Chapter 2

A COMPARISON OF SAMPLING METHODOLOGIES TO ASSESS BIOLOGICAL QUALITY IN TWO MEDITERRANEAN AREAS[1]

INTRODUCTION

Rapid Bioassessment Protocols (RBPs) have been widely used in different countries to assess biological river quality (Wright *et al.*, 1984; Plafkin *et al.*, 1989; Davies, 1994; Chessman, 1995; Growns *et al.*, 1995; Tiller & Metzeling, 1998; Chutter, 1998; Barbour *et al.*, 1999). All these methodologies intend to be efficient, effective, low in cost and easy to use (Resh & Jackson, 1993; Lenat & Barbour, 1994; Resh *et al.*, 1995), but significant differences exist between sampling procedures and metrics used. Numerous metrics are used to evaluate biological conditions (Kerans *et al.*, 1992; Lenat & Barbour, 1994; Resh, 1994; Resh *et al.*, 1995; Barbour *et al.*, 1996) but biotic indexes have been the most used around the world (e.g., Washington, 1984). Although several shortcomings in the use of indexes to assess water quality are found (Washington, 1984; Norris & Georges, 1993), they have been commonly used as metrics highly robust, sensitive, cost-effective and easy to apply and to interpret (Chessman *et al.*, 1997).

[1] This Chapter will be submitted to an international journal under the same title and authored by the following researchers: Bonada, N.; Dallas, H.; Rieradevall, M.; Day, J. & Prat, N.

The organisms more used to assess biological quality are periphyton, macroinvertebrates and fish (Plafkin *et al.*, 1989; Barbour *et al.*, 1999). Traditionally, macroinvertebrates have been the most commonly used organisms (see Rosenberg & Resh, 1993; Chessman, 1995), and a large set of biotic indexes operates around the world (Davis, 1995). These indexes have different sampling methodologies in terms of gears and mesh size used, sampling habitats, sampling intensity and/or processing of samples, but in general, a qualitative or semi-quantitative sampling is performed in the sense of RBPs (Lenat & Barbour, 1994). Several authors have studied the effect of the sampling technique used and metrics to assess water quality, and differences among them but complementary results have been reported (Barton & Metcalfe-Smith, 1992; Kerans *et al.*, 1992). Sampling habitats also vary among sampling protocols, although in the RBPs where sampling effort is kept at the minimum possible level, a single sample from "most productive habitat" have been proposed as optimum (Plafkin *et al.*, 1989). However, because of human impact can be specific to an unknown particular habitat and/or sometimes the most productive habitat is not evident, other protocols emphasize samplings in all habitats (Kerans *et al.*, 1992; Stribling *et al.*, 1993; Resh *et al.*, 1995). The processing of samples also is important in RBPs, and a large variety of methods and controversies about the fraction of sample to be used are present (see Carter & Resh, 2001). Some methods are designed to be processed in the field, when usually macroinvertebrates are identified at family level (Prat *et al.*, 2000). Taxonomical level to be used in bioassessment also have been highly discussed (Resh & Unzicker, 1975; Cranston, 1990; Marchant *et al.*, 1995; Bowman & Bailey, 1997), and although a lower taxonomical resolution implies a better precision and information (Furse *et al.*, 1984; Resh *et al.*, 1995; Stubauer & Mogg, 2000), family level shows similar distribution patters of communities than genera or species (Furse *et al.*, 1984; Ferrano & Cole, 1992; Rutt *et al.*, 1993; Marchant *et al.*, 1995; Zamora-Muñoz & Alba-Tercedor, 1996; Bowman & Bailey, 1997; Nielsen *et al.*, 1998). Consequently, numerous biotic indexes use the family level because of its simplicity and cost-effectiveness (Armitage *et al.*, 1987; Hilsenhoff, 1988; Alba-Tercedor & Sánchez-Ortega, 1988; Corkum, 1989; Prat *et al.*, 1999, 2000, Hewlett, 2000). As a consequence of this high variability in sampling techniques and processes (Carter & Resh, 2001), methods can have different bias, and thereby comparisons between biotic indexes from different areas can be difficult (Erman, 1981; Kerans *et al.*, 1992; Diamond *et al.*, 1996).

Mediterranean climate is defined in terms of precipitation (di Castri, 1973) and temperature (Aschmann, 1973) with hot and dry summers and cool and wet winters. Consequently, mediterranean rivers are subjected to a natural flow disturbance that implies the presence of seasonal floods and droughts (Molina *et al.*, 1994; Gasith & Resh, 1999). Although a high similarity is noticed in the macroinvertebrate responses to habitat, temporality and pollution (Bonada *et al.*, Chapter 3), local factors related to the microclimate, geology or substrate are the responsible of several differences in communities found between mediterranean regions. As a consequence, a RBP methodology developed in one mediterranean region could not be applied successfully in another, and therefore comparative studies to examine the applicability of methodologies in other areas are required. In that sense, Diamond *et al.* (1996) recommend a comparison of methods in reference and test sites.

The aim of this study is to compare the applicability of two RBPs methodologies used to assess biological quality in two mediterranean areas: SASS5 in South Africa (South African Scoring System vs.5) and IBMWP (Iberian Biological Monitoring Working Party) according to Guadalmed protocol in Spain (Bonada *et al.*, Chapter 1; Jaimez-Cuéllar *et al.*, in press). Both methodologies are designed to be applied in the field, identifying macroinvertebrates at family level and the metrics used to calculate the biologic index are similar. However, although both are multihabitat approaches, the habitat to be sampled, the gears used and sampling and sorting procedures are different. SASS5 (Chutter, 1998) and IBMWP (Alba-Tercedor & Sánchez-Ortega, 1988; Alba-Tercedor, 1996) are analogous to the BMWP used in Great Britain (Armitage *et al.*, 1983), FBI in United States (Hilsenhoff, 1988) and SIGNAL in Australia (Chessman, 1995, Chessman *et al.*, 1997). Both indexes have been largely applied in their respective countries giving good results and being sensitive to water pollution (Camargo, 1993; Dallas, 1995, 1997; Zamora-Muñoz *et al.*, 1995; Alba-Tercedor, 1996; Zamora-Muñoz & Alba-Tercedor, 1996; García-Criado *et al.*, 1999; Prat *et al.*, 1999; Alba-Tercedor & Pujante, 2000).

METHODOLOGY

Sampling sites

Macroinvertebrates were sampled simultaneously in a number of sites in South Western Cape (South Africa) and Catalonia (Spain) by the two former authors of this Chapter (one from South Africa –H.D.– and another from Spain –N.B.–). Each one applied their own methodology in either Spanish and South African streams and the macroinvertebrates found were used to calculate biotic indexes from each region. Both have high skills and a long experience in macroinvertebrate sampling and field identification.

All samples were collected in spring season for an appropriate comparison: October of 2001 in South Africa, and April 2002 in Spain. In South Africa, 6 sampling sites from Eerste and

Palmiet basins were selected to perform the study (Figure 1a, Table 1). Langrivier (LA), Sosyskloof (SO) and Swartoskloof (SW) are tributaries from Eerste River, and are considered headwater streams. In Eerste River one site was located in the headwaters (EM), and the other downstream (EC) before the town of Stellenbosch. The site from Palmiet River is considered a foothill-lowland river site and it belongs to the Kogelberg Nature Reserve. All sites are located in the South African mediterranean area with vegetation dominated by mountain fynbos, with *Metrosideros angustifolia* or *Brabejum stellatifolium* in the riparian area and *Prionium serratum* in river banks, although in EC some introduced trees were found (*Acacia melonoxylon*, *Quercus robur*). Headwater sites and Palmiet have brown, acid and oligotrophic waters whereas EC have a slightly higher pH and conductivity, and significant agriculture runoff has been reported (Brown & Dallas, 1995). Thereby this site is considered as impaired in contrast to the others. Substrate is dominated by boulders, large stones and bedrock in the headwaters, and stones, pebbles and coarse sand downstream. Algae are scarce in such acidic conditions, but some macrophytes and mosses are abundant as instream vegetation in the lotic habitats of SW, EM and PA.

Table 1. Geographical, physical and chemical characteristics of the rivers sampled in South Africa and Spain.

		Category	Code	Altitude m.a.s.l.	Stream Ordre at 1:250000	Conductivity µS/cm	Temperature °C	pH	O_2 mg/l	O_2 %
	EERSTE BASIN									
SOUTH AFRICA	**Eerste mountain stream**	**Mountain stream**	**EM**	390	2	27.1	15.8	6.4	9.52	93.1
	Swartboskloof	**Mountain stream**	**SW**	390	1	25.2	15.7	5.9	7.5	75.5
	Sosyskloof	**Mountain stream**	**SO**	390	1	25.2	17.9	5	7	73
	Langrivier	**Mountain stream**	**LA**	390	1	25.9	17.4	5.8	8.45	84.6
	Eerste foothill	**Foothill**	**EC**	170	3	74.9	20	6.8	8.15	85.4
	PALMIET BASIN									
	Palmiet transitional	**Foothill-lowland**	**PA**	50	5	102.2	20.3	6.4	9.21	96.3
	BESÒS BASIN									
SPAIN	**Gallifa river**	**Mountain stream**	**B24**	560	1	695	11.3	8.4	10.07	96.4
	Ripoll river	**Foothill**	**B22**	340	2	654	16.1	8.6	8.85	92.6
	Tenes river	**Mountain stream**	**B28**	570	2	734	14.7	8.4	11.44	119
	Tenes river	**Foothill**	**B25**	250	2	778	14	8.4	10.41	102
	SIURANA-EBRE BASIN									
	Montsant river	**Foothill**	**MONT**	530	2	-	-	-	-	-

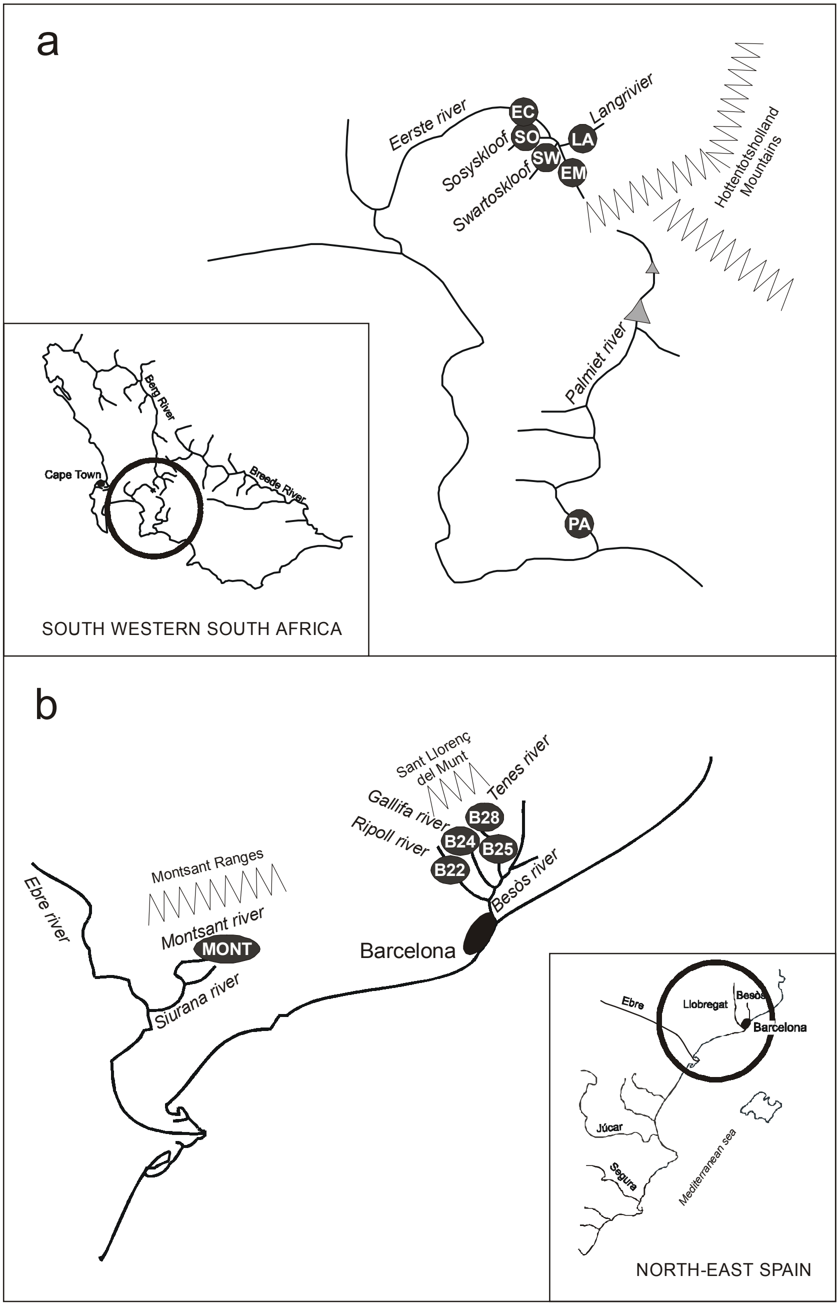

Figure 1. Sampling site location in South Africa (a) and Spain (b).

In Spain, 5 sites were sampled from Besòs and Siurana basins (Figure 1b, Table 1). Gallifa (B24), Tenes (B28, B25) and Ripoll (B22) are tributaries from Besòs River and have a calcareous and sedimentary geology. Most of the water comes from Sant Llorenç Natural Park, but only B24 and B28 could be considered as mountain streams. In these sites, the basin is forested with sclerophyllous mediterranean forest, and riparian vegetation with *Salix alba*, *Corylus avellana, Populus nigra* and *Populus alba* as dominant species. Downstream, in the foothill areas, the basin has a significant human alteration which affects water quality and riparian vegetation (e.g., presence of introduced species as *Platanus hispanica, Populus deltoides* and *Robinia pseudoacacia*) (Prat *et al.*, 1997, 1999). Montsant River is a tributary of Siurana River (tributary from Ebre River) that flows through the Montsant Natural Park with a predominant calcareous geology. MONT and B24 can be considered as pristine sites in contrast to B22, B25 and B28, influenced by human disturbances (Prat *et al.*, 1997, 1999). Instream vegetation is dominated by mosses, diatoms, zygnematales and *Cladophora* sp. Macrophytes as *Apium nodiflorum* or *Veronica* sp. are dominant in the river channel. Channel substrate is composed by bedrock, large stones and sand in headwaters and bedrock, pebbles and coarse sand in foothills.

Sampling methods

SASS5 methodology (Chutter, 1998)

A kick-net of 30x30 cm and 1 mm of mesh size is used in two groups of habitats: stones (S) and vegetation (V). Stone habitat includes stones-in-current (SIC) and stones-out-of-current (SOOC), and they are sampled in a different way. For SIC habitats a kick sampling is performed during 2 minutes if unattached stones are present or 5 minutes if not. For SOOC habitats 1 m^2 of the riverbed is sampled. Vegetation (V) includes marginal and instream vegetation, and they are sweeping with the net for 2 m. All collected material separated by habitat is poured into two different trays. Leaves, twigs and trash are removed from the tray to make easier to find the macroinvertebrates. Taxa is sorted and identified at family level except for Hydropsychidae and Baetidae for 15 minutes in the field or until no new taxa have been seen after 5 minutes of sorting. Organisms not collected but seen in the field (e.g., Heteroptera) are also included. The final SASS5 score is calculated using either stones or vegetation habitats. Number of taxa and ASPT value (i.e., SASS5/number of taxa) are also obtained. Abundances are estimated according to following ranks: 1=1, 2=2-10, 3=10-100 4=100-1000 5=>1000. In the text, SASS5 methodology will be referred as SV (stones-vegetation method).

IBMWP methodology (Alba-Tercedor & Sánchez-Ortega, 1988) according to Guadalmed Project (see Chapter 1)

In a 100 m reach, a kicking method is performed with a 250 µm mesh size net. Although all habitats must be sampled together, traditionally two groups of habitats have been identified and sampled separately, the riffles (R) and the lentic areas (L). For the lotic habitats, the net is located in front of the rock, removing the substrate and cleaning well several rocks, before the net is clogging. In lentic habitats, marginal vegetation, gravel and mud are swept. All material is put into white trays and leaves and sticks are removed. Organisms are sorted and identified in the field at family level until all collected material has been examined. The sampling procedure is repeated until no more new taxa are recorded. Organisms not collected but seen in the field (e.g., Heteroptera) are also included in the index calculation. The final IBMWP, IASPT and number of taxa are obtained using all taxa collected from both habitats. Abundances are estimated according to the following ranks: 1=1-3, 2=4-10; 3=11-100; 4=>100. Because the objective of the study was to compare both methods, and SASS5 is designed to be performed in the field, we use the Protocol 1 (see Chapter 1) for all samples. In the text, IBMWP methodology will be referred as RL (riffles-lentic method).

Table 2. Similarities and differences between SASS5 and IBMWP procedures, considering the items proposed by Resh *et al.* (1995).

Consideration	SASS5	IBMWP
1. Habitats to be examined	Stones (SIC and SOOC) and Vegetation (marginal and instream).	All habitats, separated in riffles (R) and lentic (L) areas.
2. Sampling area and intensity	Depending on the habitat.	100m reac h. Until no more new taxa are found.
3. Sampling devices	Kick -net.	Kick -net.
4. Mesh sizes	1000 ?m	250 ?m
5. Proportion examined	Time and taxa dependent.	All.
6. Taxonomic level	Family and species for Baetidae and Hydropsychidae.	Family
7. Measures used	Number of taxa, SASS5 score and ASPT score.	Number of taxa, IBMWP score and IASPT score.
8. Quality control and assurance	Samples from reference sites (H. Dallas, per. comm.).	Samples from reference sites (Bonada et al., in press).

Similarities and differences between sampling protocols are shown in Table 2. SASS5 procedure is focused in differences among physical substrates, whereas IBMWP use the flow as habitat differentiation.

Data analysis

To check for similarities and differences between communities and sites between both methods, a Non-metric Multidimensional Scaling (NMDS) was applied to the abundance's matrix. This ordination method preserves the distances between objects, plotting dissimilar objects far from the similar ones, (Legendre & Legendre, 1998). On the other hand, the NMDS method is not based on eigenvalues, and the final axes are arbitrary without enclosing the explained variability. Because of data is semiquantitative in ranks, Bray-Curtis coefficient was selected to calculate distances between variables and % of similarities between sites. PCORD program (McCune & Mefford, 1999) was used to carry out NMDS.

We next examined whether differences in macroinvertebrate community found using the Spanish and South African methods were significant or not. To perform that, a MRPP test (Multi-response Permutation Procedures) was used. This analysis is a non-parametric method that test multivariate differences among pre-defined groups (RL vs. SV —Riffles and Lentic versus Stones and Vegetation), providing the statistic A and a p-value obtained by permutation (999 runs) as result. Because its non-parametric condition this method is more appropriated than MANOVA in comparisons of data matrixes that involve species abundances including many zero values. To check for similarities and differences between biotic indexes and metrics, a non-parametric Kruskal-Wallis ANOVA by rank test was used, because of data were not normal using Shapiro-Wilk's test. The PCORD (McCune & Mefford, 1999) and STATISTICA (Stat Soft, 1999) programs were used to perform the analysis.

RESULTS

In total, 51 families were recorded in Spain and 44 in South Africa with both methodologies. The number of common taxa found by N.B. and H.D. using both methods was high, with 74.5% (38 families) of congruity in Spain and 78.3% (36 families) in South Africa (Table 3). Families found for one of the method but not the other were different in Spain and South Africa. In South Africa H.D. found 7 families not found by N.B. which collected 3 not found by H.D.; whereas in Spain, N.B. found 9 families not collected by H.D. which found 4 not found by N.B. (Table 3). In spite of these differences in methodologies, high Bray-Curtis similarities in community composition using both methods are found, with >68% in Spanish sampling and >75% in South African study.

Table 3. Macroinvertebrate families (in alphabetic order) found by each local and outsider researchers in both sampled mediterranean regions

	Only N.B. (local expert)	Only H.D.	Found by both N.B and H.D.				
SPAIN Sampling	Ancylidae	Cambaridae	Aeschnidae	Corixidae	Hydracarina	Naucoridae	Simuliidae
	Dixidae	Gammaridae	Asellidae	Culicidae	Hydraenidae	Nemouridae	Tipulidae
	Hydroptilidae	Helodidae	Baetidae	Dytiscidae	Hydrobiidae	Nepidae	
	Libellulidae	Veliidae	Bythinellidae	Elmidae	Hydrometridae	Oligochaeta	
	Lymnaeidae		Caenidae	Ephemerellidae	Hydrophilidae	Ostracoda	
	Planorbidae		Calopterygidae	Erpobdellidae	Hydropsychidae	Perlodidae	
	Polycentropodidae		Ceratopogonidae	Gerridae	Leptoceridae	Philopotamidae	
	Psychodidae		Chironomidae	Gomphidae	Leptophlebiidae	Physidae	
	Stratiomyidae		Coenagrionidae	Heptageniidae	Limnephilidae	Rhyacophilidae	

	Only H.D. (local expert)	Only N.B.	Found by both N.B and H.D.			
SOUTH AFRICAN Sampling	Aeschnidae	Gerridae	Baetidae	Dugesiidae	Hydraenidae	Philopotamidae
	Athericidae	Gomphidae	Barbarochthonida	Dytiscidae	Hydropsychidae	Pisuliidae
	Belastomatidae	Protoneuridae	Blephariceridae	Ecnomidae	Leptoceridae	Potamonautidae
	Heptageniidae		Caenidae	Elmidae	Leptophlebiidae	Simuliidae
	Hydroptilidae		Ceratopogonidae	Empididae	Libellulidae	Teloganodidae
	Naucoridae		Chironomidae	Glossosomatidae	Limnichidae	Tipulidae
	Platycnemididae		Coenagrionidae	Gyrinidae	Notonemouridae	Veliidae
			Corydalidae	Helodidae	Oligochaeta	
			Dixidae	Hydracarina	Petrothrincidae	

The NMDS analysis (Figure 2) indicates that both methods discriminate mountain streams sites from foothills. Either, Spain and South Africa present a closer assemblage among methodologies than sites, especially in the foothills in Spain and headwaters in South Africa. Foothills sites present higher distances between methods, which would indicate that not coincident macroinvertebrate assemblages are produced depending on the methodology applied (Figure 2). In South Africa, Palmiet River site displays a unique community with a similarity of 75% between RL and SV methods, whereas headwater sites have the highest similarities between methods (over than 90%). This high similarity between methods is confirmed with the MRPP analysis indicating non-significant differences in the macroinvertebrate assemblages either in Spain (A=-0.021, p=0.6814) and South Africa (A=-0.0293 and p=0.792).

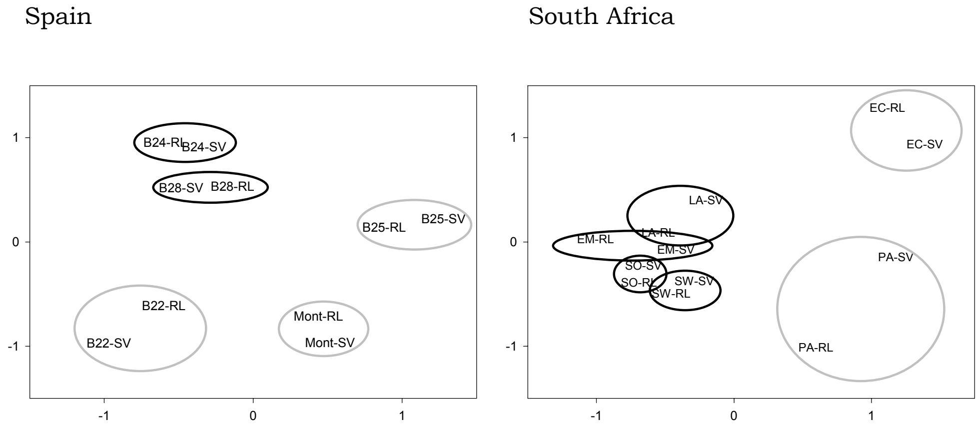

Figure 2. NMDS analysis in Spain and South Africa using SASS5 and IBMWP methods. Black circles indicate headwater sites, whereas grey ones are referred to foothills localities

Table 4. Results from the Kruskal-Wallis non-parametric tests comparing SASS5 and IBMWP methodologies in Spain and South Africa. ***p<0.01

		p-values		
		IBMWP	Taxa	IASPT
SPAIN	RL vs SV	0,5948	0,4172	0,7625
	R vs S	0,0578	0,1967	0,5271
	L vs V	0,5271	1	0,5271
	RS vs LV	0,0736	0,3711	0,3711

		p-values		
		SASS5	Taxa	ASPT
SOUTH AFRICA	RL vs SV	1	0,2207	0,2482
	R vs S	0,2482	0,079	0,2482
	L vs V	0,7401	0,621	0,3765
	RS vs LV	0.0064***	0.0001***	0,0589

Similarities between methods are also seen when values of the biological indexes and metrics are examined (Figure 3). There are not significant differences between RL and SV methods for the values of IBMWP (Spain) and SASS5 (South Africa) (Table 4). Furthermore, a high similarity is found in number of taxa or IASPT and ASPT scores, indicating that both methods provide equivalent results in both areas. The Spanish sampling sites present in average a lower IBMWP than the South African ones for both methods, but similar number of taxa, indicating that families with lower biotic scores are present providing a lower IASPT (Figure 3). Only one sample in Spain (MONT) presented a very high IBMWP score. In contrast, in South Africa only one site presented low biological quality (EC), as can be seen in Figure 3. When differences on RL and SV methodologies are analyzed by individual habitats, no difference are found between R and S or L and V (Table 4), but significant higher values in SASS5 and number of taxa is obtained comparing RS and LV in South Africa but not in Spain. A high biotic quality is observed in R and S habitats individually compared with L and V, indicating that R and S contributed more to the final score than L and V (Figure 3). In spite of these differences, ASPT remain constant among habitats. In Spain, where in average all sampling sites have a lower biological quality, these differences were not found, and a similar IBMWP, number of taxa and IASPT was recorded in all habitats for both methods. MONT site, displays a similar behavior than South African samples (except EC), with a lower IBMWP in L and V than R and S, but lower IASPT for all habitats. The site EC (the less clean site in South Africa) responds in the same way than most of Spanish sites, and no differences among habitats are observed (Figure 3).

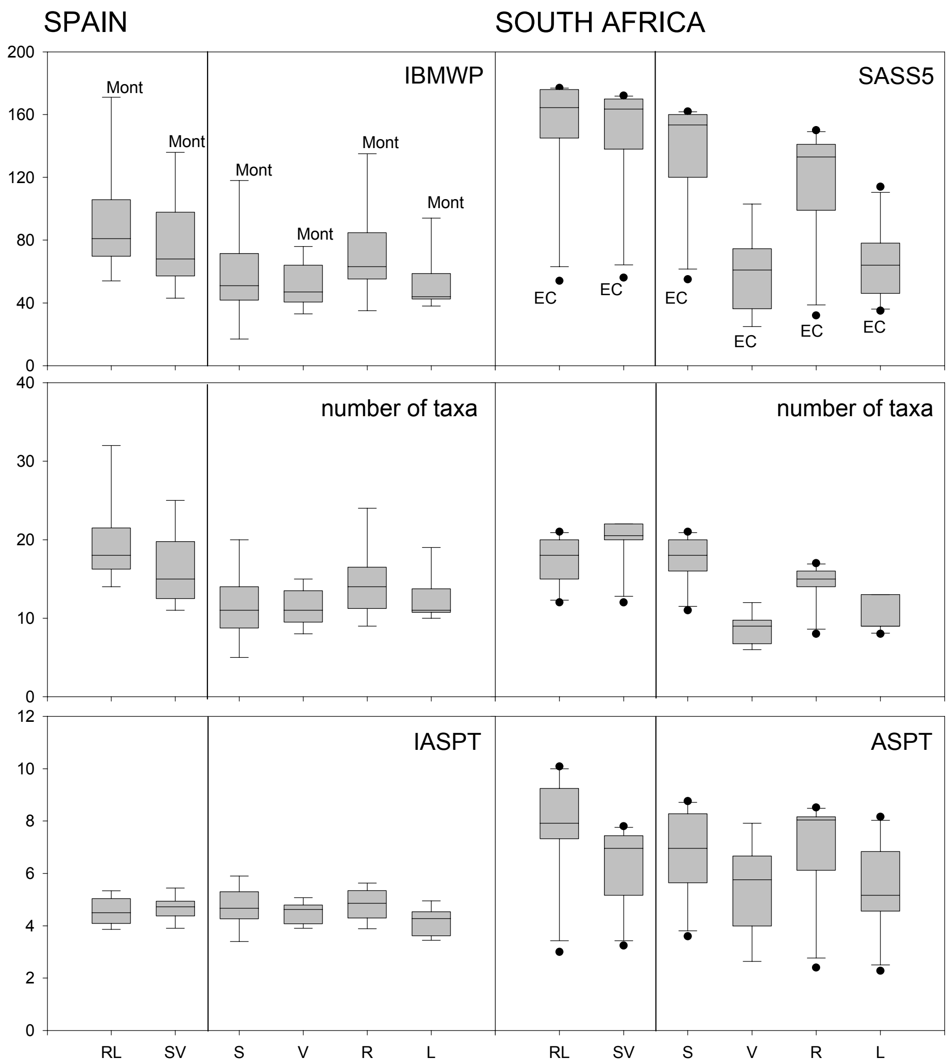

Figure 3. Box-Plot graphs from three tested metrics in Spain (IBMWP, taxa richness and IASPT) and South Africa (SASS5, taxa richness and ASPT) separated by methods and habitats. Mont (Montsant site in Spain) and EC (Eerste foothill site in South Africa) sites are indicated as extreme values in each region compared with the rest of localities. RL=riffles+lentic, SV=stones+vegetation, R=riffles, L=lentic, S=stones and V=vegetation.

DISCUSSION

There are a great variety of RBPs methods differing in sampling, subsampling, taxonomic resolution, metrics and index calculation, but all of them can yield comparable results depending of the objectives (Diamond *et al.*, 1996). However, the degree of comparability of two methods is usually unknown because no direct comparisons have been made (Diamond *et al.*, 1996). When SASS5 and IBMWP methodologies are compared, their different mesh size, sampling intensity and segregated habitats do not seem to influence on the final results, and a similar community composition and water quality is found. Because more disturbed sites were sampled in Spain than in South Africa, no coincident patterns in water quality are present between both countries, although both methods appear to work well in disturbed and undisturbed sites. For Spanish sites, only one site (MONT) may be qualified as a pristine locality with a high biotic quality with a lower IBMWP in LV habitats compared with RS habitats, which is similar to what have been found in South African samples where mostly of sites are pristine. On the other hand, in South Africa, only the site EC displays a low value of biotic index with similar values between all sampled habitats, as happen in most of the Spanish sites. Consequently, both methods are equally sensitive to water quality as they provide similar results in distinguishing high and low quality sites in Spain and South Africa, when all habitats are used. The lower quality values present in Spanish sites can be related to two factors: some pollution and poor river habitat conditions. In several studies, Prat *et al.* (1997, 1999) reported a fair biological quality in B22, B25 and B28 because the human alteration of the Tenes and Ripoll basins. On the other hand, B24 have also an impoverished macroinvertebrate assemblage although it has been considered as a reference site in Bonada *et al.* (in press). This locality has a temporary condition and a low diversity in substrate composition (with bedrock as a predominant substrate) (Prat *et al.*, 1997, 1999) that could affect to the establishment of a rich community (Lenat & Barbour, 1994). In that sense, in a nearby area, Bonada *et al.* (2000) also found low quality values in non-impaired sites because of the physical structure and temporality, but not as consequence of impaired water quality.

Although no differences are found in biotic indexes among methods, a 32% of dissimilarity (Bray-Curtis coefficient) is found between the macroinvertebrate assemblages found with Spanish RL and South African SV methods in both areas, which may be related to differences in mesh size used, sampling and sorting intensity, experience in the area or spatial variation in the macroinvertebrate distribution. In average a slightly higher number of taxa is found with RL methodology compared to SV in Spain, and lower taxa richness in South African samples (Figure 3) what could be related to the familiarity of each researcher with the macroinvertebrate fauna of her country. For example several taxa difficult to find (by size or behavior) can be missed by the non-native researcher in the foreign country, as some Psychomyiidae that live in carved sticks or other cryptic taxa living in specific microhabitats (Lenat & Barbour, 1994). In that sense, we have found that families collected only by the native researcher in its own country and not for the other are rare or infrequent (e.g., Dixidae, Belastomatidae, Psychodidae) or have been found in low abundance in the sampling period (e.g., Ancylidae, Gammaridae, Heptageniidae, Gerridae or Hydroptilidae in Spain). In other cases, because of quite cryptic families (e.g., Hydroptilidae) have been found in both countries by local researches, we can accept that the highest number of exclusive taxa found by native researchers in its own area might be by chance, and not because of their different degree of experience in each country.

The kind and number of habitats to be sampled in a RBP have been widely discussed (Resh *et al.*, 1995; Hewlett, 2000). Plafkin *et al.* (1989) proposed that the "most productive habitat" should be sampled and Lenat (1988) suggested the high current habitat with "structure". Specially in pristine sites, we found that riffles (R) or stones (S) seem to be the most productive habitats to give an optimum biotic index, and other authors have pointed out that a sampling based on riffles should be enough (Parsons & Norris, 1996) because usually these habitats provide the highest number of taxa (Carter & Resh, 2001). However, the high annual variability of mediterranean rivers implies that riffles may disappear in some cases with only pools remaining in summer (Gasith & Resh, 1999). Therefore, the use of only one habitat in these streams cannot be recommended. In that sense, a multihabitat protocol integrating all habitats, as in SASS5 and IBMWP, is preferred (Stribling *et al.*, 1993; Resh *et al.*, 1995; Bonada *et al.*, Chapter 1).

In pristine conditions, riffle habitats (R) are equivalent to stones (S) indicating a low influence of the stones-out-of-current habitat, and both contributed significantly to the final score. Dallas (1997) comparing the influence of habitat on the SASS4 scores found that stones in current represent 70% of the SASS4 of the relative percentage to the total calculated for the site, whereas stones out of current only contribute to the 46%. In impaired conditions (all sites except MONT in Spain, and EC in South Africa) differences between habitats are not significant. Number of taxa and biotic index of R and S is lower than in pristine sites, but not in L and V where similar values are found in all sites in Spain and South Africa. Consequently, in impaired conditions, R and S habitats are more affected for pollution than L and V, and the lower values of biotic indexs may be associated to the decrease of the family's biotic scores as can be seen in the IASPT and ASPT values. This phenomenon could be related with the high velocity of the water in riffles and stones that increases its vulnerability to pollutans because bounday-layer on macroinvertebrates become thinner (but see Lowell *et al.*, 1995). In fact, Logan & Brooker (1983) pointed out that the effects of pollution by solids were higher in riffles than in pools, and consequently suggest using both habitats to assess water quality.

Decisions about what sampling gear to use in a RBP also have been discussed in literature. Kick and "sweep" nets are preferred in front of Surbers or Hess samplers (Storey *et al.*, 1991; Lenat & Barbour, 1994). In that sense, kick method has been recommended in biomonitoring surveys (Storey *et al.*, 1991) providing semiquantitative or qualitative data. However, multiple methods have been used, and the most convenient should be selected according to the objectives desired in the study (see Rosenberg, 1978; Elliot & Tullett, 1978, 1983). The same happens with mesh size, as a range of size from 200 to 1000 μm has been used in biomonitoring. In our case (and contrary to many studies) because of no differences in biotic indexes are found using 250 and 1000 μm mesh size, if the objectives of the study are only to assess water quality, a more coarse mesh size may be used. An intermediate mesh size of 500 μm have been proposed by the sampling standardization normative ISO in Europe (AENOR, 1995), and is the most common used in the US (Carter & Resh, 2001). Probably, the fact that family level is the taxonomical unit used might explain similarities in results using different mesh sizes, because of the smallest animals from many families may be lost (e.g. Chironomidae) but the larger ones remain in the sample.

Sampling and sorting efforts are different between SASS5 and IBMWP. In the former, time constrains the sampling and sorting intensity, whereas in IBMWP sampling and sorting continues until no more new taxa is added and all community richness is collected, being the result a bigger sample size. However, this difference in sampling size between protocols do not affect to the biotic indexes values, which agree with the results found by Metzeling & Miller (2001) comparing SIGNAL values between different sampling sizes in Australia. Consequently, because of our results indicate that in pristine and impaired sites both methods are equally applicable, the most efficient method in time consuming could be satisfactorily used in both countries to assess water quality. In pristine conditions SASS5 could be more advantageous because its time limitation and only one sample is required. However, Dallas (1995) sampled several times using the SASS5 procedure and found that in a pristine site in one sample only 28% of total taxa was recorded, whereas in a impaired site, one sample provide 45% of taxa. The same study shows that 4 samples are required to get the 95% of taxa, and consequently, SASS5 values increase with the sampling effort. Thereby, if the objectives are to go further than a biological assessment (autoecological or faunistic studies), probably the IBMWP methodology yield better results in pristine conditions, as a best representation of all community (and sizes) is provided, including rare taxa. In that sense, Cao *et al.* (2002) demonstrated using field and simulated data sets that the total taxa richness found with a fixed sample size (e.g., using a sampling methodology constrained by time or space) varies between sites, and consequently sampling until no more taxa is added (i.e., until the highest autosimilarity between samples is achieved) imply a highest representativeness of the community.

Although, in either SASS5 or IBMWP the taxonomical resolution used is the family level, in SASS5 Baetidae or Hydropsychidae scores are disaggregated according to the different species found, as both families have tolerant and intolerant species (Chutter, 1998). However, although lower taxonomic resolution yields good information (Furse *et al.*, 1984; Resh *et al.*, 1995) field identifications of different species are usually difficult at those levels and a specific training is required to obtain good results.

A lot of data is available about biological assessment, but the different methods used make comparisons uncertain (Diamond *et al.*, 1996). Different procedures can yield similar predictions, but this must to be known to test the applicability of one method in another country, and to redesign each method depending on the objectives desired. For example, Solimini *et al.* (2000) comparing IBMWP and the Italian EBI (Extended Biotic Index) (Ghetti, 1995) found that IBMWP was more sensible to biotic quality in Tibre River (Italy) and suggest the use of it respect EBI. RBPs have been designed to be efficient, easy and rapid to apply (Resh & Jackson, 1993; Resh *et al.*, 1995). To perform that, the sampling and processing of samples is simplified without a loss of information (Resh *et al.*, 1995; Barbour & Gerritsen, 1996). SASS5 and IBMWP protocols provide similar information in South Africa and Spain, but SASS5 is a more cost-effective protocol in terms of time than IBMWP. However, Guadalmed IBMWP protocol has been designed to provide complete information of the macroinvertebrate community present to perform further autoecological studies or predictive models. Because of their similar applicability to perform bioassessment in both countries, redesigns of one method with properties from the other can be possible to get the established objectives. However, although both methods provide similar information in bioassessment in pristine and impaired sites, they should also be contrasted also in other sampling period or regions. For example, if the sampling was performed in temporary sites, just after the drought period, different mesh size could affect the final results, as the community of pools are composed of small organisms (Williams, 1987, 1996) that could escape in a coarse mesh size.

Nowadays, both countries take similar future directions. The development of the "River Health Program" in South Africa or the implementation of the "Water Frame Directive" in Europe are based on the assessment of ecological status using reference conditions and referred to ecoregions or ecotypes. These policies will provide an assurance of how aquatic ecosystems must be managed to improve their ecological status. Both RBPs methods (SASS5 and IBMWP) are adequate to fulfill these objectives.

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