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Benthic diatoms and macroinvertebrates in the assessment of the ecological status of Azorean streams

Vítor Gonçalves [1], Pedro Raposeiro [1,*] and Ana Cristina Costa [1]

[1] CIBIO-Pólo Açores e Departamento de Biologia, Universidade dos Açores, R. Mãe de Deus, 13A, 9501-801 Ponta Delgada, Portugal

* Corresponding author: raposeiro@uac.pt

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ABSTRACT

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To meet the Water Framework Directive goals all freshwater ecosystems have to be categorized by 2015. This paper analyzes the benthic diatoms and the macroinvertebrates of streams in two islands (São Miguel and Santa Maria) of the Azorean archipelago. Differences were found between the epilithic diatom communities located upstream (forested areas) and downstream (urban and agricultural) of the water's courses., The diatom-based indices revealed that 10 % of the sites presented a good/excellent state of conservation. The macroinvertebrates indices confirmed the generally poor ecological condition of these streams. However, these results must be interpreted with care due to the low biodiversity and heavy rainfall conditions in these island streams. The existence of these specialized conditions requires a different approach to correctly evaluate the ecological quality of the archipelago streams.

Key words: Water framework directive, benthic diatoms, benthic macroinvertebrates, ecological quality indices, islands, Azores.

RESUMEN

Diatomeas bentónicas y macroinvertebrados en la valoración del estado ecológico de los ríos de las Azores

Para cumplir la Directiva Marco del Agua todos los ecosistemas de agua dulce deben ser categorizados antes de 2015. En el presente trabajo se analizan las diatomeas bentónicas y los macroinvertebrados en los ríos de dos islas (San Miguel y Santa María) del archipiélago de las Azores. Se encontraron diferencias entre las comunidades de diatomeas epilíticas localizadas en la parte superior (áreas forestales) e inferior (áreas urbanas y agrícolas) de los cursos de agua. Los índices basados en las diatomeas revelaron que un 10 % de los lugares presentaban un estado de conservación bueno/excelente. Los índices de macroinvertebrados confirmaron las condiciones ecológicas generalmente pobres de estos ríos. Sin embargo, estos resultados deben ser interpretados con cuidado debido a la baja biodiversidad y el carácter torrencial de los ríos insulares. La existencia de estas condiciones especiales requiere un enfoque diferente para evaluar correctamente la calidad ecológica de los ríos del archipiélago.

Palabras clave: *Directiva marco del agua, diatomeas bentónicas, macroinvertebrados bentónicos, índices de calidad ecológica, islas, Azores.*

INTRODUCTION

The Water Framework Directive (WFD) (European Parliament & The Council of the European Union, 2000) introduced the concept of ecological status for the assessment of aquatic ecosystems, putting the emphasis on biological endpoints to assessing environmental health.

The ecological status concept overcomes the limitations of assessing ecological conditions based on physico-chemical measurements. Biotic indicators integrate environmental changes over a long time period, whereas physical-chemical measurements report anthropogenic pressures at the time of sampling (Cranston *et al.*, 1996; Giller & Malmqvist, 1998; US EPA, 2002).

The monitoring programmes, established for the purpose of estimating values of the biological quality elements for each surface category, may utilise particular species or groups of species which are representative of the quality element as a whole (European Parliament & The Council of the European Union, 2000). The comparison over time of community conditions in pristine control areas –reference conditions– with those of sites whose ecological status is under assessment is the principle under biomonitoring. The ecological status should be expressed as quality ratios representing the relationship between the values of the biological parameters observed for a given surface water body and the values for the same parameters in the reference conditions applicable to that body (European Parliament & The Council of the European Union, 2000).

Fish could be an important element in aquatic ecosystems, but their presence in Azorean inland waters is mostly limited to lentic systems and exclusively due to human introductions. In the Azores, microalgae (planktonic and benthic) and benthic macroinvertebrates are the key elements in monitoring the Azorean streams; in particular, diatoms (Kolkwitz & Marsson, 1908), have been used to assess the ecological quality of running waters. Diatoms respond quickly to environmental changes in rivers and streams (Stevenson & Pan, 1999) and are excellent indicators of local conditions (Chassé, 1997). Many water quality assessment methods with diatoms have been developed (Prygiel & Coste, 1993), including several diatom indices (Descy, 1979; Cemagreff, 1982; Sládecek, 1986; Leclercq & Maquet, 1987; Coste & Ayphassorho, 1991; Descy & Coste, 1991; Kelly & Whitton, 1995; Prygiel & Coste, 2000) that were shown to be one of the most effective tools for the evaluation of the ecological status of rivers (Eloranta & Soininen, 2002).

Benthic macroinvertebrates are common inhabitants of freshwater systems around the globe and are among the most sensitive elements of aquatic biota. They are an important food source for fishes, and participate actively in decomposition processes influencing nutrient recycling for primary production. Macroinvertebrates have been widely used as water quality bioindicators (e.g. Rosenberg & Resh, 1993; Metcalfe-Smith, 1994; Alba-Tercedor, 1996). The advantages of using this fauna component are mostly related to abundance and/or presence in different aquatic habitats, taxa selective sensitivities to a variety of pollutants and contamination levels, relatively long life cycles, and short re-colonization periods (Pujante, 1997). Moreover, the collection and identification of benthic macroinvertebrates is relatively easy and there are standard protocols for most used groups for quality indices (e.g. BWMP, ASPT, BBI). Several benthic macrofauna-based indices have been proposed and developed with the objective of environmental quality assessment of aquatic ecosystems (Rosenberg & Resh, 1993). However, the knowledge of freshwater benthic diatoms and macroinvertebrates, especially in Azorean streams, is in its infancy and mainly historical (Archer, 1874; Guerne, 1887; Barrois, 1896). Recently, their role in the streams ecology has been addressed (e.g. Gonçalves *et al.,* 2005).

The aim of the present study was to evaluate the effectiveness of the methodologies applied in Iberian lotic systems to island streams.

MATERIAL AND METHODS

Study Area

The Azores, located at 36°55′43″-39°43′2″ North, and 24°46′15″-31°16′15″ West, is an oceanic archipelago comprising nine islands and several islets (Fig. 1). Being 1 300 km apart from the nearest continental coast (Cabo da Roca-Portugal), and 1 900 km from the American Continent, it is the most remote Macaronesian archipelago. Due to their oceanic situation and volcanic origin, the freshwater systems of the Macaronesian

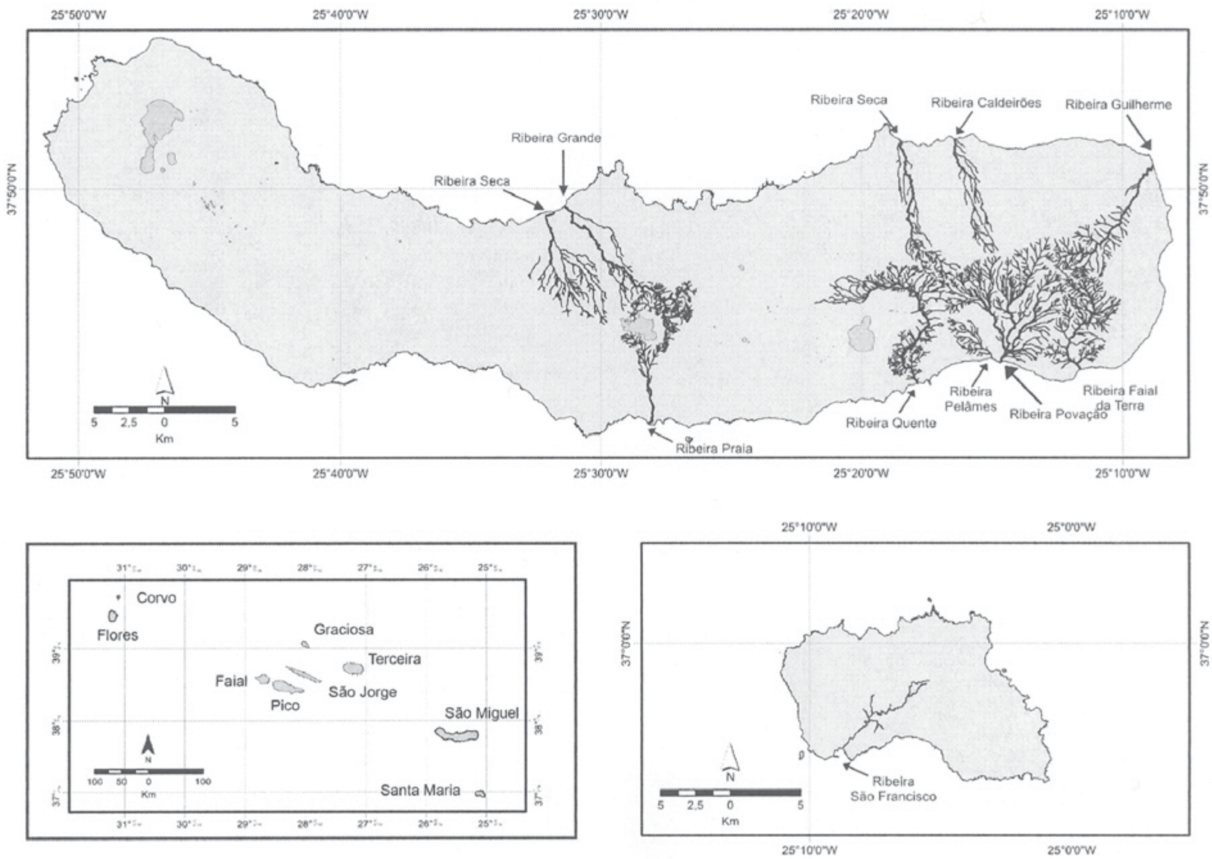

Figure 1. Location of the sampling sites in the Azorean archipelago. *Localización de los puntos de muestreo en el archipiélago de las Azores.*

Islands differ strongly from continental systems in watershed morphology and on their biotic assemblage's composition (Hughes & Malmqvist, 2005). Insular watersheds, originated from volcanic processes, are characteristically small and very steep (Hughes, 2003; Smith *et al.,* 2003). Streams drop dramatically in altitude over a very short distance and are similar to continental headwater streams, being narrow, straight and shallow with turbulent, torrential, and often seasonal flow. Substrates are coarse comprising bedrock, boulders, cobbles, and sand. The water chemical composition results mainly from three processes: i) atmospheric inputs by oceanic rains, ii) weathering of the volcanic rocks of the drainage basin, and iii) inflows of more concentrated thermal spring waters (Louvat & Allègre, 1998). According to the geological map of São Miguel Island (Zbyszewski *et al.,* 1958, 1959), the streams sampled in the present study belong to watersheds of basaltic, trachytic or intermediate rocks.

Twenty five sites, selected from 11 catchments north and south of São Miguel and Santa Maria islands (Table 1), covered all permanent freshwater lotic systems of these islands, and included a range of water quality from 'pristine' to highly organically polluted or suffering from extreme anthropogenic disturbance. The sites were sampled during 2003 (October and November) and 2004 (March and April), from upstream to downstream along a 100 m long reach. At each site physical and chemical measurements of the stream's water were taken simultaneously with the biological samples by the Instituto de Inovação Tecnológica dos Açores (INOVA). The results of the

Table 1. Sampling sites and their respective codes. *Estaciones de muestreo y sus respectivos códigos.*

Island	Stream	Local	Code
São Miguel	Guilherme	Upstream	RGU1
		Downstream	RGU2
	Caldeirões	Middlestream-Lenho	RC1
		Middlestream-Caldeirões	RC2
		Downstream	RC3
	Salga	Downstream	RSG1
	Grande	Upstream	RG1
		Midllestream	RG2
		Downstream	RG3
	Seca	Downstream	RSC1
	Praia	Upstream	RP1
	Quente	Upstream	RQ1
		Midllestream-Promineral	RQ2
		Midllestream-Central	RQ3
		Downstream	RQ4
	Pêlames	Downstream	RPL1
	Povoação	Midllestream Central	RPV1
		Midllestream West	RPV2
		Midllestream East	RPV3
		Downstream	RPV4
	Faial da Terra	Upstream	RTF1
		Downstream	RFT2
Santa Maria	São Francisco	Upstream	RSF1
		Midllestream	RSF2
		Downstream	RSF3

physical-chemical analysis are presented elsewhere (INOVA, 2005; Cymbron *et al.*, 2005). The Ribeira Seca (RSC) stream showed a strong heavy metal (chromium, arsenic, and zinc) contamination. On the other hand, the Ribeira Quente (RQ) stream had high levels of phosphorus (soluble, inorganic and total), ammonia, biochemical oxygen demand (BOD), total suspended solids, and high counts of bacteria. Ribeira de São Francisco (RSF), in Santa Maria Island, recorded high levels of sulphates, zinc, chloride, and low BOD values. The other streams were very similar in physical-chemical conditions; however, slightly higher levels of iron and manganese were found in Ribeira Grande (RG) and in RQ when compared with the remaining streams studied.

Methods

Sampling was carried out in two different time periods, one in the fall of 2003 (between October and November) and another during the spring of 2004 (March and April).

The diatom sampling was carried out according to European recommendations (Kelly *et al.,* 1998). Epilithic diatoms were collected by brushing at least five stones per sampling site, each time, and preserved in situ in 4 % formalin (v/v). Samples were treated as described by Germain (1981) using warm nitric acid and mounted in Naphrax©. Relative abundance of each taxon was determined after counting at least 400 valves in each sample (Prygel & Coste, 2000), using phase contrast microscopy at maximum magnification (total magnification = 1000 ×). The diatoms were identified to the lowest taxon possible using standard identification keys (Krammer & Lange-Bertalot, 1986, 1988, 1991a, 1991b, 2000).

Aquatic invertebrate larvae were collected from substrata as well as algae, mosses, macrophytes, and leaf litter by kick sampling using a hand net (500 µm mesh) over a 3 min period. The samples were than preserved in situ with 96 % alcohol. In addition, submersed stones from each site were brushed to obtain the epilithic organisms. At the laboratory, the organisms were sorted and identified to the family following available taxonomic literature since it is the taxonomic level used to determine faunistic indices (Garcia, 1987; Nieser *et al.*, 1994; Tachet *et al.*, 1994; González, 1997).

Non-metric multidimensional scaling was applied to the data for samples trend identification. A SIMPER analysis was used to identify individual contributions to sample grouping. An ANOSIM test was applied to check for differences between samples using the software package PRIMER (Clarke & Warwick, 2001). Pearson's Correlations between biological indices and environmental variables were calculated using SPSS vs.15.

To evaluate water quality several diatom based biotic indices (IPS-Indice de polluo-sensibilité-, IDG-Indice diatomique générique-, TDI-Trophic diatom index- and IBD-Indice Biologique diatomées-) were calculated using the software OMNIDIA vs.4.2 (Lecointe *et al.*, 1993) and the IBWMP (Iberian Biological Monitoring Working Party) as adapted by Alba-Tercedor (1996) was applied to the macroinvertebrates data.

Diatoms based indices values are classified according to five quality levels: very clean waters (higher than 16), clean waters with slight signals of stress

Figure 2. Ordination diagrams (nMDS) of diatoms from the studied sites with superimposed abundances of species contributing the most. Site codes as in table 1; **a**- refer to spring samples and **b**- to summer samples. *Diagramas de ordenación (nMDS) de diatomeas de las estaciones de estudio con superposición de abundancias de las especies con mayor contribución. Códigos como en la tabla 1; **a**- muestreos de Primavera y **b**- muestreos de Verano.*

(between 13 and 16), contaminated waters (between 9 and 12), very contaminated waters (between 5 and 8), and extremely contaminated waters (below 5). The IBWMP index was scaled according to reference conditions found in this type of rivers and five quality levels were established. These quality levels include very clean waters (values over 120), waters with stress signals (values between 61 and 100), contaminated waters (values between 36 and 61), very contaminated waters (values between 16 and 35), and extremely contaminated waters (values below 15).

RESULTS

A total of 139 diatoms *taxa* belonging to 45 genera were identified. Diatom communities were dominated by *Nitzschia* and *Navicula* species.

The non-metric multidimensional scaling (nMDS) performed on diatoms species data (Fig. 2) revealed a clear separation of RG1 and RQ2 sites. In the nMDS diagram, a clear gradient between upstream and downstream sites was evident. Diatom site distribution, according to the assemblages, follows the same pattern as the physical-chemical data assemblages obtained by PCA (Cymbron *et al.* 2005).

According to the SIMPER analysis (Table 2), *Pinnularia subcapitata*, *Eolimna minima*, *Amphora pediculus, Achnanthidium minutissimum*, and *Eunotia exigua* predominate in the upstream sites. The RG1 site (upstream site of Ribeira Grande) is the best example of this group, showing a great dominance of *P. subcapitata*, *E. exigua*, and *E. minima*. In middle and downstream sites *Nitzschia* species are dominant, with *Nitzschia frustulum*, *N. abbreviata*, *N. amphibia and N. palaea* being particularly abundant. Other characteristic species of these sites are *Navicula gregaria, Mayamaea atomus* var. *permitis* and *Luticola goeoppertiana*.

For most streams, the values obtained for diatom indices (IPS, IDG, TDI and IBD) indicate a poor to moderate water quality (Fig 3). More than 50 % of the samples indicate bad water quality, and only 7 % and 3 % correspond respectively to a good and better water quality. Therefore, results indicate high contamination at the studied streams which was generally corroborated by physical-chemical data (Cymbron *et al.,* 2005). Nevertheless, highest levels of diatom indices were reported in sites with low nutrient load and low microbiological contamination. IPS and IBD present highly significant correlations ($p < 0.001$) with soluble reactive phosphorus (SRP), inorganic phosphorus (P_{inorg}), total phosphorus (TP), and temperature. They also

Table 2. Average abundance of benthic diatom at the upstream and downstream sites, and their contribution for the dissimilarity found between sites. *Media de la abundancia de diatomeas bentónicas en las estaciones situadas en la cabecera y en el curso bajo, y contribución para la diferenciación entre estaciones.*

Taxa	*Mean Abundance*		*Dissimilarity*	*Contribution*	*Acumulated*
	Reference	*Impact*	*Mean*	*(%)*	*Contribution (%)*
Nitzschia frustulum	2.19	18.41	9.00	10.30	10.30
Pinnularia subcapitata	17.72	0.01	8.86	10.14	20.43
Eolimna minima	17.15	2.51	8.14	9.31	29.74
Nitzschia abbreviatta	2.02	14.12	7.32	8.37	38.11
Navicula gregaria	2.59	11.73	5.60	6.41	44.52
Amphora pediculus	9.96	0.85	5.07	5.80	50.32
Achnanthes minutissima	8.58	2.94	4.89	5.59	55.91
Nitzschia amphibia	1.38	7.06	3.35	3.84	59.75
Eunotia exigua	5.81	0.00	2.90	3.32	63.07
Synedra ulna	3.06	3.78	2.89	3.31	66.38
Mayamacea atomus var. permitis	0.00	5.38	2.69	3.08	69.46
Nitzschia palea	1.97	5.07	2.32	2.65	72.11
Gomphonema clavatum	4.56	0.11	2.30	2.64	74.74
Navicula goeppertiana	0.00	4.55	2.27	2.60	77.34
Navicula reichardtiana	3.52	0.68	1.71	1.95	79.30
Gomphonema parvulum	2.30	2.83	1.67	1.91	81.21

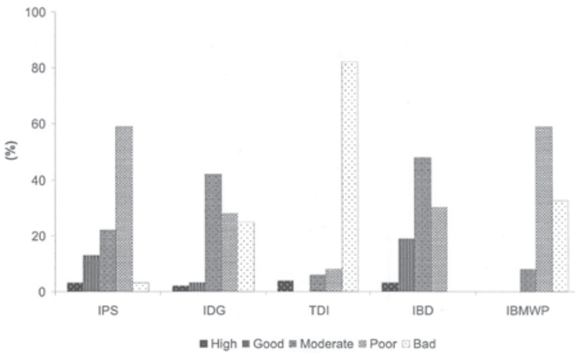

Figure 3. Results for the ecological quality for four diatom indices: IPS (Indice de polluo-sensibilité), IDG (Indice diatomique générique), TDI (Trophic diatom index), IBD (Indice Biologique diatomées) and IBMWP (Iberian Biological Monitoring Working Party). *Resultados de la calidad ecológica para cuatro índices de diatomeas: IPS (Índice de sensibilidad a la polución), IDG (Índice generalizado de diatomeas), TDI (Índice trófico de diatomeas), IBD (Índice Biológico de diatomeas) y IBMWP (Iberian Biological Monitoring Working Party).*

correlate very significantly ($p < 0.01$) with nitrate and nitrite concentrations, conductivity, as well as with coliforms and streptococcus counts. In spite of the positive response given by these indices, some limitations were detected in the discrimination of the most severe conditions (e.g. RQ2).

The ordination obtained by nMDS on the macroinvertebrates data clearly separates site RG1 from all the others (Fig. 4). Overall, downstream sites tend to be placed towards one side of the diagram and closer to each other than to the upstream ones that presented higher heterogeneity in their faunistic composition. Chironomidae, Oligochaeta, Simulidade, Hydroptilidae, Hidracarina, and Psycodidae were the *taxa* that contributed the most for the observed similarity within, and between the up, middle, and downstream site categories, as revealed by the SIMPER analysis (Table 3). By superimposing these taxa abundances on the nMDS diagram (Fig. 4) it becomes clear that Simulidae and Hidracarina are more abundant in Faial da Terra, Guillherme, and Caldeirões streams. Hydroptilidae are more abundant in Autumm at Ribeira Grande, Caldeirões, Faial da Terra, and Povoação (RPV1 e RPV2) streams. The most abundant macroinvertebrates are Chironomidae which are absent only at Ribeira Grande (RG1a) and Ribeira da Povoação (RPV3a). Oligochaeta are found mostly at RQ and in downstream sites elsewhere. A small portion of upstream and downstream sites dissimilarity (5.33 %) is due to Psycodidae taxa. These taxa are found mostly in downstream Povoação, upstream Ribeira Grande, and Faial da Terra.

DISCUSSION

The freshwater Azorean streams revealed a polluted condition or a poor ecological water quality when standard indices are applied to biological data. In fact, organic pollution mostly related with agriculture and livestock practices but also from urban origins, conditions the physical-chemical features of these ecosystems and it was reflected on its biota composition.

In relation to microalgae, their occurrence in high ecological quality or reference conditions was seldom the case. Actually, the most abundant

Table 3. Average abundance of benthic macroinvertebrate at upstream and downstream sites, and their contribution for the dissimilarity found between the sites. *Media de la abundancia de los macroinvertebrados bentónicos en las estaciones de cabecera y del curso bajo, y contribución para la diferenciación entre estaciones.*

Taxa	*Mean Abundance*		*Dissimilarity*	*Contribution*	*Acumulated*
	Reference	*Impact*	*mean*	*(%)*	*Contribution (%)*
Chironomidae	6.68	8.06	10.73	22.04	22.04
Oligochaetas	0.49	2.67	8.55	17.57	39.61
Hydroptilidae	1.66	1.07	7.77	15.97	55.58
Simulidae	2.19	1.11	7.23	14.86	70.44
Hidracarina	1.73	1.90	7.16	14.71	85.15
Psycodidae	0.40	0.59	2.69	5.53	90.69

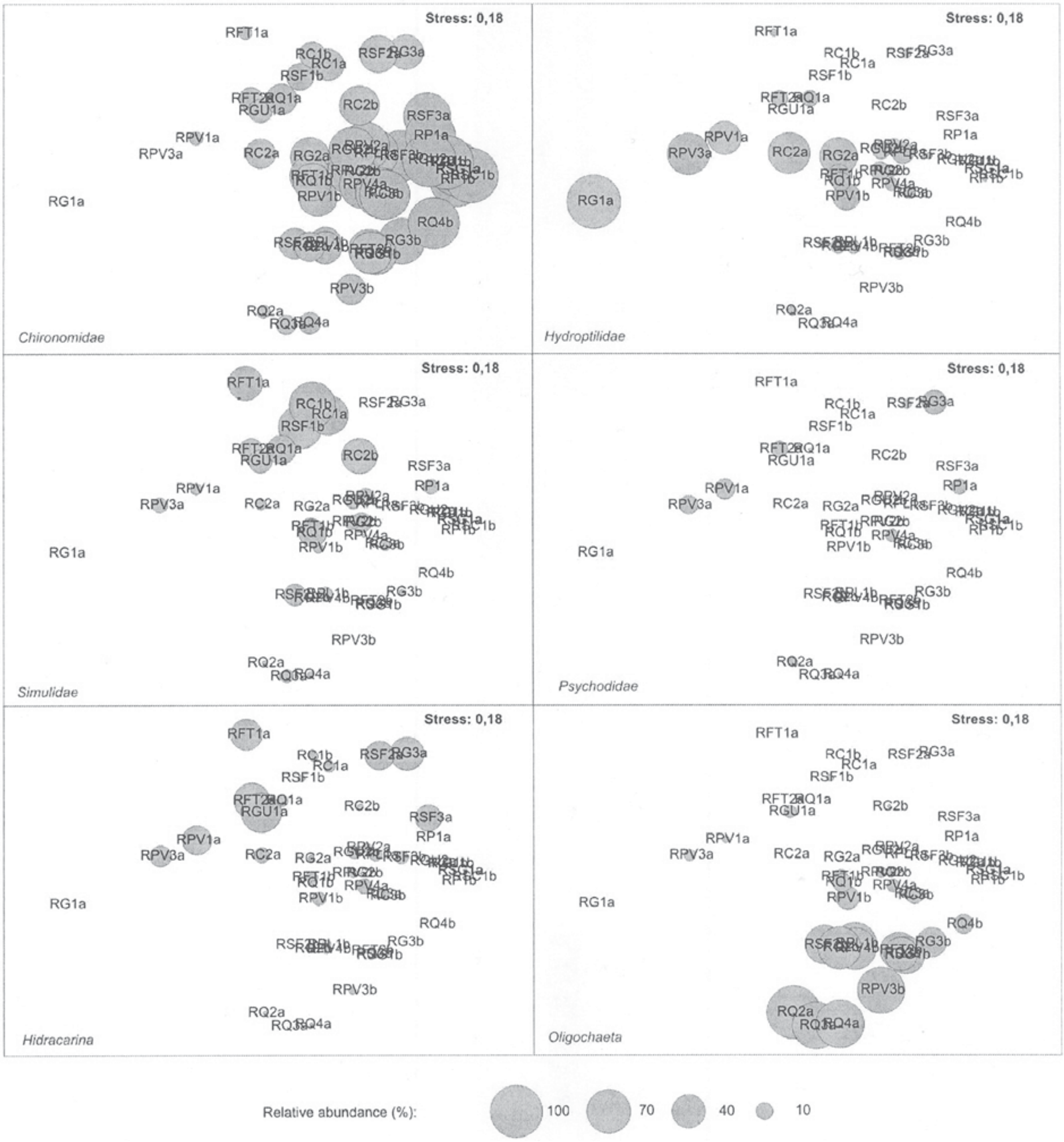

Figure 4. Ordination diagrams (nMDS) of macroinvertebrates in the studied sites with superimposed abundances for the most contributing species. Site codes as in table 1; **a**- refer to spring samples and **b**- to summer samples. *Diagramas de ordenación (nMDS) de macronivertebrados en las estaciones de estudio con superposición de abundancias de las especies con mayor contribución. Códigos como en la tabla 1;* ***a****- reportase a Primavera e* ***b****- a muestreos de Verano.*

genera in the studied streams were *Nitzchia* and *Navicula* whose species are usually associated with polluted ecosystems (Rumeau & Coste, 1988).

The usage of benthic diatoms revealed to be a good instrument to access ecological stream quality and has potential for application in routine monitoring programs in the Azores, particularly because benthic diatoms seem to strongly correlate with organic contamination, as diatom indices significantly correlate with physical-chemical parameters for these systems.

In relation to the benthic macroinvertebrate fauna, freshwater streams from São Miguel and Santa Maria had poor taxa richness when compared to continental locations. Insular conditions were determinant for this character of the fauna providing extra difficulty to data interpretation. Nevertheless, aquatic macroinvertebrate fauna in these streams was dominated by Diptera similarly to what is reported by literature for other lotic communities elsewhere (e.g. Barnes & Mann, 1991; Tachet *et al.,* 1994). Oligochaeta are favoured by organic enrichment (e.g. Rosenberg & Resh, 1993) and therefore were found in the sites where this condition was more obvious (RQ), as well as in downstream sites.

The upstream conditions revealed heterogeneity in macroinvertebrate fauna composition compromising their choice as reference sites for the Azores.

The difficulty to establish the reference conditions using macroinvertebrate fauna does not seem to be related to general degradation, as demonstrated by the physical-chemical and microalgae data, but to the particularities of these insular freshwater ecosystems reflected in low values for IBMWP index. Therefore, it might be necessary to adapt and develop a new benthic macroinvertebrate faunistic based index for monitoring purposes in the Azores, as happened in Madeira (e.g. Hughes, 2001). The next step towards this end would be to improve taxonomic expertise to species level in order to develop a species based index.

The poor results/performance revealed by the application of the currently used macroinvertebrate indices derive from particularities of local fauna, namely those related with its distinct insular character. In fact, insular freshwater fauna particularities, such as its lower diversity in relation with continental systems (Smith *et al.,* 2003) and dominance of active disperses as insects (Bilton *et al.,* 2001), result from the interplay of complex biological and geological processes (Smith *et al.,* 2003). In fact, dispersal oceanic barriers, local abiotic factors, and hydromorphological factors (e.g. highly seasonal and torrential flow regime) affect the success and establishment of colonizers (Poff, 1997; Malmqvist, 2002).

Moreover, the lower spatial heterogeneity and habitat diversity at these systems, when compared with larger continental ones, limit the pool of invertebrates able to colonize and inhabit them (Malmqvist, 2002). Nevertheless, the results herein presented demonstrate the importance of the study of macroinvertebrate fauna for water quality assessment purposes in the Azores. However, the need to develop locally adapted protocols was demonstrated. Therefore, urging ecological research has to be carried out not only to develop a reliable classification and local specific protocols, but also to prevent the environmental degradation threat already present in some of these unique and vulnerable freshwater macaronesian systems. In fact, Malmqvist *et al.* (1993) and Nilsson *et al.* (1998) clearly demonstrated the increased destruction of freshwater systems and species extinction in Macaronesia due to habitat reduction and environmental degradation.

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