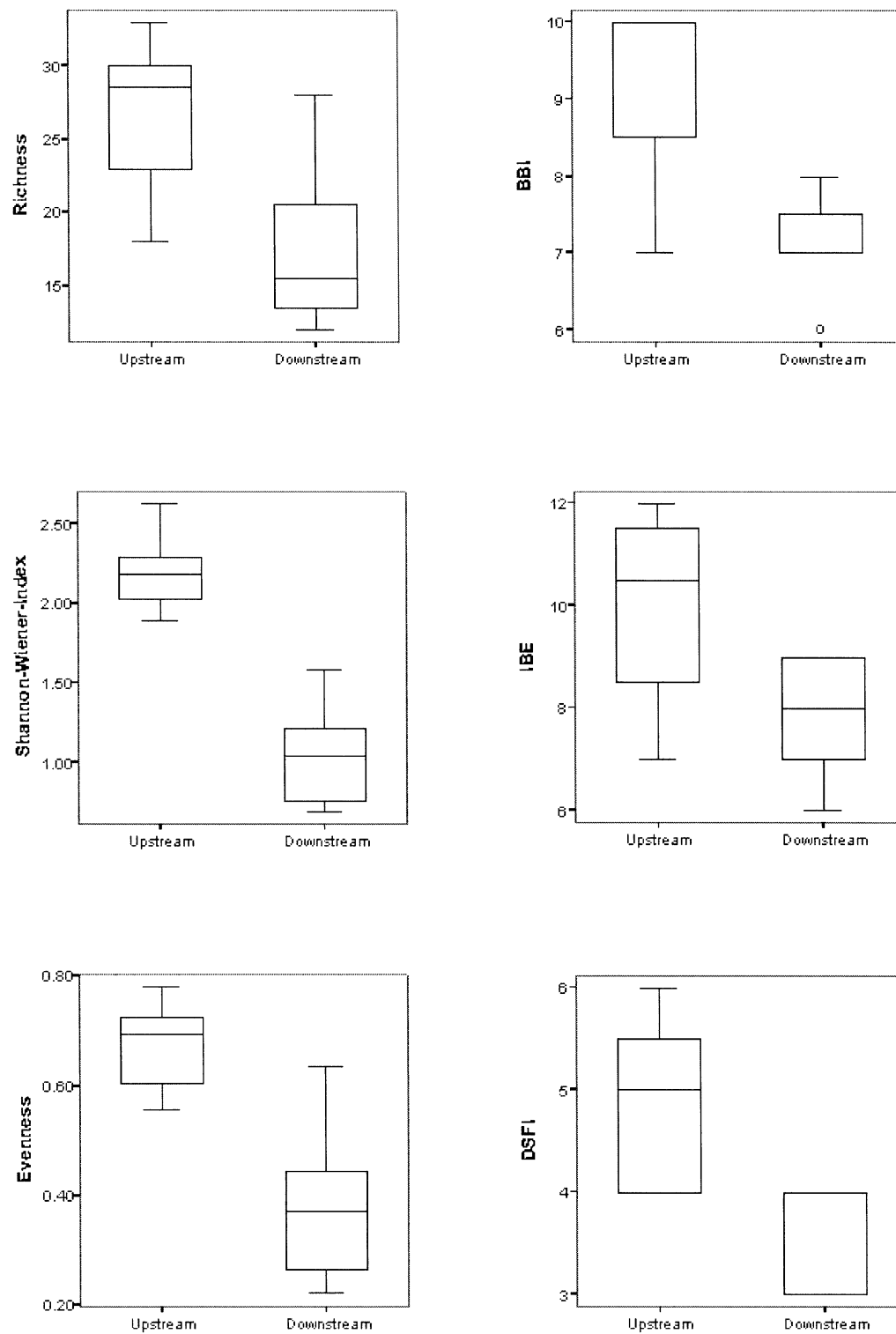


Figure 13. Discriminatory power of macroinvertebrate metric- for the two study sites from 10 February to 14 October (over the whole study period). Range bars show maximum and minimum of non-outliers; boxes are interquartile ranges (25 percentile to 75 percentile); bars in boxes are medians; small open circles are outliers.

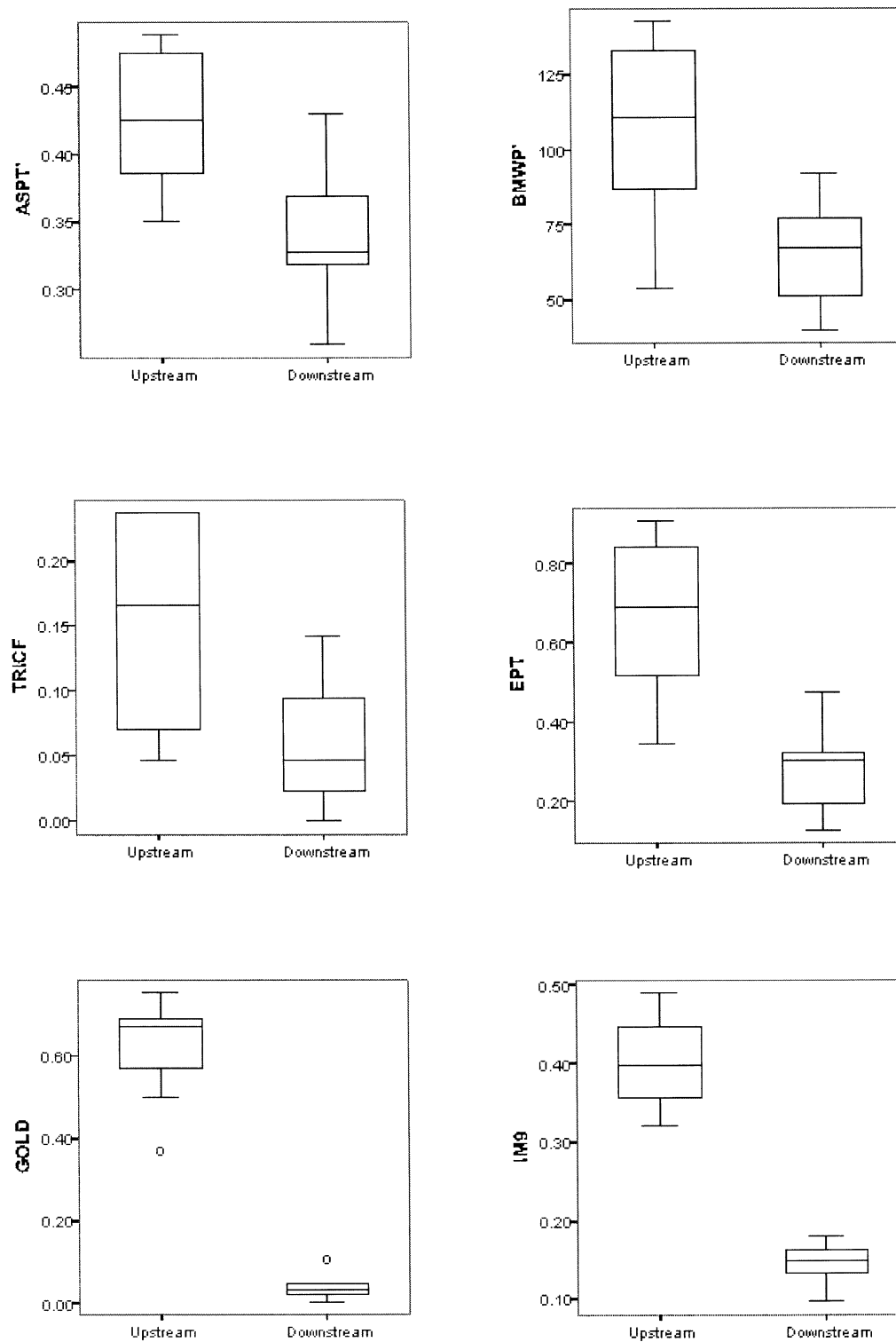


Figure 14. Discriminatory power of macroinvertebrate metric- and multimetric index for the two study sites from 10 February to 14 October (over the whole study period). Range bars show maximum and minimum of non-outliers; boxes are interquartile ranges (25 percentile to 75 percentile); bars in boxes are medians; small open circles are outliers.

ent from the number of taxa. For this reason ASPT' is more useful in assessing temporary streams.

Stepwise regressions showed that SRP influenced 50% of the tested metrics and index, with high coefficients of determination (range from 0.28 to 0.78). Despite the great variation in discharge over the study period, discharge is only included in two models (ASPT' and DSFI), and as the last of the independent variables. These results suggest that, for 77% of tested metrics (H, E, BBI, TRICF, EPT, GOLD, IM9), hydrological variability is not a predictive variable for assessing quality status.

The attributes for a good assessment methodology are: stability under natural community fluctuations; capacity to discriminate quality classes; applicability for a large period of time (Barbour et al., 1999; Boulton, 1999; Linke et al., 1999; Barbour & Yoder, 2000). For this reason, metrics with overlapping scores between sites with different quality status must be rejected.

Box-and-whiskers plots for metrics and multimetric index covering the period from February to May (excluding summer and autumn samples) showed few overlaps between the two study sites. However, for most metrics, the range of variation for each site would be more separated, according to first principal component ordination, which completely splits the two sites. GOLD, EPT and IM9 presented the more separate ranges of variation between the two sites, presenting higher performance to discriminate two contrasting quality statuses. The same analysis performed for the whole study period, showed a higher degree of overlap. This could be explained by the fact that contrasting hydrological situations are included, with changes in community structure as expressed in the second principal component of ordination. In this period, only GOLD and IM9 maintained their ranges of variation completely split. For this reason, GOLD and IM9 were considered the best methodologies for assessing temporary Mediterranean streams. The Shannon-Wiener index also presents separated ranges of variation, but not so divided as GOLD and IM9. As expected, IM9 presented the lowest score magnitude fluctuations, because it is a multimetric index composed of three metric categories (ASPT'-tolerance, GOLD-composition and TRICF-richness), which compensates for the less discriminatory power of each component metric. Those results confirmed previous studies about better performances of multimetric indices when compared to single metrics

(Paller & Specht, 1997; Maxted et al., 2000; Klemm et al., 2002)

The multimetric index IM9, developed for temporary southern Portuguese streams (Pinto et al., 2003) confirmed its robustness when compared with common European metrics (AQEM consortium, 2002) along a wider period of time which included an unpredicted flood and summer flow interruption.

Future research should be done covering the complete organic degradation gradient and extended to temporary streams from other latitudes (Olson & Soderstrom, 1978; Sommerhauser et al., 1997; Meyer & Meyer, 2000).

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