

Environmental factors – spatial and temporal variation of chironomid communities in oceanic island streams (Azores archipelago)

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Abstract – Freshwater systems on volcanic oceanic islands have very particular characteristics as a result of their geological origins, relatively small size, distances from source areas for colonizers, and distinct catchment morphology. These factors result in freshwater communities that are clearly distinct from continental systems. Chironomid spatial and temporal distribution was investigated in chironomid assemblages at 21 sites across the Azores Archipelago. Results using PERMANOVA, a permutational multivariate analysis of variance, indicated significant longitudinal differences in assemblages but none between islands or over time.

Links between hydromorphological and physicochemical variables and the community assemblage were assessed using DISTLM, a linear model for distance-based multivariate analysis. The percentage of variation explained by hydromorphological factors (31%) was slightly higher than that explained by physicochemical (28%) factors. Descriptors of land use (forest area, scrub area, natural area of catchment) and stream slope were found to be the best environmental predictors of chironomid assemblages in Azores. Physicochemical variables such as temperature, pH, nitrite, iron and conductivity were the principal drivers of change in chironomid composition in stream locations. Headwater sites, characterized by lower temperature, acid to neutral pH values, low conductivity, nutrient and metal concentrations were dominated by *Rheocricotopus aripes*. Mid-section sites, located in agricultural areas were dominated by *Thienemanniella clavicornis*. Urbanized lower reaches were characterized by higher temperature, pH, conductivity and nutrient levels and were dominated by *Cricotopus* sp. These results give essential information that allows us to predict the response of different chironomid species to hydromorphological and physicochemical gradients across the archipelago's streams contributing providing the basis for the development of tools for the implementation of the Water Frame Directive.

Key words: Chironomidae / environmental gradient / oceanic islands / spatial and temporal variation / insular streams / Azores

Introduction

Freshwater systems on volcanic oceanic islands differ from their continental counterparts due to their oceanic location (isolation), comparatively small size and geological youth. At a more local scale, catchment morphology and often a more extreme torrential seasonal flow regime also shape these systems. These biogeographical filters operate over a range of spatial scales, influencing colonizing organisms and events that shape the local freshwater

assemblage. Even the absence of given taxon provides evidence on mechanisms governing these processes (Bilton *et al.*, 2001; Covich, 2006; Cowie and Brenden, 2006). Freshwater communities occurring on these islands tend to be depauperate but highly endemic (Borges and Brown, 1999; Walter, 2004; Hughes, 2006; Covich, 2009; Raposeiro and Costa, 2009). Oceanic island ecosystems tend to be simpler and offer great opportunities for the study of environmental factors determining the distribution of freshwater faunal elements such as the Chironomidae, the most abundant and diverse freshwater taxa in Azores archipelago (Raposeiro and Costa, 2009;

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Borges *et al.*, 2010). Such studies provide a better understanding of the key environmental drivers of change in freshwater communities, essential for the development and implementation of regionally appropriate conservation measures and ecological monitoring programmes.

Many lotic ecological assessment protocols are based on benthic macroinvertebrates, one of the four biological elements specified in Water Frame Directive (WFD; European and The Council of the European, 2000) for assessing ecological status. However, many metrics, developed for continental streams and rivers, cannot be directly applied to insular systems, because of the distinct nature of their biotic assemblages [see Hughes (2005) for an overview of ecological monitoring on the Macaronesian islands]. For example, the commonly used EPT (Ephemeroptera, Plecoptera, Trichoptera) metric is inappropriate for Azores due to the absence of Plecoptera and the low diversity of both Ephemeroptera (one species reported) and Trichoptera (four species reported). Over twenty well-known metrics used to assess the suitability of macroinvertebrates in defining the ecological quality of Madeiran lotic systems based on perceived environmental impacts, were found to perform badly for the island of Madeira (Hughes, 2003). A similar situation was reported by Gonçalves *et al.* (2008) for the Azores.

A possible alternative approach is the study of the Chironomidae (Insecta, Diptera, Nematocera) as potential bioindicators of freshwater ecological status in Azorean streams, given their ubiquity, abundance and diversity. However, most macroinvertebrate based protocols only identify chironomids to family or sub-family or even omit them [*e.g.* Family Biotic Index – FBI (Hilsenhoff, 1988); Biological Monitoring Working Party – BMWP (Armitage *et al.*, 1983)]. Chironomids are often excluded because of the difficult larval taxonomy that involves time consuming preparation of slides for subsequent identification and the large numbers of larvae that tend to occur in benthic samples. This is unfortunate, since chironomid diversity can match or even exceed that of other aquatic group insects combined (Heino and Paasivirta, 2008). Methods such as the Chironomid Pupal Exuviae Technique – CPET (Coffman, 1973; Wilson and Bright, 1973; Ruse and Wilson, 1995; Wilson and Ruse, 2005) largely overcome these difficulties because field and laboratorial protocols are well established and exuvial taxonomy is relatively easy, allowing identification to genus or even species. CPET is based upon the premise that the diversity and perceived tolerance of members of the aquatic chironomid larvae reflect local environmental conditions and that the winged adults ensure effective dispersal and colonisation of other aquatic habitats.

Situated in the North Atlantic, the Azores constitute the most remote archipelago of the Palaearctic sub region of Macaronesia, which also comprises the archipelagos of the Madeira, Canary and Cape Verde. The Azorean freshwater fauna is dominated by insects, in particular the Diptera (Gonçalves *et al.*, 2008; Raposeiro and Costa, 2009; Raposeiro *et al.*, 2009) and especially the Chironomidae, or non-biting midges (Borges, 2005;

Raposeiro and Costa, 2009). The Azorean chironomid fauna has been studied by several workers during the last century (*e.g.* Frey, 1945; Stora, 1945; Freeman, 1959). More recent records of Chironomidae were provided by Murray *et al.* (2004) and Raposeiro *et al.* (2009) who gave a total of 44 dipteran to the Azores and Raposeiro (2010) and Raposeiro *et al.* (in press) who updated the list.

Chironomid larvae inhabit almost every kind of aquatic habitat (Pinder, 1986; Ashe *et al.*, 1987; Armitage *et al.*, 1995a; Osborne *et al.*, 2000). As a consequence they have been used as biological indicators in freshwater ecosystems (*e.g.* Rosenberg and Resh, 1993; Wilson and Ruse, 2005; Raunio *et al.*, 2007b; Raunio *et al.*, 2007a; Heino and Paasivirta, 2008). Further, studies relating environmental variables to the spatial and/or temporal patterns of chironomid distribution are reported from Australia (Hardwick *et al.*, 1995; Dimitriadis and Cranston, 2007), Finland (Raunio *et al.*, 2007b; Heino and Paasivirta, 2008), the United Kingdom (Ruse and Wilson, 1995; Ruse, 2000; Ruse and Davison, 2000), Spain (Calle-Martínez and Casas, 2006; Puntí *et al.*, 2009) and Japan (Inoue *et al.*, 2005). The association between the environmental variables that determine chironomid community structure is complex, determined by physical, chemical and biological processes that vary both temporally and spatially in the lotic environment.

The principal aims of this study were to characterize chironomid communities of Azorean streams, using chironomid pupal exuviae to (i) determine if there are differences among chironomid assemblages between two islands of the Azores archipelago and (ii) identify environmental and chemical factors that determine chironomid assemblage structure both temporally and spatially.

These results will contribute to characterising chironomid distribution patterns along these particular lotic systems and provide information on factors determining the distribution of potential chironomid bioindicators across a quality gradient, ranging from reference to degraded conditions, paving the way to the development of regionally appropriate metric to describe ecological status, in accordance with the demands of the WFD (Hughes and Malmqvist, 2005; Hughes, 2006).

Materials and Methods

Study area and sampling procedure

The Azores is an oceanic archipelago, comprising 9 volcanic islands in three geographical groups (eastern, central and western groups), located between the coordinates 37 to 40°N, 25 to 31°W (Fig. 1). The oceanic and temperate azorean climate is characterized by low thermal amplitude, high levels of precipitation, high air humidity, and persistent wind (Cruz, 2003), with mean annual temperatures of 14–18 °C and mean annual precipitation of 740–2400 mm. Most annual precipitation (65–70%) occurs between October and March (Bettencourt, 1979).

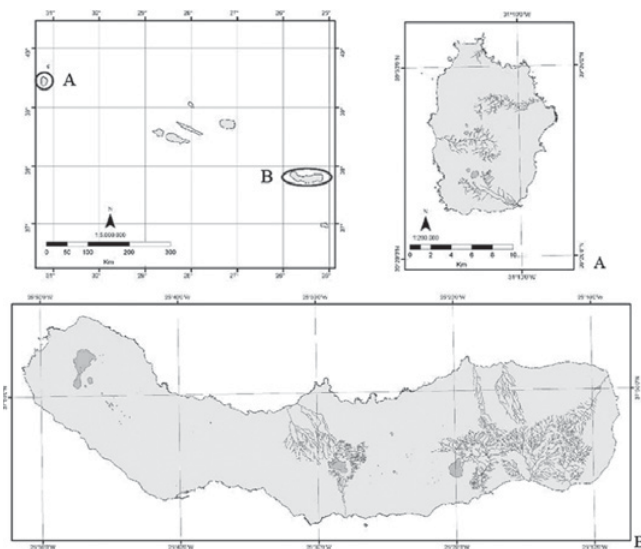


Fig. 1. The Azores archipelago: A) Flores island; B) São Miguel Island.

Azorean stream drainage networks are typically narrow and short with very steep, shallow channels often characterized by turbulent, torrential, and seasonal flow. Substrates are predominantly coarse, comprising bedrock, boulders, cobbles and sand. Background stream water chemistry is determined by (i) atmospheric input from oceanic-influenced precipitation, (ii) weathering of volcanic substrates and (iii) geothermal activity and spring water (Louvât and Allegre, 1998).

Forested areas (native and non-native) dominate the higher reaches of most catchments while agricultural and urban areas occupy the more accessible lower reaches. This study was conducted from early January to late November 2008, on the islands of São Miguel (eastern group, Fig. 1) and Flores (western group, Fig. 1). A total of 21 sites (seven in the headwater reaches, close to source; seven in mid-section reaches and seven in the lower reaches, close to the river mouth) along 10 permanent streams (eight in São Miguel and two in Flores) were selected and sampled to ensure adequate representation along both the altitudinal and anthropogenic gradient (Tab. 1). Although permanent streams, fed by lakes or spring waters, exist in Santa Maria, São Miguel, São Jorge, Faial and Flores islands (DROTRH and INAG, 2001), we selected São Miguel and Flores because they are the only islands where more than one permanent stream over 10 km² in catchment area can be found.

Pupal exuviae were collected seasonally (winter, spring, summer and autumn) with a 250 µm hand net over a 20 minute period along the stream margins, focusing on areas where flotsam (foam and floating material) accumulated behind obstacles such as exposed rocks and boulders (see Ferrington *et al.*, 1991; Wilson and Ruse, 2005) in order to guarantee that a minimum of 200 exuviae were available for analysis (Wilson and Ruse, 2005). Collected material was placed in a 250 µm sieve, washed and preserved in 96% ethanol. In the laboratory the material

was sorted and identified to the lowest possible taxonomic level (species or species-group) using keys by Langton (1991) and Langton & Visser (2003). Approximately 200 exuviae were subsampled randomly from each collection to provide an unbiased, representative sample (Ruse, 1993).

Physicochemical variables were measured at each site prior to biological sampling. Water temperature (°C), pH and dissolved oxygen (%) were measured *in situ* with electronic field probes (DKK, model WQC-24). Water samples were collected and then transported to the laboratory in a cool box and analyzed using certified methods within 24 hours. Analysis determined concentrations of sulphate (mg SO₄ L⁻¹), phosphorus (µg P L⁻¹), Ammonium (µg NH₄ L⁻¹), Nitrite (µg NO₂ L⁻¹), Nitrate (µg NO₃ L⁻¹), chloride (mg Cl L⁻¹), total iron (mg Fe L⁻¹), zinc (mg Zn L⁻¹), chromium (µg Cr L⁻¹), manganese (µg Mn L⁻¹), mercury (µg Hg L⁻¹), arsenic (µg As L⁻¹), cadmium (µg Cd L⁻¹), copper (mg Cu L⁻¹), sodium (mg Na L⁻¹) and hydrocarbons (mg/L). Conductivity (µS/cm), total suspended solids (mg L⁻¹), biological oxygen demand (mg O₂ L⁻¹), chemical oxygen demand (mg O₂ L⁻¹), total and fecal coliforms (ufc/100 mL) were also determined in laboratory. Hydromorphological factors were obtained in ArcGis 9 (ESRI) using 1:25000 topographic maps (altitude, latitude, longitude, stream order, distance to mouth, distance to source and slope) for each site. Land use categories and areas (non-native forest, scrub, agriculture area, natural vegetation and urban areas) were obtained from Regional Planning Department of the Azores (Quatenaire-Portugal, 2008); a circular buffer area (200 m radius) was created around each sampling site. All described variables were obtained for each site, sample site and sampling season, with the exception of cartographic variables.

Data analyses

Several indices of species diversity (Magurran, 2004), such as Pielou's evenness, Fisher's α and Shannon-Wiener were calculated from the biological data. Non-redundant subsets of environmental and pressure parameters were obtained using the Pearson correlation coefficients to find and compare highly correlated parameters. If two environmental or pressure variables were highly correlated (threshold value of $r \geq 0.6$), then the variable with a highest overall mean correlation coefficient was excluded from subsequent analyses (Hering *et al.*, 2006; Feld and Hering, 2007). A total of 7 environmental and 14 chemical variables were retained from an initial list of 13 environmental and 28 chemical variables (Tab. 2). Analyses were carried out using Primer 6.0 (Clarke and Gorley, 2006).

A Principal Components Analysis (PCA) was performed to identify abiotic gradients and patterns between streams. Permutational multivariate analysis of variance – PERMANOVA (Anderson, 2001; McArdle and Anderson, 2001) was used to determine associations between the chemical variables and chironomid assemblages. PERMANOVA partitions the total sum of squares based

Table 1. Location of sampling sites, respective sampling codes and UTM coordinates.

Island	Stream	Location	Code	UTM	
Flores	Grande	Headwater sites	RGR0	25S 654026/4366597	
		Headwater sites	RGR1	25S 652188/4368056	
		Mid-section sites	RGR2	25S 651256/4366471	
		Lower reaches sites	RGR3	25S 649839/4366831	
	Badanela	Headwater sites	RBD0	25S 654106/4370554	
		Headwater sites	RBD1	25S 654126/4370547	
		Lower reaches sites	RBD2	25S 659820/4370524	
	São Miguel	Guilherme	Headwater sites	RGU0	26S 658164/4184939
			Mid-section sites	RGU1	26S 660669/4186486
			Lower reaches sites	RGU2	26S 662931/4189980
Faial da Terra		Headwater sites	RFT0	26S 658356/4183665	
		Mid-section sites	RFT1	26S 659753/4182525	
		Lower reaches sites	RFT2	26S 658977/4178911	
Grande		Headwater sites	RG1	26S 635636/4182114	
		Lower reaches sites	RG3	26S 630243/4187075	
	Praia	Mid-section sites	RP0	26S 634841/4178145	
	Teixeira	Mid-section sites	RTX2	26S 632100/4183838	
	Povoação	Lower reaches sites	RPV4	26S 654812/4179468	
	Caldeirões	Mid-section sites	RC2	26S 651830/4190893	
Quente	Mid-section sites	RQ2	26S 649998/4182002		
	Lower reaches sites	RQ4	26S 654812/4179468		

on the full experimental design (given below), and calculates a distance based pseudo- F statistic for each term in the model, based on the expectations of the mean squares. P -values are obtained using a permutation procedure for each term (Anderson *et al.*, 2008), or *via* Monte Carlo random drawn from an asymptotic permutation distribution if too few permutations are available for a given test (Anderson and Robinson, 2003).

We tested for two spatial and one temporal distribution patterns using PERMANOVA: (i) between islands (fixed, two levels; Flores and São Miguel), (ii) over time (random, four levels: Winter, Spring, Summer and Autumn) and (iii) along the lotic longitudinal gradient (fixed, three levels: headwater sites; mid-section sites and lower reaches sites). Because chemical and environmental data have different measurement scales and similarity methods, we normalised data, according to Clarke and Gorley (2001). The relative abundance of the 21 species was $\log(x + 1)$ transformed to reduce differences in scale (Clarke and Green, 1988). For PERMANOVA, we used 9999 permutations of the data (an appropriate distribution for a pseudo- F statistic under a true null hypothesis is obtained by using a permutation test. According to Anderson and Braak (2003) the programme permutes the units identified by the denominator term of the F -ratio).

SIMPER analysis was used to identify species' relative contribution to the groups resulting from PERMANOVA analyses. Bray-Curtis dissimilarities were calculated for all pairs of observations for the analyses below.

Links between the non-redundant set of hydromorphological and physicochemical variables (see Tab. 2) and the community assemblage were assessed using distance-based multivariate analysis for a linear model – DISTLM (Legendre and Anderson, 1999; McArdle and Anderson,

2001). DISTLM analyses and models the relationship between the multivariate data cloud, for one or more predictor variables (Anderson *et al.*, 2008). DISTLM allows predictor variables to be fitted individually or together in user specified sets. The DistLM routine was based on the AIC model selection criterion (Burnham and Anderson, 2004) using a Step-wise selection procedure. The AIC model (Akaike, 1974; Akaike, 1973) was used to select the model that explained more variation, and therefore the 'best' model for that particular data set. Primer 6.0 and PERMANOVA + for PRIMER software were used (Clarke and Gorley, 2006; Anderson *et al.*, 2008). For visual interpretation of the resulting model in multi-dimensional space, we used distance based redundancy analysis (dbRDA) to investigate the relationship between location, environmental and chemical variables (Anderson *et al.*, 2008). Each vector begins at the centre of the circle (the origin) and ends in the coordinates (x and y). The length and direction of each vector indicates the strength and sign of the relationship between the given variable and the dbRDA axes. The overlaying vector illustrates the relationship between the eight variables identified by the DistLM procedure.

Results

Physicochemical characterization

PERMANOVA results (Tab. 3) showed significant temporal (Time – $F_{3,57} = 2.31$, $P < 0.002$) and spatial variation along longitudinal gradient (Location – $F_{2,57} = 5.49$, $P < 0.007$), but not between islands ($P > 0.1$). The PCA ordination also reflected this pattern (Fig. 2).

Table 2. Hydromorphological and physicochemical (variables excluded from subsequent analyses in bold).

Variable	Headwater sites	Mid-section sites	Lower reaches
<i>Hydromorphological</i>			
Production forest area (%)	31.84 ± 43.41	45.08 ± 42.55	0.00 ± 0.00
Scrub area (%)	18.19 ± 35.7	18.20 ± 36.13	6.21 ± 13.83
Agriculture area (%)	3.33 ± 8.13	17.15 ± 36.14	28.02 ± 36.34
Natural vegetation area (%)	46.65 ± 48.88	19.56 ± 35.18	2.29 ± 5.09
Urban area (%)	0.00 ± 0.00	0.00 ± 0.00	63.49 ± 47.55
Strahler Order	2.74 ± 0.71	3.73 ± 0.45	5.00 ± 0.60
Altitude (m)	605.48 ± 104.07	227.15 ± 94.56	13.78 ± 7.38
Distance to source (m)	1777.03 ± 818.87	4914.74 ± 2030.97	9946.97 ± 2212.69
Distance to mouth (m)	7329.49 ± 1550.71	4172.66 ± 1951.03	298.66 ± 234.69
Slope (%)	10.06 ± 8.53	7.10 ± 7.43	5.28 ± 3.69
Latitude	567290 ± 214208	649042 ± 10544	650514 ± 10162
Longitude	4293730 ± 92521	4204560 ± 59840	4246894 ± 91057
<i>Physicochemical</i>			
Temperature (°C)	14.63 ± 3.14	15.20 ± 2.02	17.74 ± 2.88
pH	6.74 ± 0.99	7.45 ± 0.45	7.95 ± 0.42
Conductivity (µS/cm)	76.71 ± 32.07	142.28 ± 65.39	225.31 ± 103.35
Total Suspended Solids (mg L ⁻¹)	3.04 ± 1.65	11.88 ± 30.13	6.04 ± 10.43
Dissolved Oxygen (%)	104.74 ± 7.85	104.96 ± 7.06	103.35 ± 8.15
Biological Oxygen Demand (mg O ₂ L ⁻¹)	1.28 ± 0.91	1.25 ± 1.00	1.70 ± 1.15
Chemical Oxygen Demand (mg O₂ L⁻¹)	11.00 ± 9.43	4.27 ± 1.91	5.09 ± 4.07
Ammonium (µg NH ₄ L ⁻¹)	19.56 ± 5.45	27.88 ± 27.93	30.22 ± 23.18
Nitrite (µg NO ₂ L ⁻¹)	4.17 ± 5.51	10.24 ± 14.83	11.39 ± 10.76
Nitrate (mg NO ₃ L ⁻¹)	0.35 ± 0.25	1.36 ± 1.63	3.34 ± 2.35
Chloride (mg Cl L⁻¹)	12.37 ± 2.65	19.56 ± 13.18	21.78 ± 5.05
Sulfate (mg SO₄ L⁻¹)	2.47 ± 0.98	9.82 ± 27.93	5.43 ± 2.49
Soluble Phosphate (µg P₂O₅ L⁻¹)	32.52 ± 27.45	125 ± 65.47	143.13 ± 53.81
Inorganic Phosphorus (µg P L⁻¹)	25.56 ± 18.77	76.15 ± 46.21	74.52 ± 44.56
Total Phosphorus (µg P L⁻¹)	30.07 ± 21.09	85.38 ± 52.82	94.13 ± 63.03
Chromium (µg Cr L⁻¹)	0.64 ± 0.12	0.60 ± 0.02	0.66 ± 0.16
Manganese (µg Mn L ⁻¹)	56.67 ± 52.33	55.69 ± 31.56	91.74 ± 104.87
Iron (mg Fe L ⁻¹)	0.52 ± 0.77	0.21 ± 0.22	0.42 ± 0.61
Copper (mg Cu L⁻¹)	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Zinc (mg Zn L ⁻¹)	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0
Cadmium (µg Cd L⁻¹)	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00
Mercury (µg Hg L ⁻¹)	0.30 ± 0.00	0.31 ± 0.04	0.31 ± 0.06
Lead (µg Pb L⁻¹)	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Arsenic (µg As L⁻¹)	3.00 ± 0.00	3.46 ± 1.42	3.72 ± 1.51
Sodium (mg Na L⁻¹)	10.41 ± 6.26	18.46 ± 10.54	32.46 ± 22.42
Hydrocarbons (mg L ⁻¹)	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Total Coliforms (ufc/100 mL)	177.04 ± 323.36	1034.62 ± 2410.57	4591.74 ± 4562.55
Fecal Coliforms (ufc/100 mL)	63.7 ± 100.73	716.92 ± 2090.45	2283.91 ± 3408.51

Table 3. Results of three-factor permutational multivariate analysis of variance (PERMANOVA) on normalized chemical data: Island (Is); Season (Se); Location (Lo); Time (Ti); and Residual (Res). **Bold F-values** indicate significant *P*-values (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

Source of variation	<i>df</i>	SS	MS	Pseudo- <i>F</i>	<i>P(perm)</i>	Unique perms
Is	1	25.522	25.522	1.9428	0.1266	7867
Se	3	79.355	26.452	2.3102	0.0015**	9907
Lo	2	99.093	49.546	5.4868	0.0069**	9949
IsxSe	3	40.073	13.358	1.1666	0.2602	9894
IsxLo	2	55.548	27.774	2.8566	0.0094**	9933
SexLo	6	52.053	8.6755	0.75769	0.8589	9900
IsxSexLo**	5	48.246	9.6492	0.84274	0.7036	9891
Res	57	652.64	11.45			
Total	79	1106				

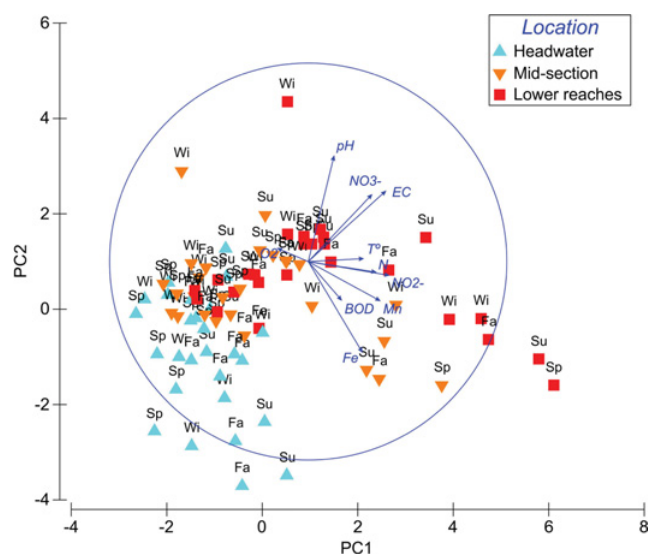


Fig. 2. Diagram of Principal Component Analysis of the chemical variables. Location are represented by: Headwater sites; Mid-section sites; and Lower reach sites. Time is presented by: Wi – Winter; Sp – Spring; Su – Summer and Fa – Fall. Solid arrows represent chemical variables: the length of the arrow indicates the importance of the variable while the direction of the arrowhead indicates the direction of increasing influence.

The first two PCA axes explained 39% of the variation and cumulatively 49% with the inclusion of the third axis. Temperature, conductivity, ammonium, nitrite, nitrate and phosphate compounds increased along the longitudinal gradient (Table 2) while dissolved oxygen, biological and chemical oxygen demand decreased. At most sites, dissolved oxygen values and pH were generally high during spring but decreased from summer and fall as a result of baseflow conditions.

Azorean chironomids communities

A total of 24 taxa were identified from 3 subfamilies and 5 tribes. The Orthocladiinae (15 taxa) showed the greatest richness, followed by Chironominae (6 taxa) and Tanypodinae (3 taxa). The Orthocladiinae was the dominant subfamily with a mean composition of 96%. Orthocladiinae exuviae comprised between 27% and 100% of the total exuvias found.

Distinct spatial chironomid distribution patterns were apparent. *Cricotopus sylvestris*, *Polypedilum nubeculosum* and *Pseudorthocladus curtistylus* were exclusive to São Miguel Island, while *Psectrocladius limbatellus*, *Pseudosmittia* sp1 and *Telmatopelopia nemorum* were only collected from streams on Flores. *T. nemorum* and *Zavelimyia nubila* occurred only at headwater sites, while *Chironomus riparius*, *C. cingulatus*, *C. sylvestris*, *C. ornatus* and *P. nubeculosum* were consistently absent from such sites (Appendix I). The most dominant species were *Thienemanniella clavicornis* and *Orthocladus fuscimanus*,

Table 4. Average species number and species diversity indices in headwater sites, in mid-section sites and lower reaches sites.

Diversity indices	Headwater sites	Mid-section sites	Lower reaches sites
Number of taxa	5	7	7
Pielou's evenness	0.55	0.63	0.61
Fisher's α	0.45	0.56	0.57
Shannon-Wiener Diversity Index	0.87	1.16	1.09

with mean composition per site of 39% and 17%, respectively. These species were detected at least once for all sampling sites. *Cardiocladius freyii* was present at all sampling sites, with mean composition of 10% per site, with the exception of RFT1. *Thienemanniella clavicornis*, *Orthocladus (Eudactylocladius) fuscimanus*, *C. freyii*, *Parametrioctenus stylatus* and *R. atripes* occurred at more than 60% of the sampling sites. Species diversity indices varied considerably among sites (Tab. 4). The highest number of taxa was at RPV4 ($n = 13$) during the winter while the lowest number of taxa was at RGU2 and RFT0 in autumn, and RTX2 and RG1 in winter ($n = 2$).

Spatial and temporal distribution patterns

PERMANOVA results (Tab. 5) showed that chironomid assemblages differed significantly along the longitudinal gradient (Location – $F_{2,56} = 12.42$, $P = 0.004$), but not between islands ($P > 0.05$ on all factors and interactions) or over time ($P > 0.05$ on all factors and interactions). SIMPER results (Tab. 6) of longitudinal location indicated lower levels of dissimilarity between sites situated on mid-section reaches and headwater sites (42%) and between headwater sites and mid-section sites (59%), than that observed between headwater and lower reaches sites (64%). *R. atripes*, *T. clavicornis*, *Cardiocladius freyii* and *O. fuscimanus* were identified as the principal species contributing to the observed dissimilarity (> 50%) between the headwater sites and mid-section sites and between headwater sites and sites along the lower reaches. SIMPER results revealed longitudinal changes in percent abundance; in *R. atripes* (2.6% – headwater sites; 1.4% – mid-section sites; 0.6% – lower reaches sites), *Cardiocladius freyii* (0.7% – headwater sites; 2.0% – mid-section sites; 2.1% – lower reaches sites), *O. fuscimanus* (1.8% – headwater sites; 2.3% – mid-section sites; 2.6% – lower reaches sites) and *P. stylatus* (0.3% – headwater sites; 1.5% – mid-section sites; 1.7% – lower reaches sites).

Hydromorphological and physicochemical determinants of chironomids assemblages

The most important hydromorphological variables explaining differences in stream location were altitude

Table 5. Results of three-factor permutational multivariate analysis of variance (PERMANOVA) on relative abundance of chironomid: Island (Is); Time (Ti); Location (Lo) and Residual (Res). **Bold F-values** indicate significant *P*-values (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

Source of variation	<i>df</i>	SS	MS	Pseudo- <i>F</i>	<i>P</i> (<i>perm</i>)	Unique perms
Is	1	5758.4	5758.4	2.75	0.086	7827
Ti	3	5818.3	1939.4	1.81	0.053	9920
Lo	2	24483.0	12241.0	12.42	0.004**	9941
IsxTi	3	5686.1	1895.4	1.77	0.060	9921
IsxLo	2	2968.2	1484.1	1.71	0.170	9952
TixLo	6	5834.3	972.4	0.91	0.577	9908
IsxTixLo	5	4275.4	854.4	0.80	0.694	9920
Res	56	59851.0	1068.8			
Total	78	122690.0				

Table 6. Summary of SIMPER analysis results on the occurrence and relative abundance of chironomid taxa (> 10%).

Species	Average abundance	Average abundance	Average dissimilarity	% Contribution to dissimilarity	Cumulative % contribution
Average dissimilarity = 58.65%					
	Headwater sites	Mid-section sites			
<i>Rheocricotopus atripes</i>	2.62	1.38	8.52	14.52	14.52
<i>Thienemanniella clavicornis</i>	2.43	3.50	7.77	13.25	27.78
<i>Cardiocladius freyii</i>	0.66	2.03	7.18	12.24	40.02
<i>Orthocladius fuscimanus</i>	1.78	2.27	6.81	11.60	51.62
Average dissimilarity = 63.76%					
	Headwater sites	Lower reaches sites			
<i>Rheocricotopus atripes</i>	2.62	0.55	10.12	15.80	15.80
<i>Thienemanniella clavicornis</i>	2.43	3.19	8.28	12.93	28.74
<i>Cardiocladius freyii</i>	0.66	2.06	7.60	11.87	40.61
<i>Orthocladius fuscimanus</i>	1.78	2.61	7.41	11.58	52.18
<i>Parametrioecnemus stylatus</i>	0.27	1.77	7.16	11.19	63.37
Average dissimilarity = 41.58%					
	Mid-section sites	Lower reaches sites			
<i>Cricotopus sylvestris</i>	0.86	1.13	5.26	12.57	12.57
<i>Orthocladius fuscimanus</i>	2.27	2.61	5.24	12.52	25.09
<i>Cardiocladius freyii</i>	2.03	2.06	5.19	12.41	37.50
<i>Rheocricotopus atripes</i>	1.38	0.55	5.14	12.28	49.78
<i>Thienemanniella clavicornis</i>	3.50	3.19	5.14	12.28	62.06

(*P* < 0.001), natural vegetation area (*P* = 0.002) and agricultural land use (*P* = 0.003), followed by urban area (*P* = 0.004), forest production (*P* = 0.005), slope (*P* = 0.013) and scrub vegetation areas (*P* > 0.05) identified in DistLM procedure (Tab. 7).

The Step-wise procedure selected altitude, production forest area, scrub area, natural vegetation area, slope and agriculture area as variables that determined chironomid composition in stream location (AIC = 561.43; R² = 0.34; number of variables = 6).

The dbRDA ordination (Fig. 3) illustrated how sites were clearly separated by their location along the longitudinal gradient and the principal contributing environmental variables. The first two dbRDA axes explained 89% of the relationship between the chironomid assemblage and the measured hydromorphological variables, and 31% of the total variability in the community data.

Table 7. Summary of DistLM procedure for hydro-morphological variables.

Variable	SS (trace)	Pseudo- <i>F</i>	<i>P</i>
Production forest area	6113.4	4.01	0.005
Scrub area	3044.9	1.92	0.079
Agriculture area	7910.0	5.27	0.003
Natural vegetation area	8489.7.9	5.68	0.002
Urban area	6994.7	4.62	0.004
Altitude	26163	20.69	0.001
Slope	4949.9	3.21	0.013

The first dbRDA axis was strongly related to altitude while the second axis was related to land use (production forest, agriculture and natural vegetation area).

The most important chemical variables identified in DistLM routine were water temperature (*P* = 0.001),

Table 8. Summary of DistLM procedure for physicochemical variables.

Variable	SS (trace)	Pseudo- <i>F</i>	<i>P</i>
Water temperature pH	12389	8.6487	0.001
Conductivity	16962	12.353	0.001
Total suspended solids	14317	10.172	0.001
Dissolved oxygen	607.98	0.38347	0.839
Biological Oxygen Demand	758.6	0.47906	0.767
Ammonium	1138.7	0.72134	0.605
Nitrite	2617.3	1.6784	0.127
Nitrate	7085.9	4.7197	0.006
Manganese	9959.5	6.8028	0.001
Iron	3211.6	2.0698	0.080
Zinc	5236.3	3.4328	0.010
Mercury	1225.6	0.77694	0.532
Hydrocarbons	910.34	0.57559	0.668
	1116	0.70683	0.446

pH ($P=0.001$), conductivity ($P=0.001$) and nitrate ($P=0.001$) as the principal determinants of chironomid composition along the longitudinal gradient. Nitrite ($P=0.006$) and total iron ($P=0.01$) were also important parameters (Tab. 8). The Step-wise procedure selected temperature, pH, nitrite, iron and conductivity as the strongest parameters determining chironomid composition in relation to stream location (AIC = 562.16; $R^2 = 0.32$; n° of variables = 5) (Tab. 8).

The dbRDA for chemical variables (Fig. 4) also showed a clear longitudinal separation of stream sampling sites and the principal contributing chemical variables (pH, nitrite, iron and temperature). The first two dbRDA axes captured 87% of the variability in the fitted model, and 28% of the total variation in the location. The vector overlays showed the relationship between the five variables found by the DistLM procedure. The first dbRDA axis was negative in relation to pH and nitrite and positive in relation to iron. The second axis showed a strong positive relationship with temperature.

Discussion

The number of chironomid taxa recorded in Azorean streams was low (24) compared to typologically similar continental rivers (*e.g.* mountain rivers) and other macaronesian streams. For example, 71 taxa were recorded at Aude River (Gendron and Laville, 1995) in France, 107 taxa at Pang River (Ruse and Wilson, 1995), in United Kingdom, 54 taxa in Tenerife Island (Armitage *et al.*, 1995b) and 46 taxa on the island of Madeira (Hughes, 2003). Similar to findings from Madeira (Hughes, 2003) and Tenerife (Armitage *et al.*, 1994), the *Orthocladiinae* and *Chironominae* were the most frequently observed subfamilies in Azorean streams (Murray *et al.*, 2004; Raposeiro *et al.*, 2009). Similar to Tenerife (Armitage *et al.*, 1994), *Thienemaniella clavicornis* is the most dominant species in Azorean streams, but appears to be far rarer on Madeira (Hughes, 2003).

Bilton *et al.* (2001) described active and passive dispersal mechanisms for success in the colonization of

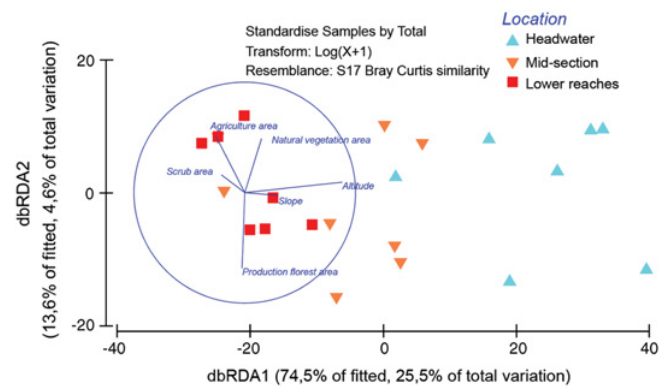


Fig. 3. Distance-based RDA ordination of first and second fitted axes relating the environmental variables to chironomid composition on different stream location. Vectors projections are given for the environmental variables selected by the DistLM routine. Length and direction of the vectors represent the strength and direction of the relationship.

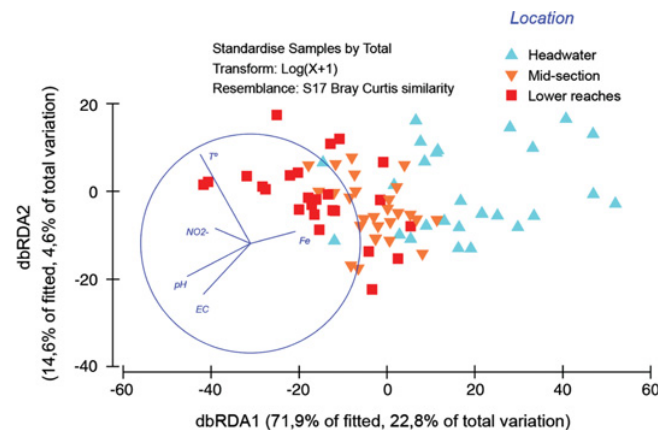


Fig. 4. Distance-based RDA ordination of first and second fitted axes relating the chemical variables to chironomid composition on different stream location. Vectors projections are given for the chemical variables selected by the DistLM routine. Length and direction of the vectors represent the strength and direction of the relationship.

freshwater systems. Actively flying chironomids adults can disperse over large areas (Massaferro and Brooks, 2002), allowing the colonization of numerous freshwater ecosystems on the same island or even in neighbouring islands, which partly explains the high levels of similarity between the two islands in this study. In a setting such as the Azorean archipelago, active dispersal methods are important for the “stepping stone” type freshwater habitat colonization between islands (Whittaker and Fernandez-Palacios, 2007).

Spatial variability: hydromorphological and physicochemical factors

The present study provides information about the physicochemical and hydromorphological variables

determining chironomid assemblage distribution patterns in Azorean streams. An understanding of both local and large-scale environmental factors is vital in order to assess the importance of their role in structuring chironomid communities (Punti *et al.*, 2007; Calle-Martínez and Casas, 2006; Punti *et al.*, 2009). Differences in chironomid assemblage structure between islands were not apparent ($P = 0.068$) but were significantly different along the lotic longitudinal gradient ($P = 0.004$).

Physicochemical and hydromorphological variables explained 58.5% of the variance in chironomid assemblage composition. These values are similar to those determined in several previous studies on benthic diatoms (Soininen *et al.*, 2004), macroinvertebrates (Mykrä *et al.*, 2007; Heino and Paasivirta, 2008) and fish (Magalhaes *et al.*, 2002). The percentage of variance in chironomids assemblages explained only by hydromorphological variables in our study was considerable (30.8%) and similar to the percentage of variation explained by physicochemical variables (27.7%). The relatively high percentage of unexplained variation (41.5%) in our study is typical of noisy data sets with many 0 values (Borcard *et al.*, 1992), and could be result of abiotic and biotic factors not considered in this study, such as species' interactions (Kohler, 1992), food availability (Peeters *et al.*, 2004), reproduction, competition and predation (MacNeil *et al.*, 1999).

The similarity of the faunal assemblages between islands appears to be related to the physical and chemical similarities of the islands' streams coupled with the chironomid dispersal abilities. PERMANOVA results did not detect significant spatial differences in physicochemical composition between islands ($P > 0.1$), but did detect significant differences along the lotic longitudinal gradient ($P < 0.007$). This emphasizes the importance of local, lower scale physicochemical and hydromorphological in determining the longitudinal distribution patterns of the chironomid community following colonization.

Habitat complexity also influences the composition of the freshwater assemblages (Heino, 2000; Tolonen *et al.*, 2001; White and Irvine, 2003). Coffman (1995, 1989) proposed ecological heterogeneity as one of the main factors influencing chironomid species richness. To date, no detailed data on habitat complexity in Azorean streams is available. However, field observations indicate that key drivers such as substrate and flow are very similar between islands. Several authors (Malmqvist, 2002; Hughes and Malmqvist, 2005) have reported low spatial heterogeneity and low habitat diversity in Macaronesian systems (almost all streams are narrow, steep, with coarse substrate and typical step-pool flow patterns). The similar chemical composition and low spatial heterogeneity appears to be reflected in the expected high similarity of chironomids assemblages found in both islands.

T. clavicornis, *O. fuscimanus* and *R. atripes* dominated sampling sites on both São Miguel and Flores islands. These species are known to possess a high dispersion ability and are well represented in the Palaearctic (Saether and Spies, 2009) and Macaronesian regions (Oromí and Báez, 2001; Hughes, 2008).

The islands under study have suffered dramatic changes in land use following human colonization in the fifteenth century when the native vegetation at low and middle altitudes was cleared or became gradually extinct. In the 20th century, an increase in land dedicated to pasture has further depleted small fragments of native vegetation at mid range altitudes (Silva and Smith, 2004). Nowadays, indigenous forested areas are limited to mid to high altitude (approximately > 600 m). However mid-range reaches are considerably altered by human intervention including agriculture, canalization, flow diversion and organic discharge and almost all lower reaches are affected by urbanization. This study has clearly shown that changes in land use exerted a significant effect on longitudinal changes in chironomid assemblage composition along the stream represented by the secondary axes of the dbRDA.

Many authors highlight agriculture as a major cause of degradation in stream habitats (Allan and Flecker, 1993; Allan, 2004), and that the biological impacts of physical alterations from this activity can last for decades (Harding *et al.*, 1998). This type of impact contrasts with the impacts of urbanization which are mainly related to direct effects of point source pollution, hydromorphological and biological impacts (Brasher, 2003; Feld and Hering, 2007; Mykrä *et al.*, 2007; Munné and Prat, 2009; Weijters *et al.*, 2009). The physicochemical gradient of temperature, pH and conductivity in this study illustrates the indirect, combined effect of multiple mechanisms operating over several spatial scales describing natural biotic and abiotic gradients, changes in land use an organic enrichment (Hughes *et al.*, 2009). Most of the retained variables *e.g.* increase of nitrogen and phosphate compounds, increase of coliforms also described consequences of changes of the land use along the longitudinal gradient (see Tab. 2).

The dbRDA ordinations revealed scale dependent environmental and chemical variables with potentially high levels of covariance. In fact, the history of colonization of the archipelago (the Azores have suffered dramatic changes in land due to colonization, remaining indigenous forested mostly in high altitude) combined with the natural spatial and temporal patterns of insular streams could obscure the ready distinction of these sources of variation. Similar situations were reported by authors for the Mediterranean systems (Díaz *et al.*, 2008; Hughes *et al.*, 2009). The increase in nutrient levels along the longitudinal gradient observed in the Azorean streams is consistent other studies identifying chironomid composition changes along the river continuum, in association with altitude and stream order (Lindegaard and Brodersen, 1995; Heino, 2005; Inoue *et al.*, 2005; Heino and Paasivirta, 2008).

Chironomid species richness, evenness and diversity tended to be lower at headwater sites (2nd–3rd order), increasing to maximum values at sites midway along the longitudinal gradient (3rd–4th order), before decreasing again at sites further downstream (5th–6th order). These findings partly agree with Coffman (1989), who found maximum richness of lotic chironomids in 3rd order streams (*e.g.* RC2; RQ2) as a result of higher ecological

heterogeneity. The patterns revealed by the three diversity indices are also consistent with the Connell's intermediate disturbance hypothesis, (Connell, 1978), which describes maximal species richness at intermediate levels of disturbance (Tab. 7). Vannote *et al.* (1980) and Giller and Malmqvist (1998) attributed the increase in diversity from headwaters to mid section of freshwater courses to increasing temperature, improved light conditions and broader stream channels. Moore and Palmer (2005) also reported higher invertebrate biodiversity in agricultural streams which then progressively declined along a land use gradient towards urbanization. Three groups of chironomids (headwaters, mid-section and low reaches) were discernible in relation to the influence of environmental variables [identified in PERMANOVA test ($F_{2,54} = 3.21$, $P = 0.004$)]. The dbRDA results clearly indicated a strong environmental framework structuring the characteristics of both the sites and taxa.

The right-hand side of the dbRDA ordination was occupied mainly by headwater sites in semi-pristine conditions, located in scrub or natural vegetation. These sites were characterized by low temperatures, circum-neutral pH, low conductivity, nutrients and metals. *R. atripes* was the most abundant species at headwater sites; *Z. nubila* and *Paramerina cingulata* were restricted to these habitats, indicated by SIMPER analysis results. According to Vallenduuk and Moller Pillot (2007), these latter two Tanypod species tend to occur in the water surface layer of soil or plants, or in pools. These features indicate more stable habitats, when compared with bed-rock habitats, with low levels of disturbance (Townsend and Hildrew, 1994). Milner and Petts (1994) proposed stream channel stability as a major environmental factor determining stream community structure. The left-hand of the dbRDA ordinations were occupied by samples from stream sites located in areas subject to high levels of human intervention. These sites were lower lying, located close to urbanized areas and displayed higher temperature, pH, conductivity and nutrient levels. *C. sylvestris* and *P. stylatus* typically occurred at lower reaches locations. Several studies have reported that *Cricotopus* is abundant at nutrient enriched sites (e.g. Davies and Hawkes, 1981; Wilson, 1992; Calle-Martínez and Casas, 2006; Maasri *et al.*, 2008). Increased nutrient levels at mid-section sites adjacent to agriculture and areas dedicated to grazing of livestock appeared to augment chironomid abundance. *T. clavicornis* and *Synorthocladius semivirens* were more abundant at such sites while *Pseudorthocladius curtistylatus* and *Polypedilum nubeculosum* were exclusive to these sites. *T. clavicornis*, *Cardiocladius freyii* and *O. fuscimanus* were the most abundant species and are tolerant to a wide range of conditions.

Temporal variability

Despite the distinct seasonal climatic patterns, PERMANOVA results did not detect significant temporal patterns ($P > 0.05$) in chironomid composition (Tab. 5)

but did detect significant differences in chemical composition (Time – $F_{3,56} = 23814$, $P = 0.002$). These findings indicate seasonal persistence in community patterns. SIMPER analysis results showed that *T. clavicornis* consistently contributed over 30% to group similarity, followed by *O. fuscimanus* (contribution > 18%) and *Cardiocladius freyii* (contribution > 8%). These species predominated in the sampled streams throughout the entire sampling period.

Persistence and stability of the 3 most abundant taxa were consistently high across the entire community, suggesting a temporally persistent pool of common taxa in Azorean streams, similar to other studies in continental streams (Townsend *et al.*, 1987; Winterbourn, 1997). However, further research is required to understand the strategies employed by these core chironomid taxa. Seasonal persistence of the chironomid communities is consistent with the results of previous studies of macro-invertebrates communities in Mediterranean streams (Mellado, 2005; Bêche *et al.*, 2006). Observed persistence in Azorean chironomid communities in this study could be the result of naturally high disturbance levels which are typical of oceanic islands (Hughes, 2005; Covich, 2006; Covich, 2009) and act as selective filters favouring species adapted to such conditions.

Chironomid emergence patterns vary both temporally and spatially across streams and voltinism varies both interspatially and intraspatially (Wrubleski and Rosenberg, 1990). Most chironomid lotic species are multivoltine (three or more generations per year) with peaks in emergence occurring between spring and autumn (Tokeshi *et al.*, 1995). The presence of several species across the four sampling seasons could be explained by the continuous emergence of species from overlapping generations or cohorts. Small sized species such *T.* are multivoltine and often observed to possess closely overlapping generations (Tokeshi *et al.*, 1995). Multivoltine species are better adapted to exploit situations of environmental change, especially when rapid fluctuations occur (Delettre, 1995), such as rapid runoff and highly torrential flow regimes which are common after rainfall on the Azores.

Final remarks

Relatively reduced habitat diversity, low spatial heterogeneity, distance from sources of colonization and the geological youth of the Azores archipelago, limit the pool of invertebrates able to colonize and inhabit Azorean streams, a phenomenon common to other Macaronesian islands (Hughes and Malmqvist, 2005; Hughes, 2005; Hughes, 2006).

Local changes in the physical environment due to changes in land use (agriculture and forestry, urbanization) alter local habitat quality, influencing community composition and biodiversity. Our results confirm that chironomids constitute a promising candidate group for ecological assessment in Azorean lotic systems, but larger

data sets are required to better analyze intraannual and interannual variability and the effect of habitat scale variables. A better understanding of the ecological requirements of chironomid communities in Azorean streams and their biology is essential for developing water-quality monitoring protocols suitable for insular streams. More detailed of life cycles and emergence patterns over several seasons will provide more information on chironomid phenology. All of these findings would contribute to testing and possibly adapting the Chironomid Pupal Exuviae Technique (CPET) to Azorean surface waters.

The information from this study will help support current and future research of Azorean stream ecosystems and future freshwater management issues such as conservation planning, management measures, implementing biomonitoring programmes, and predicting of how human alterations will affect lotic ecosystems, allowing the development and implementation of sustainable and effective natural resource management across the archipelago.

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