Benthic macroinvertebrate based indices for assessing the ecological status of freshwaters on oceanic islands

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Following the Water Framework Directive (2000/60/EC), macroinvertebrates are required biological elements for monitoring European aquatic ecosystems. Several efforts have been made towards establishing a biomonitoring programme for the Azores freshwater systems using benthic macroinvertebrates. However, little was previously known concerning Azorean freshwater macroinvertebrate fauna. Data from a major ongoing survey of macroinvertebrate freshwater fauna for two islands are presented and the use of macroinvertebrates as water quality indicators for the Azorean streams is evaluated. The upper, middle and lower reaches of streams longer than 10 km from São Miguel (10) and Santa Maria (1) were surveyed (a total of 33 samples). A total of 21 taxa were collected; Diptera, in particular chironomidae, were dominant; chironomids were collected from all sampling sites. The Azorean lotic fauna is characterized by low levels of abundance and the absence of macroinvertebrate groups commonly associated with continental systems. Traditional biotic indices, used to classify ecological quality, yielded poor to bad classifications despite little or no environmental impacts at the sampling sites. The paucity of macroinvertebrate fauna is probably due to the result of geological and physicochemical processes, the oceanic character of the islands, their volcanic origin, small size and geological youth and climate related factors such as seasonal peaks in rainfall. Considering these constraints, we demonstrate that watershed age plays a determinant role in shaping Azorean macroinvertebrate freshwater communities which can confound the water quality classification via the use of traditional biotic indices.

Key words: Azores, chironomidae, lotic systems, stream, water quality

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INTRODUCTION

The use of benthic macroinvertebrates as environmental indicators is based on their ability to respond to a variety of environmental variables such as sediment quality, water quality, hydrological conditions, shading and biological factors (Rosenberg & Resh 1993; Chessman 1995; Bonada et al. 2006). As result, benthic macroinvertebrates have become the most commonly used biological indicators in freshwater systems (Resh & Jackson 1993). The

Water Framework Directive (2000/60/EC) (WFD) demands the establishment of bio-monitoring programmes for European aquatic ecosystems and includes macroinvertebrates as one of the biological elements to be monitored. In the Azores, we have taken the initial steps towards monitoring freshwater systems using benthic macroinvertebrates. Apart from pre-liminary studies (Murray et al. 2004; Gonçalves et al. 2005, 2008; Raposeiro et al. 2006; Malhão et al. 2007; Raposeiro et al. 2007; Raposeiro & Costa 2008; Raposeiro et al. 2009a; Raposeiro
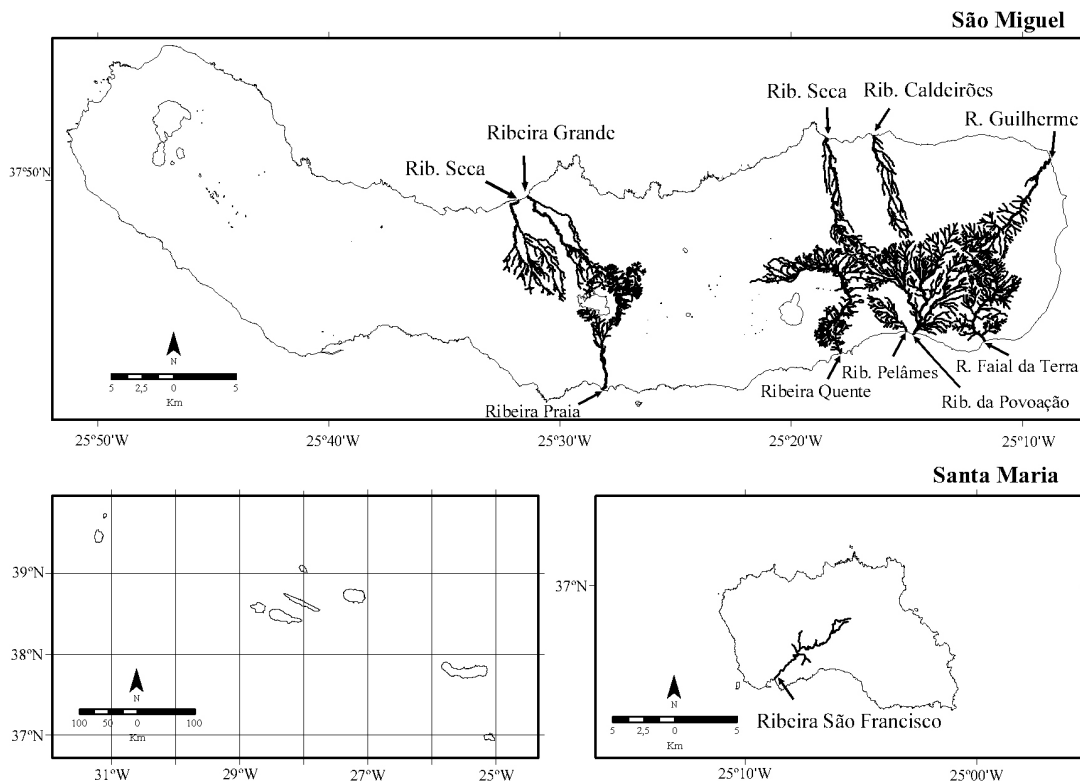
Fig. 1. Location of the archipelago of the Azores, São Miguel Island (above) and Santa Maria Island (below), with sampled streams.

et al. 2009b) nothing has been published on Azorean lotic freshwater benthic macroinvertebrates. Reference conditions, that represent an unimpacted ecological state, still remain to be established in Azorean lotic systems. Freshwater systems of the Macaronesian Islands differ strongly from continental systems in watershed morphology and biotic assemblages, due to their isolated location within the Atlantic Ocean, and also due to the volcanic origin of the islands. Streams of oceanic islands freshwater systems are characteristically narrow, short and very steep (Smith et al. 2003; Hughes 2005; Hughes & Malmqvist 2005). Physically they can resemble continental headwater streams, being narrow, straight and shallow with turbulent, torrential, and often seasonal flow. Substrates are coarse, comprising bedrock, boulders, cobbles and sand. According to Brasher (2003), effects of habitat alteration can occur simultaneously at different scales, such as watershed, stream reach, and

microhabitat. Urbanization (watershed) is typically accompanied by canalization and the removal of riparian canopy cover (a reach-scale process), resulting in higher water temperatures, increased siltation, and decreased substrate size (microhabitat-scale processes; Brasher 2003). Sandin & Johnson (2004) described a continuous change in macroinvertebrate community composition across a north-south gradient and showed the importance of local variables such as substratum, in-stream and riparian vegetation, and some chemical variables in explaining the among-site variance of benthic macroinvertebrate assemblages.

The Azores archipelago is located in the North-Atlantic Ocean, between 37° N and 40° N latitude and 25° W and 31° W longitude, about 1500 km from the closest point of mainland Europe (Cabo da Roca, Portugal). It is formed by nine volcanic islands, aligned on a WNW-ESE trend, which are distributed in three groups: the Western, the

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central and the Eastern (Fig. 1). The largest island is S. Miguel (745 km^2) and the smallest is Corvo (17 km^2). Santa Maria is the southern and easternmost island (37° N, 25° W) with an area of 97 km^2. The three island groups are separated by 1,000 to 2,000 m deep sea channels.

Geologically, the Azores comprises a 20-36 Myr old volcanic plateau; the oldest rocks emerged 8.12 Myr ago (Santa Maria Island) while the youngest (Pico Island) is about 0.25 Myr old (Borges & Brown 1999; Quartau 2007). The eastern part of all Azorean Islands is geologically the oldest, which is the result of particular sismovolcanic mechanisms of this archipelago. The islands are formed via processes of coalescence and sequential eruptions, resulting in different complexes and ages within each one. Moore (1990) recognised six volcanic zones for the island of São Miguel. The oldest unit, the Nordeste volcanic complex, in the easternmost part of São Miguel, was built of mafic alkali basalt during the last 4 million years. An eruptive volume increase, a few hundreds of thousand years ago, led to the growth of the trachytic strato-volcanoes of Sete Citades, Água de Pau and Furnas. During latest Pleistocene and Holocene several vulcanic eruptions occurred in the areas between these three volcanoes. The youngest area is located between Sete Cidades and Água de Pau volcanoes, the Picos volcanic complex. Santa Maria is located at the eastern and southern extreme of the archipelago and is undoubtedly the oldest of all the Azorean Islands. Santa Maria has been formed by several volcanic eruptions (Serralheiro 2003), but the last eruptive events that occurred in the island during the Pliocene probably led to the extinction of some fauna and flora. In Quaternary Santa Maria was uplifted progressively (Serralheiro & Madeira 1993).

The climate of the Azores archipelago is oceanic and temperate with mean annual temperatures of 14–18 °C and mean annual precipitation of 740–2400 millimetres. Most annual rainfall (65–70%) is produced between October and March (Bettencourt 1979).

Wind patterns differ among the different groups of islands. South and Southwest winds prevails in Western and Central groups whereas in São Miguel and Santa Maria winds pre-dominate from North and Northeast directions. Strongest winds blow from South and West largely surpassing the wind average speed of 16,8km/h (Porteiro 2000).

These environmental features, together with the torrential flow regime and habitat type, limit colonisation by freshwater organisms. Thus, Azorean freshwater biota diversity is very poor compared with continental systems. Insect orders, particularly Diptera are dominant while Plecoptera are absent (Raposeiro et al. 2008).

Quantitative methods for assessing ecological status have been developed for all of the biological elements. Most macroinvertebrate monitoring methods are either: a) pollution indices (e.g. BioMonitoring Working Party index (BMWP) (Armitage et al. 1983), b) diversity indices (e.g. Shannon-Wiener), c) comparative indices (e.g. Jaccard's) (Magurran 2004), d) multimetric approaches who consider each taxon to be a variable and the presence or abundance of each taxon as an attribute of a site or a time point (Norris & Georges 1993) or e) predictive models (*e.g.* United Kingdom's RIVPACS, Australia's AUSRIVAS and Canada's BEAST; Wrigth et al. 2000). All of these have limitations and differ in the calculation methods, but all are based on the comparison of the detectable changes in communities subjected to particular conditions.

Freshwater monitoring programs have been developed in oceanic islands outside the European Community (EC) *e.g.* in Hawaii (Stephen et al. 2004). Within the EC, a new macroinvertebrate index was developed specifically for the island of Madeira (Hughes & Furse 2001), though a monitoring program remains to be established. In the Canary Islands, the other Atlantic European and Macaronesian archipelago, the lack of permanent surface waters precludes the application of monitoring programs. The purpose of the present work was to test the applicability of the benthic macroinvertebrate biotic indexes for water quality assessments and monitorization in the Azores archipelago.

MATERIAL AND METHODS

To characterize the aquatic benthic macro-invertebrates of the Azores, one stream on Santa

Maria (the only permanent stream on this island) and ten streams in São Miguel were selected and sampled during the spring of 2004 (Fig. 1). Each stream was sampled (i) close to source, (ii) along the middle and (iii) lower reaches (sampled codes as in Table 1). Benthic invertebrate communities were collected using a semi quantitative technique (kick-sampling with a D-frame hand-net with a 500 μm mesh net) using dipping, kicking and sweeping over a 3 minute period. These collections were supplemented by manually turning over three cobble sized stones in the sampling area, scrubbing them with a brush to

Table 1. Sampling sites, C.L. Channel length (in kilometres), Reach/ Locality (Downstream as DownS) and their respective codes.

Island & Stream		C.L	Reach/Loc	Code
São Miguel	Guilherme	8.50	Upstream	RGU1
			DownS	RGU2
	Caldeirões	7.85	Midsection Lenho	RC1
			Midsection Caldeirões	RC2
			DownS	RC3
	Salga	7.03	DownS	RSG1
	Grande	13.30	Upstream	RG1
			Midsection	RG2
			DownS	RG3
	Seca	6.93	DownS	RSC1
	Praia	5.92	Upstream	RP1
	Quente	14.40	Upstream	RQ1
			Midsection Promineral	RQ2
			Midsection Central	RQ3
			DownS	RQ4
	Pelames	3.65	DownS	RPL1
	Povoação	8.90	Midsection Central	RPV1
			Midsection West	RPV2
			Midsection East	RPV3
			DownS	RPV4
	Faial da Terra	8.30	Upstream	RFT1
			DownS	RFT2
Santa Maria	São Francisco	5.36	Upstream	RSF1
			Midsection	RSF2
			DownS	RSF3

remove invertebrates and then inspecting them for any remaining attached macroinvertebrates (which were removed by hand). All samples were sieved through a 500 μm mesh and preserved in 96% ethyl alcohol. Samples were sorted and all organisms were identified to family level.

DATA ANALYSIS

Macroinvertebrate abundance (total number of individuals belonging to a *taxon* in a sample = N), and *taxa* richness (number of *taxa* in a sample = S) were calculated as descriptive measures of the benthic macroinvertebrate community for each site. The BMWP index and the derived Average Score Per Taxon index (ASPT) (Armitage et al. 1983) were used to assess water quality.

Data were (square root) transformed and Bray-Curtis dissimilarities were calculated for all pairs of observations for these analyses. Non metric multidimensional scaling was applied to data for trend identification in samples; SIMPER analysis (species contribution to similarity) to identify relevant taxa at each location (software package PRIMER 6.0; Clarke & Gorley 2006). Ordination plots produced by non-metric multidimensional scaling (nMDS) analyses were used to graphically represent the patterns of community similarity at each location.

In order to illustrate that natural constraints influence on these lotic communities composition a relationship between *taxa* richness (S) from different aged volcanic complexes was established. A Spearman rank correlation between *taxa* richness (S) from different aged volcanic complexes was additionally determined (maximum ages of aerial rocks as given by Nunes 1999, and by Quartau 2007).

RESULTS

A total of 6576 individuals from 21 *taxa* were identified for all the samples, with a total of 15 *taxa* from Ribeira de São Francisco (2) and 1 *taxon* from Ribeira Seca. The samples were dominated by Diptera (81%), in particular chironomids (53%) followed by Oligochaeta (Naididae, 10%).

The most important *taxa* in Azorean streams are Simulidae, Chironomidae, Naididae, Hidra-

carina and Hydroptilidae. A low abundance and diversity for most groups as well as the absence of some traditional groups (*e.g.* Ephemeroptera, Plecoptera) were observed. The most abundant *taxa* in upstream sites are Simuliidae and Orthocladiinae (Table 2). In downstream sites the most abundant *taxa* are Naididae, Simuliidae and Orthocladiinae (Table 2). Chironomidae (Ortho-

cladiinae) are present in all sampled sites. The highest water quality class attained using the BMWP index was "moderate" water quality. Only 8% of the sampling sites were classified as "moderate", whereas 54% of the sites were classified as "poor" or "bad" (38%) water quality (Fig. 2).

Table 2. Average abundance of benthic macroinvertebrate at upstream and downstream sites, and their contribution for the dissimilarity found between the sites.

Upstream Group	Av. similarity: 51.94				
Taxa	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum%
Orthocladiinae	7.23	35.59	2.13	68.53	68.53
Simulidae	4.84	13.13	0.77	25.28	93.81

Downstream Group	Av. similarity: 54.35				
Taxa	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum%
Orthocladiinae	8.16	43.74	2.16	80.48	80.48
Simulidae	2.16	4.86	0.66	8.95	89.43
Naididae	1.54	1.7	0.37	3.12	92.55

| Upstream & Downstream Groups	Av. dissimilarity: 46.05					
	Upstream Group	Down-stream Grp.				
Taxa	Av. Abund	Av. Abund	Av. Sim	Sim/SD	Cont%	Cum%
Simulidae	4.84	2.16	12.05	1.46	26.16	26.16
Orthocladiinae	7.23	8.16	8.02	1.28	17.42	43.57
Naididae	0	1.54	4.7	0.61	10.21	53.78
Hydrachnidae	0.94	0.67	3.4	0.92	7.38	61.16
Haplotaxidae	0.62	0.44	2.63	0.88	5.71	66.87
Hidracarina	0.68	0.33	2.23	0.99	4.84	71.7
Hydroptilidae	0.5	0.6	2.1	1.14	4.55	76.25
Chironominae	0	0.72	1.9	0.58	4.12	80.38
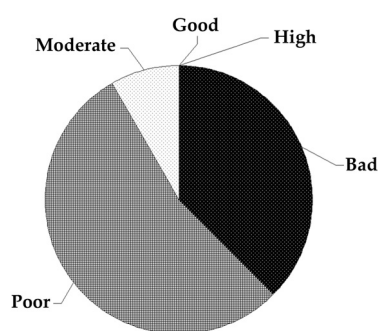
Fig. 2. Results for the ecological quality for BMWP index (Biological Monitoring Working Party): Poor (54%), Moderate (8%), Good (0%), High (0%) and Bad (38%).

The nMDS ordination showed poor separation of upstream, middle and downstream sites (Fig.3a), although upstream samples form a smaller clustering in the middle of the diagram, revealing a higher degree of similarity between upstream samples, while downstream samples were more widely spread in the ordination space. Superimposed ASPT values revealed (Fig.3b) a quality gradient with low quality sites in the bottom left of the diagram to high quality in the top right. A SIMPER analysis singled out the Simuliidae and Orthocladiinae as the principal *taxa* responsible for separating sites along the river continuum. A positive and highly significant regression (F= 43.4; R^2=0.95, p=<0.05) between
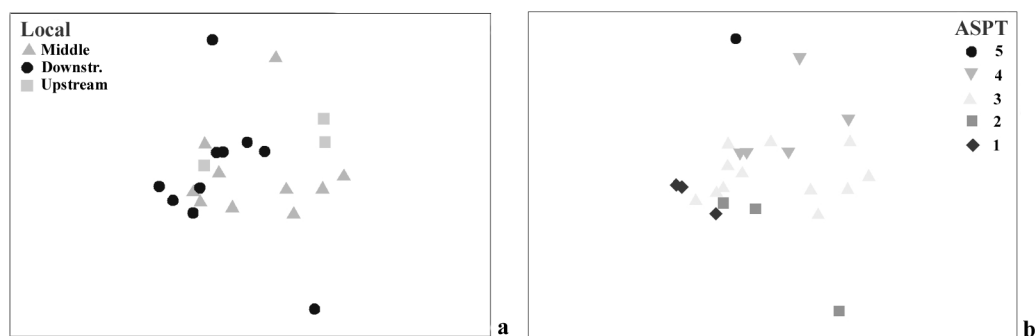
Fig. 3 a) Non-metric multidimensional scaling (nMDS) separation between upstream, middle and downstream samples; b) Score Per Taxon index (ASPT) superimposed on the diagram (Standardize samples by Total; transform square root; Resemblance S17 Bray Curtis similarity).
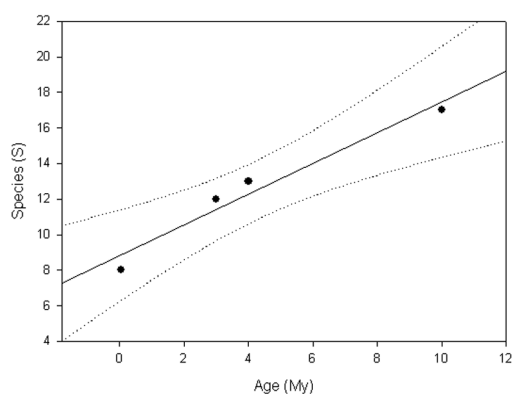

Fig. 4. Relationship between geological age (volcanic complexes) against macroinvertebrate taxa richness.

taxa richness (S) and volcanic complexes age was also found (Fig. 4).

DISCUSSION

Azorean freshwater macroinvertebrate diversity and abundance are very low when compared to continental communities, i.e. 21 *taxa* were found in this study compared to 45 *taxa* found in a single Mediterranean freshwater stream of similar size (Muñoz 2003), and 44 *taxa* for Canaries (Nilsson et al. 1998), another Macaronesian archipelago. A similar pattern was observed in Hawaii (Brasher et al. 2004), where the native stream fauna is relatively depauperate when compared to that of continental streams. Brasher et al. (2004) also stated that diversity in Hawaiian

streams would be naturally low in pristine conditions. More diverse orders of insects such as Ephemeroptera (mayflies), Plecoptera (stoneflies) are absent from the native stream biota both in Hawaii (Howard & Polhenuts 1991) and in the Azores. However, Trichoptera species were found in the Azores but have not yet been recorded from Hawaii. Volcanism within the Azores results in active hydrological vents and on stream substrate of a pumice nature. The absence of some groups can be related to this loose substratum and/or to chemical (metal) water enrichment. There is evidence of heavy metals in some sites *e.g.* Ribeira Grande (Louvat & Allègre 1998).

As in many previous studies, there was a marked reduction in the richness and total abundance of macroinvertebrates where metal concentrations were increased (Malmqvist & Hoffsten 1999; Clements et al. 2000; Watanabe et al. 2000, Hirst et al. 2002). This was one of the sites that revealed to be biologically poor.

Isolation and time for speciation will be important issues for diversification of groups with low dispersal ability (Borges & Hortal 2009). The limited arrival of lineages from the colonization source will result in a poor diversity and a stronger (and negative) relationship between diversity and distance to the colonising source (Santa Maria Island, in the Azores, being the closest to the mainland source). Limited dispersal abilities might also result in higher isolation of different populations within the same island, limiting colonization as well as the time for colonization. This may explain some differences

within-island, which would be observed as an increase in heterogeneity along different watersheds (in different age complexes) in São Miguel Island.

According to the island biogeography Species-Area Relationship (SAR) there is a positive correlation between increasing richness and increasing area (MacArthur & Wilson 1967). Following SAR logic, Santa Maria should have lower number of *taxa* than São Miguel, since Santa Maria is a smaller island. However, this is not the case not only for freshwater invertebrate species diversity but terrestrial organisms such as land molluscs when compared with the remaining Azorean Islands. T*axa* diversity is only slightly lower than the adjacent island of São Miguel (Borges et al. 2005) and it is likely the relative age of the islands explains some of the observed differences in freshwater faunal composition. The relation found between bedrock age and number of *taxa* appears to supports classical biogeographical theory (Fig. 4). However, the Azorean Islands and the watersheds within each island have different ages due to the sequence of eruption events that took place over millions of years. The difference between Santa Maria and São Miguel macroinvertebrate communities can be used to indicate that the time available for colonization would be an important factor for species colonization and might prevail over the existing barriers (e.g. distance, dominant winds, currents) derived from the island effect. Time, as an important factor shaping the oceanic island faunas, has recently been stressed in the development of the "General Dynamic Model of Oceanic Island Biogeography" by Whittaker & Fernandez-Palacios (2007) and Whittaker et al. (2008). According to Borges et al. (2009) dispersal limitation may be viewed as one of the main forces that shaped the Azorean native biota. They suggest that a non-stochastic pattern of island colonization for the Azores, as older *taxa* inhabit the oldest island of Santa Maria and the first to be colonized. Species immigration to a new oceanic island will be dominated by colonists from other islands within the group, but due to the isolation of the islands this is assumed to occur at a fairly low rate (Whittaker & Fernandez-Palacios 2007). According to Borges & Brown (1999), Santa Maria has acted as a

refuge for older biota, so it can be assumed that most lineages have re-colonized the rest of the islands from this starting point (Borges & Hortal 2009).

The poor results derived from selected traditional macroinvertebrate indices are a direct result of the distinct insular character of the local fauna. This situation has already been discussed by Hughes & Furse (2001), Hughes (2003), Brasher et al. (2004), Hughes (2005), Hughes and Malmqvist (2005) and recently by Gonçalves et al. (2007) and Raposeiro et al. (2009a) who state that invertebrate metrics development for streams in continental settings may not be appropriate for oceanic islands. For example, the commonly used EPT (Ephemeroptera, Plecoptera, Tricoptera) metric based upon the number of Ephemeroptera, Plecoptera and Trichoptera *taxa* under the assumption that these *taxa* are pollution sensitive, is inappropriate for Azores, Madeira (Hughes 2005) and Hawai (Brasher et al. 2004), given the absence of Plecoptera and the reduced diversity of Ephemeroptera and Trichoptera. In fact, according to Hughes (2003), over twenty well-known metrics, used to assess the suitability of macroinvertebrates in defining the ecological quality of Madeira archipelago lotic systems based on perceived environmental impacts, were found to perform badly for Madeira Island.

Water quality degradation is evidently not the only cause for absence of some *taxa*, as Ribeira do Guilherme, in São Miguel Island, presents consistently excellent physicochemical water quality (INOVA 2005; 2006), but scores very low with BMWP. Probably the absence of some high scoring families, for this index, due to geographic constraints (age) and physiographic (torrential regime), prevent the BMWP to classify this stream as good quality. However, the proportions between different non sensitive/sensitive taxa will in this case provide more useful information evident in a better result of the ASPT that weights families than middle of the sensitivity scale.

This may explain the better behaviour of ASPT index that considers a weighting factor for each family, compared to BMWP, a metrics based only presence/absence of *taxa*. The instability and torrential nature of the short and high slope streams of these oceanic islands are also drawbacks for the application of these faunal

indices since opportunistic *taxa* e.g. Oligochaeta are likely to be the most abundant after the frequent periods of high discharge.

The Chironomidae is by far the most diverse and abundant macroinvertebrate family occurring across the Azores (Gonçalves et al. 2008), being present in all samples. The development of chironomid based metrics should be considered for ecological monitoring of freshwater streams across the archipelago. The ubiquity and abundance of chironomids should be used in the appraisal of ecological quality in Azorean freshwater systems to assess the suitability of the Chironomid Pupal Exuviae Technique (CPET) (Wilson & Wilson 1983; Wilson & Ruse 2005). According to Hughes (2003), this approach was tested in Madeira with good results.

ACKNOWLEDGEMENTS

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