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Environmental factors, spatial variation, and specific requirements of Chironomidae in Mediterranean reference streams

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Abstract. Chironomidae spatial distribution was investigated at 63 near-pristine sites in 22 catchments of the Iberian Mediterranean coast. We used partial redundancy analysis to study Chironomidae community responses to a number of environmental factors acting at several spatial scales. The percentage of variation explained by local factors (23.3%) was higher than that explained by geographical (8.5%) or regional factors (8%). Catchment area, longitude, pH, % siliceous rocks in the catchment, and altitude were the best predictors of Chironomidae assemblages. We used a *k*-means cluster analysis to classified sites into 3 major groups based on Chironomidae assemblages. These groups were explained mainly by longitudinal zonation and geographical position, and were defined as 1) siliceous headwater streams, 2) mid-altitude streams with small catchment areas, and 3) medium-sized calcareous streams. Distinct species assemblages with associated indicator taxa were established for each stream category using IndVal analysis. Species responses to previously identified key environmental variables were determined, and optima and tolerances were established by weighted average regression. Distinct ecological requirements were observed among genera and among species of the same genus. Some genera were restricted to headwater systems (e.g., *Diamesa*), whereas others (e.g., *Eukiefferiella*) had wider ecological preferences but with distinct distributions among congenerics. In the present period of climate change, optima and tolerances of species might be a useful tool to predict responses of different species to changes in significant environmental variables, such as temperature and hydrology.

Key words: Chironomidae assemblages, environmental gradient, optima and tolerances, autoecology, spatial variation, partitioning variance.

One of the focal points of aquatic community ecology is to identify factors (i.e., habitat, competition) that determine community composition in streams and to study how these factors influence biotic diversity and abundance (Allan 1995). Aquatic macroinvertebrate communities respond to multiple environmental gradients, many of which are scale-related (Vinson and Hawkins 1998). Therefore, communities are shaped by both local-scale processes and broad-scale constraints, such as geology and climate (Menge and Olson 1990, Poff 1997). Several recent studies examined the relationships between freshwater communities and environmental factors measured at distinct spatial scales (Johnson et al. 2007, Mykra et al. 2007). The relative importance given to factors that affected community structure of benthic macroinvertebrates differed among studies, but most authors reported that local-scale factors exerted the greatest influence (e.g., Death and Joy 2004, Sandin and Johnson 2004).

Chironomidae are the most broadly distributed, species-rich, and often the most abundant family of benthic macroinvertebrates in fresh waters (Pinder 1986). They are a heterogeneous group of species with variable responses to environmental gradients (Lencioni and Rossaro 2005). Ecological information on chironomids is still fragmentary, especially for larvae, because species identification is time-consuming and requires sound taxonomic expertise. Chironomidae are widely used in bioassessment as indicators of lake trophic conditions (Saether 1979) and organic pollution in running waters (Orendt 1999). This family also is used in paleolimnological studies for environmental reconstruction (Walker 2001).

Several studies have identified spatial assemblage patterns and significant environmental factors contributing to Chironomidae assemblage structure in tem-

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perate streams (e.g., Lindegaard and Brodersen 1995, Lencioni and Rossaro 2005). One important environmental gradient is longitudinal zonation. Only a few studies have addressed Chironomidae in Mediterranean streams (González et al. 1985, Casas and Vílchez-Quero 1993). These studies were conducted along relatively small spatial gradients, were based mostly on pupal exuviae, and were not done exclusively in reference conditions. They reported that altitudinal gradient strongly influenced Chironomidae assemblage composition.

Biological responses to environmental factors can be studied using an autecological approach at the population level (Tokeshi 1999). For example, estimation of optima and tolerances for each species is an excellent way to obtain autecological information on relevant environmental conditions. However, autecological characterization requires species identification because species of the same genus might have different responses to environmental factors (Rossaro et al. 2006). Autecological characterization also requires much data covering a wide range of spatial and temporal variation. Only a few studies report the specific ecological requirements of Chironomidae taxa in near-pristine Mediterranean streams (but see Calle-Martínez and Casas 2006).

According to the Water Framework Directive (European Commission 2000), a prerequisite for effective management of water systems is information on the state of freshwater biodiversity in near-pristine ecosystems. In our study, the ecological requirements of the most frequent chironomid species were analyzed in relation to important environmental gradients present in Mediterranean catchments of the Iberian Peninsula. Sites in reference (or the least-disturbed) condition in middle and lower sections of the catchments that included a range of stream types with different geological, morphological, and physicochemical features (Sánchez-Montoya et al. 2007) were used for a large-scale examination of Chironomidae assemblages. These related characteristics should affect the composition of Chironomidae assemblages with the result that Chironomidae assemblages should be distinctly different among stream types. The specific aims of our study were to 1) assess the contribution of environmental factors at different spatial scales (geographical, regional, and local) to the structure of Chironomidae assemblages, 2) identify the environmental factors most strongly related to assemblage structure, 3) determine assemblage groups in different Mediterranean reference streams and their representative indicator species, and 4) define the optima and tolerances of Chironomidae taxa to relevant environmental factors influencing assemblage composition.

Methods

Study area

The study area (Fig. 1) covered ~78,560 km^2 of the Iberian Mediterranean coast and included large (e.g., Júcar: 18,136 km^2) and small catchments (e.g., Chillar: 54 km^2) (Appendix 1). Thermal, pluviometric, and altitudinal gradients were present from north to south and from the mountains to the coast (usually west to east). The annual range in temperature is −2 to 42°C, and annual precipitation ranges from 280 to 1000 mm. Strong storms often cause flooding during spring and autumn (MIMAM 2000). The Mediterranean climate has hot dry summers and cool wet winters. Rivers show high seasonality, with high annual and interannual variability in discharge, and frequent and predictable periods of flooding and drying (Gasith and Resh 1999). Limestone and other sedimentary rocks dominate along the coast, although some siliceous areas are present in the Sierra Nevada (south), Montseny, and Pyrenees (north). Sclerophylous and evergreen trees and shrubs are dominant, but deciduous forests are found in some areas.

Only samples taken in spring were considered in our study because streams in Mediterranean zones have a high probability of drying during part of the year (usually in summer). Restricting sampling to spring ensured that water flows and biological assemblages were comparable because when streams are reduced to pools, macroinvertebrate communities (Bonada et al. 2006), and Chironomidae assemblages (Puntí et al. 2007) might change. Sixty-three sites in 22 river catchments were sampled during spring 2003 (Fig. 1). The sites ranged widely in altitude (12–1940 m above sea level [asl]) and latitude (from Muga stream in the northeast to Guadiaro in southern Spain) (Appendix 2; see Robles et al. 2004 for a description of the catchments).

Only minimally disturbed sites were used to ensure that they represented near-pristine conditions (i.e., most headwater streams) or least-disturbed sites (most mid-reaches). The site network consisted of very small streams at high altitude to mid-reaches of several medium-sized streams because no minimally disturbed large streams were present in the area. Sites were selected on the basis of 18 criteria used to establish reference conditions in Mediterranean streams (Sánchez-Montoya et al. 2005). A reference site was identified in relation to features at 3 spatial scales: 1) catchment (e.g., no canalization or water abstraction, natural land uses in catchments >70%), 2) site (e.g., natural riparian vegetation appropriate to the type, absence of point and diffuse pollution source), and 3) instream (e.g., no transversal structures (dams),

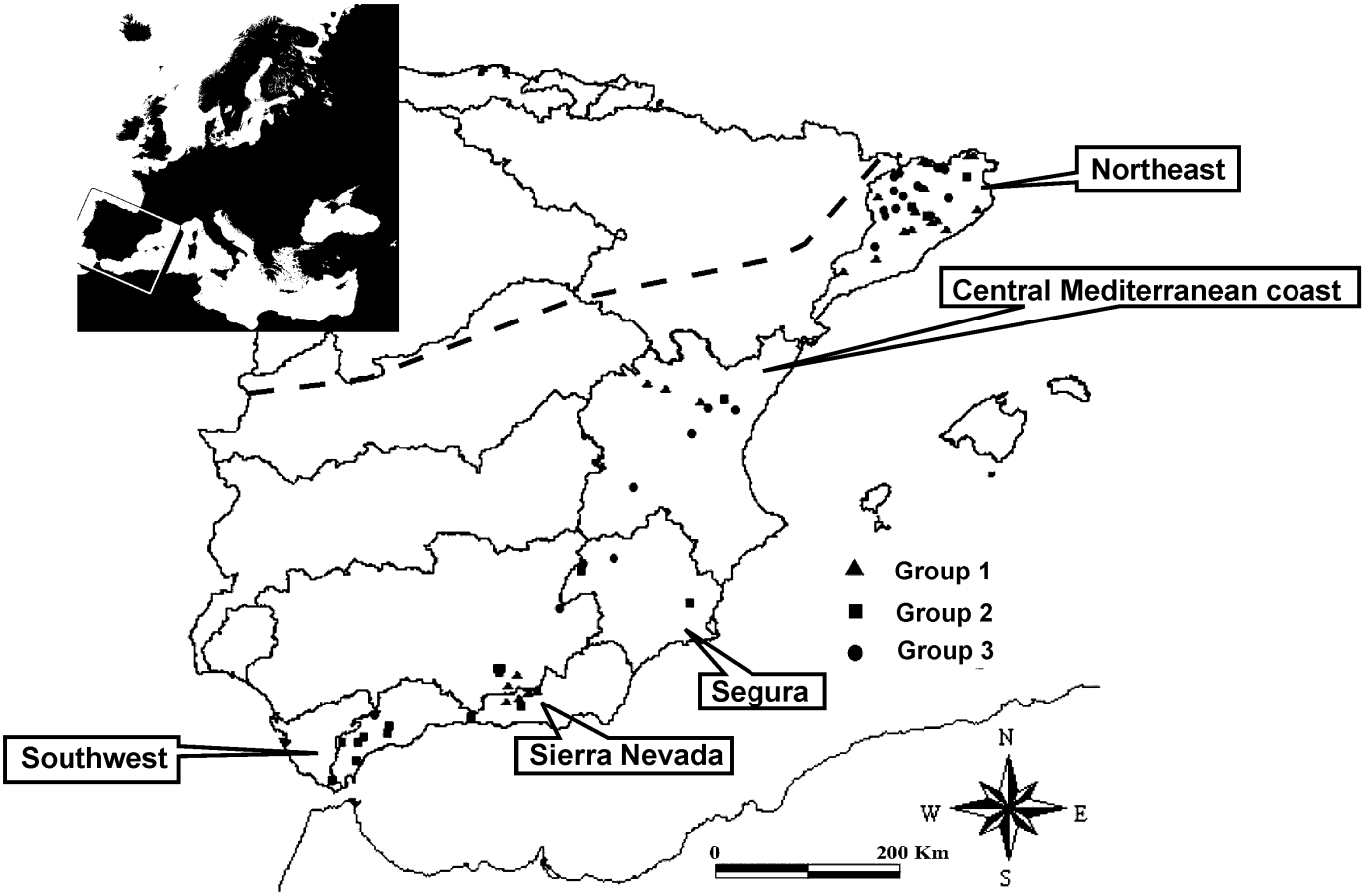

FIG. 1. Study sites along the Mediterranean coast of the Iberian Peninsula. The dashed line shows the boundary of the Mediterranean climate (Köppen 1931), and the solid lines represent hydrological boundaries between Spanish Water Authorities. Sites were classified by *k*-means analysis into 3 groups (see *Data analysis* for explanation).

no sand and gravel extraction). Most selected sites fulfilled the 18 criteria. A few mid-reach sites fit all but one criterion and were retained in the analysis.

Environmental descriptors

The environmental variables (41 variables) were divided into 3 groups based on the spatial scale of the variable: geographical (site coordinates), regional (geological characteristics, catchment area, land use), and local (e.g., water chemistry and habitat) (Appendix 2). Geographical variables were calculated by including all terms of a cubic trend surface regression (i.e., x, y, x^2, xy, y^2, x^3, x^2y, xy^2, and y^3) with x (latitude) and y (longitude) and using a similar approach to that of Borcard et al. (1992). Use of this geographical component in the analysis allows inclusion of large-scale spatial structure in the data set (Meot et al. 1998). The geographical component explains patterns in the species data not shared by any of the other environmental variables measured. It is an indirect synthetic descriptor of other unmeasured biological or environmental factors (see Magalhaes et al. 2002, Johnson et al. 2007).

Geological characteristics and catchment area were calculated from a digital terrain model (DTM; 30 × 30 m; Centro Geográfico del Ejército, Ministerio de Defensa, Spain, 2005) and Arc/Info software (version 9.0; Environmental Systems Research Institute, Redlands, California). Classification of catchment land cover was obtained from CORINE LAND COVER (Instituto Geográfico Nacional, Madrid, Spain; 2000). Local variables included riparian characteristics (Munné et al. 2003) and bedform variables that indicated habitat condition (Pardo et al. 2004). Physicochemical variables (e.g., conductivity, pH, temperature, O_2, and discharge) were measured in situ with portable meters. Water samples were analyzed in the laboratory for alkalinity, Cl^-, and SO_4^{2-} following standard procedures (APHA 1992). Other local vari-

ables, such as altitude, stream order, and percentage of dry period were derived from geographical information system (GIS) data available from the database of CEDEX (Centro de Estudios Hidrográficos, Spain).

Biological sampling

Benthic macroinvertebrates were sampled with the protocol established in the GUADALMED project (Jáimez-Cuéllar et al. 2004). This protocol has been used in several benthic studies (e.g., Bonada et al. 2005, 2006, Sánchez-Montoya et al. 2007) and provides a standardized data set. At each site, a multihabitat sample was collected from all available habitats with a kick net (250-μm mesh size). The collected material was placed in trays, and organisms were identified to family level (except for Hydracarina, Oligochaeta, and Ostracoda). Sampling ended when no new taxa were recorded. Samples were preserved in the field using 10% formalin. Chironomidae were sorted in the laboratory. All chironomids collected in our study were larvae, which were sorted, counted, and mounted on slides for identification with high power magnification to the highest taxonomic resolution possible. Larvae were first grouped by morphological appearance (shape of the head capsule, color, body setae, and size) under a stereomicroscope, and all (if <10 individuals of each morphological type) or part (if >10 individuals of each type) of the larvae in each group were mounted on slides (Pinder 1983). In total, 12,409 larvae were examined (4347 mounted specimens).

We used identification keys and species descriptions selected from the European literature, including Wiederholm (1983), Nocentini (1985), Schmid (1993), and Rieradevall and Brooks (2001). For some genera (e.g., *Corynoneura*, *Micropsectra*, and *Tanytarsus*), the authors' own experiences in the identification of larvae and reference collections were used. In some cases, a chironomid larva could not be identified to species because of small size of individuals (2^{nd} or 3^{rd} instars) or difficulty in differentiating some groups (e.g., *Orthocladius–Cricotopus*) at the larval stage. Therefore, in the final biological matrix, a number of taxonomical levels were mixed. The relative abundance of Chironomidae (percentage of each taxon per sampling site) was calculated and used in multivariate analysis.

Data analysis

Detrended correspondence analysis (DCA) (Hill and Gauch 1980) of taxon relative abundances was done to assess the degree of taxonomic turnover across ecological gradients and to determine the gradient length in the biological data set. The gradient lengths of the first 2 axes were 3.0 and 2.7 standard deviation (SD) units, respectively, indicating that either a linear and unimodal species response model should perform reasonably well (Lepŝ and Ŝmilauer 2003). Methods based on a linear response model were best suited to our data (variance explained by redundancy analysis [RDA] = 64%, variance explained by canonical correspondence analysis [CCA] = 59%). Therefore, RDA was used to examine the relationship between Chironomidae assemblages and the explanatory variables. RDA is a constrained form of the linear ordination method of principal components analysis (Legendre and Legendre 1998). All analyses were run on 4^{th}-root-transformed Chironomidae abundance data. When necessary, environmental variables were $\log(x)$ or $\arcsin(\sqrt{[x]})$-transformed to approximate normally distributed random errors (Appendix 2). No consensus exists regarding whether rare taxa should be removed from a data set when multivariate analysis is used (Cao et al. 2001). In our case, taxa occurring in ≥ 2 samples and with relative abundance $\geq 2\%$ in ≥ 1 sample were included in the multivariate analysis to prevent a disproportionate effect of Chironomidae taxa with low occurrence on the results (Gauch 1982). All ordinations were run with CANOCO (version 4.5; Microcomputer Power, Ithaca, New York).

Direct gradient analysis (partial constrained ordination or partition of variance [pRDA]) was used to estimate the fraction of variance in community composition explained by the 3 groups of explanatory variables (geographical, regional, and local). pRDA allows examination of relationships between desired environmental variables and biological variables by removing the effects of known factors of no interest. The same variable can be used both as a covariable and as an environmental variable in different parts of the same analysis. In variation partitioning, covariables are useful for distinguishing the relative contributions of groups of variables to explain species composition (Legendre and Legendre 1998).

First, constrained ordinations were run to determine the significant ($p < 0.05$) environmental variables. Only significant variables were considered as environmental variables in the pRDA. Variables included in the 3 groups (geographical, regional, and local) and the individual effects explained by each variable ($\lambda 1$ or marginal effects) are shown in Appendix 2. A series of pRDAs was run for Chironomidae assemblages (Borcard et al. 1992). pRDAs were carried out in the following steps: 1) RDA with species data and all 3 groups of environmental variables as explanatory variables and no covariables was used to determine the total amount of variation explained (TVE) by the 3 environmental groups; 2) pRDA with 1 of the 3

environmental variable groups as explanatory variables and the other 2 groups together as covariables was used to obtain single effects for each group of variables; 3) calculation of the variation shared by several combinations between groups of variables (interaction effects); and 4) calculation of the unexplained proportion of variation (1 – TVE).

RDA with forward selection was run to detect the main environmental variables that could best explain the variability of the analyzed data set. Bonferroni-adjusted forward selection was used to reduce redundancy between variables. The significance of each remaining variable was tested with Monte Carlo permutation (9999 permutations, $p < 0.05$). The significance level was set to α/n for each variable tested to compensate for the number of statistical tests (Legendre and Legendre 1998). Environmental variables were chosen only when their addition did not cause any variation inflation factor >20. Pearson correlations among the first 4 canonical axes and environmental variables were used to interpret the meaning of these axes and their significance.

Groups of Chironomidae assemblages were obtained by clustering samples based on their projections onto the first 2 ordination axes with a *k*-means method (SPSS for Windows, version 10.6; SPSS, Chicago, Illinois). The indicator value method (IndVal) (Dufrêne and Legendre 1997) was applied to determine the most representative Chironomidae taxa among the groups of *k*-means obtained (PC-ORD for Windows, version 4.20; MjM Software, Glendenen Beach, Oregon; McCune and Mefford 1999). IndVal is based on the comparison of relative abundances and relative frequencies of taxa in different predetermined groups of sites. Each taxon is associated with an indicator value (IV) that varies between 0 and 100, and a *p*-value obtained by Monte Carlo permutations (9999 runs).

Last, a Weighted Average (WA) regression (C2 programme, CALIBRATE version 1.3; Department of Geography, Newcastle, UK) was used with independent environmental variables to calculate the optima and tolerances of several species of chironomids. This analysis estimates the optimum of an environmental variable for each species based on the average of the values of the variable in sites where taxa are present, weighted by species' relative abundances. WA regression assumes that each taxon has a Gaussian response to an environmental variable; therefore, the species optimum (the mode) and tolerance (standard deviation from the optimum) can be calculated (Birks et al. 1990). WA regression has been widely applied in paleolimnology to infer environmental conditions using optima and tolerances of Chironomidae species (Brodersen and Anderson 2002).

TABLE 1. Percentage of variation explained (pure and shared effect) for each group of variables classified by scale.

Effect	Variation explained (%)
Pure effect: geographical	8.5
Pure effect: regional	8.0
Pure effect: local	23.3
Shared effect: geographical and regional	–0.2
Shared effect: geographical and local	1.5
Shared effect: regional and local	3.1
Shared effect: geographical, regional, and local	4.1
Total variance explained	48.3
Unexplained	51.7
Total variance	100

Results

Relative importance of geographical, regional, and local variables

In total, 141 taxa of Chironomidae in 73 genera were identified from the 63 sites (Appendix 3). Only 117 taxa had relative abundances >2% and were included in multivariate analyses. TVE was 48.3% for the first RDA (3 groups of environmental variables, no covariables) (Table 1). pRDA showed that the single effect of local variables accounted for 23.3%, whereas the single effects of geographical and regional variables accounted for 8.5 and 8%, respectively, of the total variance (Table 1). Thus, local-scale variables explained substantially more of the among-site variance in community composition than did regional- or geographical-scale variables. The total shared variance of the 3 groups of environmental variables accounted for 4.1%, whereas the total shared variance of regional and local variables accounted for 3.1%, and total shared variance of the geographical and local environmental variables accounted for 1.5% of the total variance. The total shared variance of geographical and regional environmental variables was –0.2%. This negative value indicated that the variance explained by the geographical × regional term was substantially lower than the unique variance explained by the geographical and regional variables separately. The single effects of the 3 variable groups accounted for 82.4% and interaction terms accounted for the remaining 17.6% of the TVE.

Best predictors of Chironomidae assemblages

The first 4 axes of the RDA explained 19.2% of the total variation of the 117 Chironomidae taxa in the 63 sites. Five environmental variables were included in

TABLE 2. Summary statistics of RDA using forward selection of variables. Pearson correlations between significant environmental variables and the canonical axes are shown. *$p < 0.05$, **$p < 0.01$.

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalue	0.08	0.043	0.029	0.024
Species–environment correlations	0.828	0.781	0.836	0.806
Cumulative % variance of species data	8.0	12.3	15.2	17.6
Cumulative % variance of species–environment relationship	41.8	64.0	79.1	91.8
Correlations with first 4 axes				
pH	0.596**	0.061	–0.323*	–0.131
Altitude	–0.546**	–0.267*	0.304*	0.243
Catchment area	0.481**	–0.252*	0.063	0.095
% siliceous rocks	–0.500**	–0.044	–0.361**	0.507**
Longitude	–0.075	–0.513**	–0.457**	–0.410**

the model after applying Bonferroni-corrected forward selection. Catchment area was the 1st variable selected (6.6% of the total variance), followed by longitude (3.4%), pH (3.4%), altitude (2.9%), and % siliceous rocks in the catchment (2.8%) (Table 2). These results showed the combination of geographical, regional, and local environmental variables could best explain the variation in among-site differences in Chironomidae assemblages, even though in the pRDA, local variables explained the highest percentage of TVE.

A low percentage of Chironomidae variability was explained by the RDA (Table 2), but canonical axes were significant in relation to the set of variables used (Monte Carlo tests, 999 permutations; $F = 1.23$, $p < 0.01$). Based on the 5 significant variables, the 1st axis explained 8% of the total variability in the species data. This axis was positively correlated with pH and catchment area and negatively correlated with altitude and % siliceous rocks in the catchment (Table 2). It differentiated sites in mainly siliceous headwater streams with lower pH and small catchment area from sites in mid-altitude streams with larger catchment areas and higher pH. The 2nd axis explained 4.3% of total variability in the species data and was negatively related to longitude, altitude, and catchment area. It differentiated lower altitude sites in the southeast from higher altitude sites in the southwest and northwest where high peaks are found (Fig. 2). Species–environment correlations were high for all axes, despite the low cumulative percentage of variability explained.

Chironomidae assemblages

The first 2 canonical axes were used in the classification of sites by *k*-means clustering because they included the maximum variability expressed by environmental variables (Table 2). As a result, 3 groups of sites with distinct Chironomidae assemblages were identified: 1) siliceous headwater streams, 2) mid-altitude streams with small basin areas (mixed siliceous and calcareous), and 3) medium-sized calcareous streams (Fig. 2).

Group 1 consisted of 25 headwater sites mainly from the catchments in the northeast (Pyrenees and Montseny ranges) and southeast (Sierra Nevada basins) (Fig. 1) and was characterized by the highest % siliceous rocks (61.3 ± 47.5%) and altitudes (942.6 ± 506.2 m asl), and the lowest values of catchment area (33.7 ± 48.2 km^2) and pH (7.63 ± 0.65). These sites were differentiated by 12 indicator taxa (Table 3) that generally were associated with low-temperature habitats and included *Eukiefferiella brevicalcar*, *Tvetenia discoloripes*, *Tvetenia bavarica-calvescens*, *Trissopelopia* spp., and *Thienemanniella partita*. Group 2 consisted of 18 sites from the southwest and central area (e.g., Guadiaro, Guadalhorce, and Segura catchments). They had intermediate altitudes (484.9 ± 436.3 m asl) with intermediate catchment areas (168.6 ± 370.9 km^2) and a low % siliceous rocks (38.5 ± 38.6%). These sites were differentiated by 7 indicator taxa (Table 3), including *Rheocricotopus chalybeatus* group, *Rheotanytarsus* spp., and *Ablabesmyia longystila*. Group 3 consisted of 20 sites, mainly calcareous, in the northeast and the central Mediterranean coast (Ter, Llobregat, Palancia, and Segura catchments). They had greater catchment areas (812.9 ± 1270.4 km^2) and pH values (8.33 ± 0.38) and intermediate altitudes (558 ± 261.0 m asl). These sites were differentiated by 8 indicator taxa (Table 3), including *Orthocladius–Cricotopus*, *Microtendipes pedellus* group, *Eukiefferiella ilkleyensis*, and *Cricotopus sylvestris* group, that were generally associated with highly mineralized waters. IVs of most indicator taxa in all groups was >25 (Table 3), values that showed that these species were present in ≥50% of sites in one group and that their relative abundance in that group was ≥50% (Dufrêne and Legendre 1997).

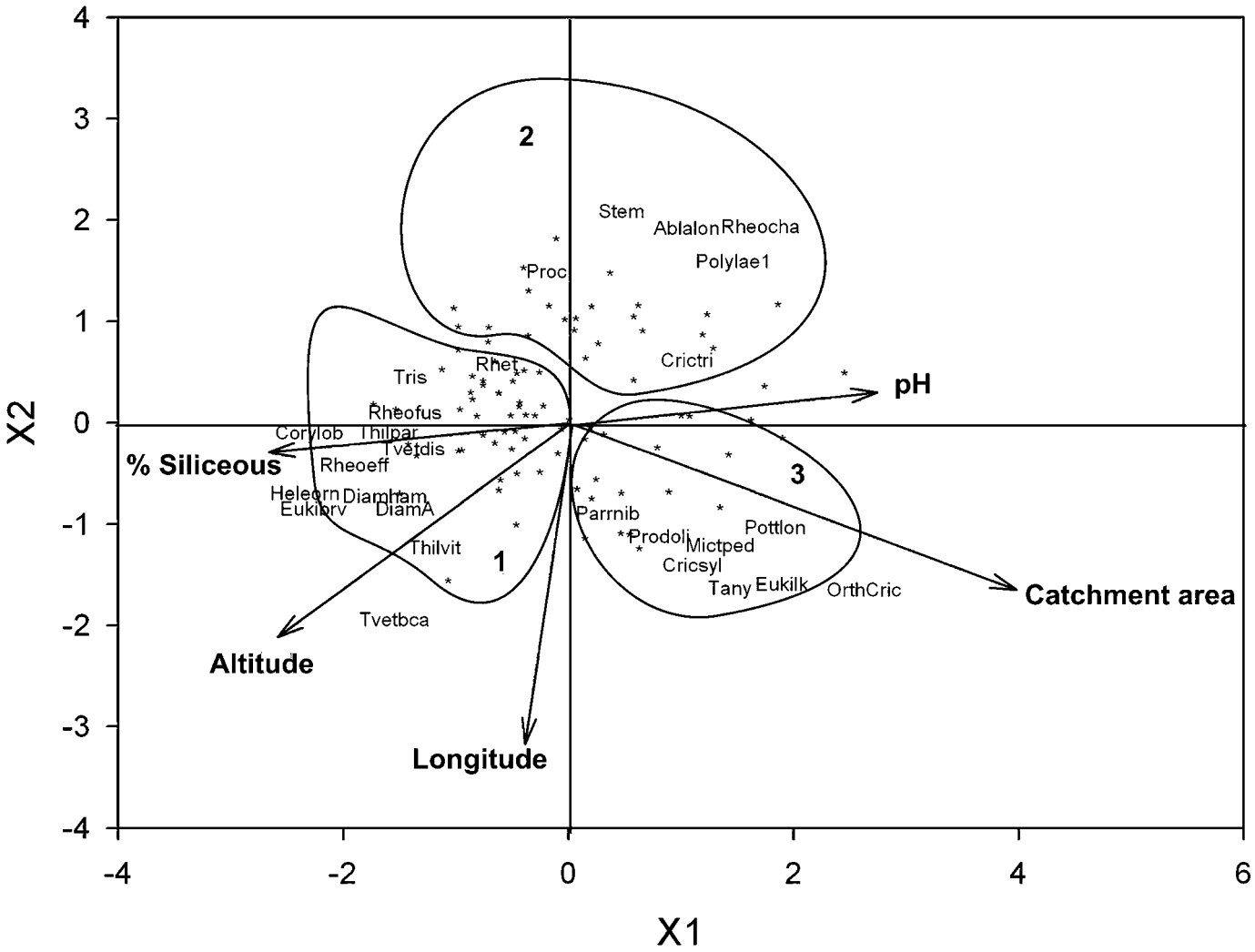

FIG. 2. Ordination biplot (redundancy analysis) of Chironomidae assemblages in 63 streams in Mediterranean Spain. Environmental variables were selected using forward selection and Monte Carlo permutation tests. Chironomidae taxa are represented by stars, but taxa that are indicators for a site group are shown with standardized codes for species names (Schnell et al. 1999; see Appendix 3 for codes). Numbers indicate groups of sites identified by *k*-means clustering. Hand-drawn shapes show the distribution limits among the 3 groups of sites for the first 2 ordination axes (X1 and X2). Group 1 sites were in siliceous headwater streams, group 2 sites were in mid-altitude streams with small catchment areas, and group 3 streams were medium-sized calcareous streams.

Optima and tolerances

We assumed that taxa would be most abundant in streams with values of environmental variables near their optima. Altitude and catchment area were the major environmental gradients relevant in our study area, but optima and tolerance were calculated for many other factors. Optima and tolerances of altitude and surface catchment area are presented for the 59 most frequent taxa collected in the study area and that occurred in ≥10 samples (Figs 3, 4). Of the selected taxa, *Heleniella ornaticollis*, *Diamesa* sp. A, and *Eukiefferiella brevicalcar* had the highest optimum for altitude (>1000 m), whereas *Phaenopsectra* spp., *Virgatanytarsus* spp., *Cricotopus* group *sylvestris*, and *Paramerina* spp. were restricted to lower altitudes (<500 m) (Fig. 3). In general, taxa that had lower optima for catchment area (<200 km^2), such as *Stempellinella* spp., *Corynoneura lobata*, and *Paratrissocladius excerptus*, had narrow values of tolerances for this variable, indicating that these taxa were restricted to small catchments (Fig. 4). In contrast, taxa with higher optima values for catchment area (>800 km^2), such as *Orthocladius rivulorum*, *Microtendipes pedellus* group, and *Virgatanytarsus* spp., had wider tolerances and preferred mid-reaches (Fig. 4).

TABLE 3. Indicator values (IVs) of Chironomidae taxa for site group. Sites were classified by *k*-means analysis of Chironomidae assemblages into 3 groups (see *Data analysis* for explanation). Group 1 sites were in siliceous headwater streams, group 2 sites were in mid-altitude streams with small catchment areas, and group 3 streams were medium-sized calcareous streams.

Group 1		Group 2		Group 3	
Taxa	IV	Taxa	IV	Taxa	IV
Eukiefferiella brevicalcar	72.5	*Rheocricotopus chalybeatus* group	57.8	*Orthocladius-Cricotopus*	76.4
Tvetenia discoloripes	53.7	*Rheotanytarsus* spp.	47.0	*Microtendipes pedellus* group	37.2
Tvetenia bavarica-calvescens	52.3	*Ablabesmyia longistyla*	35.1	*Eukiefferiella ilkleyensis*	37.2
Trissopelopia spp.	37.9	*Polypedilum laetum* group sp. 1	29.2	*Cricotopus* (*Isocladius*) *sylvestris* group	20.2
Thienemanniella partita	36.8	*Procladius* spp.	27.0	*Potthastia longimana*	20.0
Rheocricotopus fuscipes	36.5	*Cricotopus* (*Cricotopus*) *trifascia*	19.3	*Paracricotopus niger*	19.6
Thienemanniella vittata	31.4	*Stempellina* spp.	16.2	*Prodiamesa olivacea*	15.9
Heleniella ornaticollis	31.0			*Tanytarsus* spp.	14.7
Corynoneura lobata	26.0				
Rheocricotopus effusus	25.0				
Diamesa sp. A	22.7				
Diamesa hamaticornis type	16.0				

Optima and tolerance values for some of the environmental variables identified previously in the RDA as relevant for chironomid assemblage composition (altitude, % siliceous rocks, catchment area, pH, temperature, and discharge) provided information regarding niche specificity of some congeneric species (Table 4). In the genus *Corynoneura*, *C. lobata* and *C. scutellata* groups occurred in headwater (mid-high altitudes), mainly siliceous streams with low temperatures. *Corynoneura coronata* occurred in streams at intermediate altitudes with higher temperature, discharge, and percentage of carbonates and had a wide tolerance for catchment area. The 6 *Eukiefferiella* taxa had variable optima and tolerances. *Eukiefferiella*

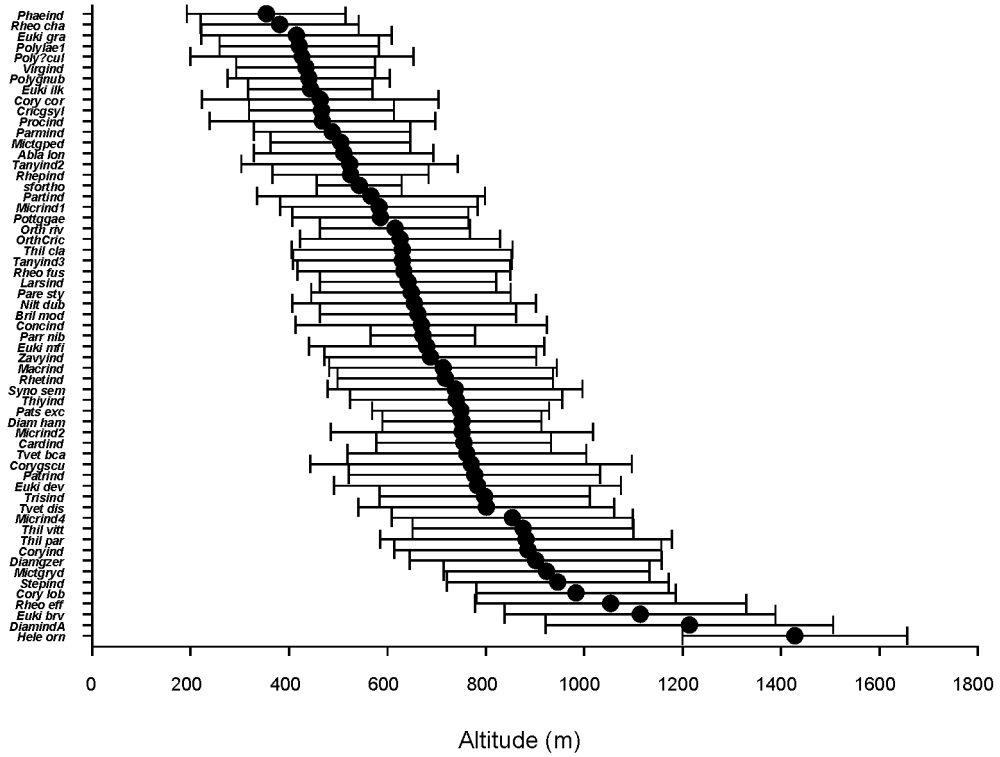

FIG. 3. Optima (modes) and tolerances (error bars) for altitude for the 59 most frequent taxa (occurred in ≥10 sites) in streams in Mediterranean Spain. Taxa are arranged on the *y*-axis in order of increasing optima for altitude. Taxa are listed by standardized codes for species names (Schnell et al. 1999; see Appendix 3 for codes).

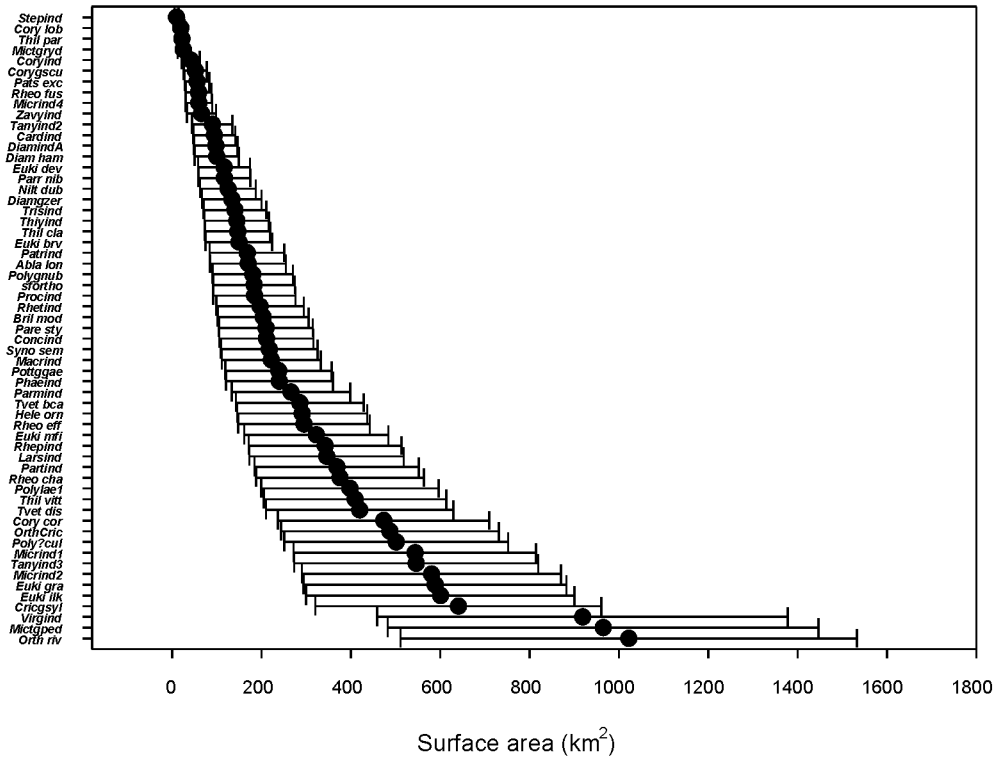

FIG. 4. Optima (modes) and tolerances (error bars) for catchment area for the 59 most frequent taxa (occurred in $\geq$10 sites) in streams in Mediterranean Spain. Taxa are arranged on the *y*-axis in order of increasing optima for catchment area. Taxa are listed by standardized codes for species names (Schnell et al. 1999; see Appendix 3 for codes).

brevicalcar and *E. coerulescens* was found mostly at higher altitudes, mainly in siliceous catchments. *Eukiefferiella brevicalcar* was restricted to fast-flowing streams but had wide tolerances for catchment area, whereas *E. devonica* and *E. minor-fittkaui* larvae inhabited mid-altitude, not exclusively calcareous streams. *Eukiefferiella gracei* and *E. ilkleyensis* were clearly differentiated from other *Eukiefferiella* taxa by their distribution in relatively low-altitude streams with a higher percentage of carbonates and higher temperature, discharge, and catchment area. In the genus *Rheocricotopus*, *R. effusus* were found in headwaters of siliceous streams and had a wide tolerance for catchment area, whereas *R. fuscipes* was more restricted to small, mid-altitude, mineralized, and slow-flowing streams. *Rheocricotopus chalybeatus* group was found in fast-flowing lower altitude streams that were mainly calcareous with variable catchment areas.

Discussion

Scale-dependent effects on community composition

Establishing the effects of coarse-scale and local environmental factors on species distribution is a prerequisite for a comprehensive understanding of processes that determine structural and functional features of stream communities (Sandin and Johnson 2004). Several factors, such as dispersal capacity, historical effects, climatic constraints, and spatial variation in local environmental conditions, determine the structure of biological communities (Minshall 1988, Bonada et al. 2005). Our study examined Chironomidae distributions across the Mediterranean region of the Iberian Peninsula, over a large area with strong environmental gradients and allowed analysis of the contribution of environmental factors structuring Chironomidae communities in near-pristine streams. Our finding that local environmental variables explained the highest amount of variance (23.3%) in Chironomidae community structure is consistent with the results of a number of previous studies of groups of organisms, such as benthic diatoms (Soininen et al. 2004), macroinvertebrates (Death and Joy 2004, Mykra et al. 2007), fish (Magalhaes et al. 2002), and macrophytes (Johnson et al. 2007). In contrast, other authors have reported that large-scale factors are the best predictors of stream communities (Richards et al. 1996, Urban et al. 2006). These disagreements regarding the importance of local or large-scale variables in stream communities might also result from differences

TABLE 4. Optima (O) and tolerances (T) of congeneric Chironomidae taxa for 6 environmental variables.

		Altitude (m)		Siliceous (%)		Temperature (°C)		Area (km^2)	
Genus	Taxon	O	T	O	T	O	T	O	T
Diamesa	*Diamesa hamaticornis*	751.5	323.1	32.57	43.18	10.18	2.50	99.2	222.5
	Diamesa hamaticornis	1582.1	331.2	100.00	37.91	6.35	1.24	44.4	32.3
	Diamesa zernyi group	901.2	511.5	53.57	45.35	9.44	2.75	133.1	247.7
	Diamesa sp. A sensu Schmid	1213.7	584.7	90.99	32.01	7.51	3.63	97.5	246.2
Corynoneura	*Corynoneura coronata*	463.4	480.9	35.68	35.76	14.41	4.90	473.2	553.9
	Corynoneura lobata	983.2	405.0	61.44	45.56	9.59	3.01	18.7	26.7
	Corynoneura scutellata group	770.1	653.0	61.39	46.53	10.54	4.40	51.4	76.6
Eukiefferiella	*Eukiefferiella brevivalcar*	1113.5	550.3	76.30	44.05	9.05	3.92	149.3	718.4
	Eukiefferiella coerulesencs	988.7	451.6	89.54	30.21	8.75	2.62	15.9	20.7
	Eukiefferiella devonica	782.8	583.5	39.94	48.85	12.03	4.53	116.0	272.3
	Eukiefferiella gracei	414.8	386.9	18.02	12.70	13.63	4.76	588.3	522.3
	Eukiefferiella ilkleyensis	443.2	252.9	28.42	32.91	12.60	3.38	600.3	1020.1
	Eukiefferiella minor-fittkaui	679.4	478.5	39.67	41.11	11.80	4.04	322.4	926.8
Rheocricotopus	*Rheocricotopus chalybeatus* group	380.9	320.8	33.94	37.88	14.98	4.26	375.2	833.4
	Rheocricotopus effusus	1053.5	551.4	83.99	34.26	8.83	3.36	294.9	1017.0
	Rheocricotopus fuscipes	633.4	432.7	60.90	44.79	11.53	3.21	58.8	189.1
Thienemanniella	*Thienemanniella clavicornis*	630.2	448.4	73.73	41.93	14.48	4.41	146.5	318.1
	Thienemanniella partita	881.6	593.5	57.88	46.98	10.89	3.82	21.2	20.2
	Thienemanniella vittata	875.5	448.5	64.93	46.61	10.34	2.95	409.1	1069.8
	Thienemanniella sp. 1	415.7	95.3	41.67	45.87	13.71	3.36	199.7	363.1
Microtendipes	*Microtendipes pedellus* group	504.9	284.3	22.44	32.80	12.92	2.89	964.5	1481.9
	Microtendipes rydalensis group	923.2	417.9	71.60	29.08	9.82	5.47	24.2	28.4
Polypedilum	*Polypedilum pedestre*	974.3	683.8	75.16	59.59	10.22	6.77	208.2	419.2
	Polypedilum cf. *cultellatum*	426.3	453.8	38.22	40.79	15.26	5.30	501.2	570.1
	Polypedilum breviantenatum	362.1	325.4	17.23	30.98	15.88	3.68	1119.9	1237.3
	Polypedilum nubeculosum group	440.3	330.0	63.49	46.29	13.27	4.25	179.9	355.8
	Polypedilum laetum group sp. 1	420.6	323.7	32.51	34.86	14.72	4.88	397.5	922.7

in the study design or may be caused by criteria used in the classification of variables at distinct spatial scales (Sandin and Johnson 2004).

The percentage of variation in Chironomidae assemblages explained by geographical variables in our study was considerable (8.5%) and was similar to the percentage of variation explained by regional variables (8%). The geographical pattern in distributions might reflect historical and climatic factors that are largely independent of present-day environmental variables (Sandin and Johnson 2000). However, a low percentage of the explained variability (4.1%) was described by the interaction of the 3 explanatory variable groups. Thus, the groups of variables used in our study were less related among themselves in comparison with other studies (Sandin and Johnson 2004). TVE (48.3%) also was higher in our study than in other studies. For example, TVE was 24.8% in a study of caddisfly communities in the streams that we studied (Bonada et al. 2005). In contrast, the relatively high percentage of unexplained variation (51.7%) in our study is typical of noisy data sets with many taxa and many 0 values (Borcard et al. 1992). Unexplained variation could be the result of unmeasured variables, such as species interactions, food resources, dispersal, sampling variability, or measurement errors. Overall, our results show that different groups of variables act at local and regional spatial scales to affect community composition (habitat filters sensu Poff 1997).

Variables that affect Chironomidae composition and distribution

Little information is available on environmental factors and mechanisms that regulate assemblage composition and distribution of Chironomidae taxa in Mediterranean streams (Calle-Martínez and Casas 2006, Puntí et al. 2007). Our data indicate that longitudinal zonation is the strongest environmental gradient underlying distribution patterns in Iberian Mediterranean streams. Geographical position was next in importance and was closely related to community patterns along the secondary axes of the RDA. pH also was an important driver of community assembly and is directly related to other regional variables, such as catchment geology. This pattern is consistent with the findings of other authors, who have demonstrated that Chironomidae composition changes along the river continuum, in association with

TABLE 4. Extended.

	Discharge (L/s)		pH	
Genus	O	T	O	T
Diamesa	0.88	2.18	8.10	0.71
	4.67	3.43	6.64	0.65
	2.30	3.35	7.80	0.93
	1.86	3.31	7.40	0.76
Corynoneura	4.70	4.34	7.98	0.44
	0.51	1.56	7.60	0.63
	0.16	0.21	7.72	0.65
Eukiefferiella	2.48	3.23	7.42	0.69
	0.44	0.58	7.99	0.59
	1.59	2.51	7.75	0.69
	5.38	4.18	8.31	0.34
	2.29	2.94	8.28	0.33
	1.00	1.33	8.06	0.58
Rheocricotopus	2.46	3.45	8.19	0.47
	1.71	2.12	7.41	0.62
	0.23	1.25	7.82	0.47
Thienemanniella	1.03	2.61	8.07	0.46
	0.48	0.82	7.60	0.76
	0.87	1.25	7.98	0.57
	0.77	0.95	8.54	0.31
Microtendipes	2.10	2.05	8.03	0.49
	1.01	2.00	7.93	0.57
Polypedilum	5.34	4.13	7.59	1.0
	4.41	4.33	8.42	0.23
	3.97	2.79	8.27	0.32
	0.20	0.37	8.04	0.56
	1.83	2.95	8.32	0.38

altitude, stream order, and channel width (Ward and Williams 1986, Lindegaard and Brodersen 1995).

Altitudinal gradients affect distributions of other organisms in the Mediterranean region and in other parts of the world (Coffman 1989, Casas and Vílchez-Quero 1993), and altitude strongly influenced Chironomidae assemblages in our study. For example, Chironomidae assemblages in headwater siliceous streams in the Pyrenees and the Sierra Nevada were similar despite the geographical distances between these mountains. Differences in altitude can result in considerable differences in local climate and other physical conditions, thereby affecting assemblage structure. However, molecular taxonomic techniques might show that populations of the same morphological species (such as *Diamesa* or *Eukiefferiella*) that are separated by great distances (Pyrenees and Sierra Nevada) actually differ. Future studies based on molecular taxonomic techniques might help clarify the importance of mountain isolation.

Chironomidae as indicators for reference condition

Chironomids have many adaptations for dispersal and colonization (Armitage 1995), but many species have regionally restricted distributions and ecological preferences. Our data show that 3 distinct Chironomidae assemblages provided a broadly meaningful ecological interpretation for reference conditions in Mediterranean streams.

Indicators for headwater streams (group 1) were a diverse group of taxa. Several taxa, such as *T. bavarica-calvescens*, *H. ornaticollis*, and *R. effusus*, typically are associated with low-temperature torrential mountain streams. These taxa, and *Brillia bifida* and *P. excerptus*, occur in the Sierra Nevada (Casas and Vílchez-Quero 1993) and Pyrenees (Prat et al. 1983, Puntí et al. 2007). They are representative of headwater systems but are not restricted to upper altitudes. In contrast, *Diamesa* is regarded as a characteristic genus with a narrow ecological niche. The genus consists mainly of cold-stenothermal species (Maiolini and Lencioni 2001) that inhabit siliceous headwater streams. However, even in this cold-stenothermal genus, differences in optima and tolerances were observed at the species level. *Diamesa zernyi-thienemanni* group and *Diamesa hamaticornis* were found in headwater streams at lower altitudes and were not restricted to siliceous geology, whereas *Pseudodiamesa branickii* and *Diamesa bertrami* are typical of nonglacial alpine streams (Lods-Crozet et al. 2001). Diamesinae maintain relatively dense populations at mean water temperatures of ~5°C (Maiolini and Lencioni 2001). Our results indicate that many *Diamesa* species have a higher temperature optima than are reported for alpine streams, but pH optima similar to those described by Rossaro et al. (2006).

Most species of *Eukiefferiella* were widely distributed along the altitudinal gradient, but *E. brevicalcar*, *E. devonica*, and *E. coerulescens* were found at higher altitudes, and *E. brevicalcar* was an indicator for group 1 streams. Casas and Vílchez-Quero (1993) analyzed the altitudinal distribution of Chironomidae in the Sierra Nevada Mountains and found that *Eukiefferiella* was one of the richest and most numerically dominant genera in headwater streams. Many of our headwater streams were at relatively low altitudes, and optima and tolerances for altitude for some *Eukiefferiella* taxa were lower than values reported in other studies (Laville and Vinçon 1991, Casas and Vílchez-Quero 1993).

Assemblages in mid-altitude streams (group 2) were characterized by more ubiquitous species with short life cycles. Many of these species were tolerant of slow-flow conditions and were warm-water adapted. Chironominae and Tanypodinae were dominant in this group (García and Laville 2000). Chironominae are more abundant when water temperature increases (Maiolini and Lencioni 2001). For example, most

Polypedilum species recorded were found mid-altitude mountain and foothill streams.

Assemblages in medium-sized calcareous streams (group 3) were characterized by *Orthocladius* and *Cricotopus*, which are tolerant and opportunistic genera generally associated with mineralized waters (Calle-Martínez and Casas 2006). Their presence or absence was not related to a well-defined range of environmental variables.

Our data confirm the importance of species-level identification to provide information about the ecological requirements of chironomids in reference streams. Our results are consistent with the observation that species belonging to the same genus often have clearly different ecological niches. However, large data sets are required to determine species autoecology when optima and tolerances are obtained from field data because a weak sampling effort might not define the full range of conditions in which some species exist. Mediterranean streams are strongly seasonal. Thus, samples from all seasons are required to obtain large data sets that integrate space and time and include intra- and interannual variability. Predicted climate changes might increase the number of ephemeral streams and decrease cold-water habitats (Rossaro et al. 2006). Therefore, a better understanding of the ecological requirements of chironomids in Mediterranean regions will help us understand the potential consequences of climate change in these highly diverse ecosystems.

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Literature Cited

ALLAN, J. D. 1995. Stream ecology. Structure and function of running waters. Chapman and Hall, London, UK.

APHA (AMERICAN PUBLIC HEALTH ASSOCIATION). 1992. Standard methods for the examination of water and wastewater. 18th edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

ARMITAGE, P. D. 1995. Behaviour and ecology of adults. Pages 195–224 *in* D. P. Armitage, P. S. Cranston, and L. C. V. Pinder (editors). The Chironomidae. The biology and ecology of non-biting midges. Chapman and Hall, London, UK.

BIRKS, H. J. B., J. M. LINE, S. JUGGINS, A. C. STEVENSON, AND C. J. F. TER BRAAK. 1990. Diatoms and pH reconstruction. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 327:263–278.

BONADA, N., C. ZAMORA-MUÑOZ, M. RIERADEVALL, AND N. PRAT. 2005. Ecological and historical filters constraining spatial caddisfly distribution in Mediterranean rivers. Freshwater Biology 50:781–797.

BONADA, N., M. RIERADEVALL, N. PRAT, AND V. H. RESH. 2006. Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean-climate streams of northern California. Journal of the North American Benthological Society 25:32–43.

BORCARD, D., P. LEGENDRE, AND P. DRAPEAU. 1992. Partialling out the spatial component of ecological variation. Ecology 73:1045–1055.

BRODERSEN, K. P., AND N. J. ANDERSON. 2002. Distribution of chironomids (Diptera) in low arctic West Greenland lakes: trophic conditions, temperature and environmental reconstruction. Freshwater Biology 47:1137–1157.

CALLE-MARTÍNEZ, D., AND J. CASAS. 2006. Chironomid species, stream classification, and water-quality assessment: the case of 2 Iberian Mediterranean mountain regions. Journal of the North American Benthological Society 25:465–476.

CAO, Y., D. P. LARSEN, AND R. S. THORNE. 2001. Rare species in multivariate analysis for bioassessment: some considerations. Journal of the North American Benthological Society 20:144–153.

CASAS, J., AND A. VÍLCHEZ-QUERO. 1993. Altitudinal distribution of lotic chironomid (Diptera) communities in the Sierra Nevada Mountains (southern Spain). Annales de Limnologie 29:175–187.

COFFMAN, W. P. 1989. Factors that determine the species richness of lotic communities of Chironomidae. Acta Biologica Debrecina, Supplementum Oecologica Hungarica 3:95–100.

DEATH, R. G., AND M. K. JOY. 2004. Invertebrate community structure in streams of the Manawatu-Wanganui region, New Zealand: the roles of catchment versus reach scale influences. Freshwater Biology 49:982–997.

DUFRÊNE, M., AND P. LEGENDRE. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345–366.

EUROPEAN COMMISSION. 2000. Directive 2000/60/EC of the European Parliament of the Council of 23rd October 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities L327:1–72.

GARCÍA, X. F., AND H. LAVILLE. 2000. First inventory and faunistic particularities of the chironomid population from a 6th order section of the sandy River Loire (France). Archiv für Hydrobiologie 147:465–484.

GASITH, A., AND V. H. RESH. 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annual Review of Ecology and Systematics 30:51–81.

GAUCH, H. G. 1982. Multivariate analysis in community ecology. Cambridge University Press, Cambridge, UK.

GONZÁLEZ, G., X. MILLET, N. PRAT, AND M. A. PUIG. 1985. Patterns of macroinvertebrate distribution in the Llobregat river basin (NE Spain). Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie 22:2081–2086.

HILL, M. O., AND H. G. GAUCH. 1980. Detrended correspondence analysis, an improvised ordination technique. Vegetatio 42:47–58.

JÁIMEZ-CUÉLLAR, P., S. VIVAS, N. BONADA, S. ROBLES, A. MELLADO, M. ÁLVAREZ, J. AVILÉS, J. CASAS, M. ORTEGA, I. PARDO, N. PRAT, M. RIERADEVALL, C. E. SÁINZ-CANTERO, A. SÁNCHEZ-ORTEGA, M. L. SUÁREZ, M. TORO, M. R. VIDAL-ABARCA, C. ZAMORA-MUÑOZ, AND J. ALBA-TERCEDOR. 2004. Protocolo Guadalmed (PRECE). Limnetica 21:187–204.

JOHNSON, R. K., M. T. FURSE, D. HERING, AND L. SANDIN. 2007. Ecological relationships between stream communities and spatial scale: implications for designing catchment-level monitoring programmes. Freshwater Biology 52: 939–958.

KÖPPEN, W. 1931. Grundis der Klimakunde. Walter de Gruyter, Berlin, Germany.

LAVILLE, H., AND G. VINÇON. 1991. A typological study of Pyrenean streams: comparative analysis of the Chironomidae (Diptera) communities in the Ossau and Aure Valleys. Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie 24:1775–1784.

LEGENDRE, P., AND L. LEGENDRE. 1998. Numerical ecology. Elsevier Science, Amsterdam, The Netherlands.

LENCIONI, V., AND B. ROSSARO. 2005. Microdistribution of chironomids (Diptera: Chironomidae) in Alpine streams: an autoecological perspective. Hydrobiologia 533:61–76.

LEPŠ, J., AND P. ŠMILAUER. 2003. Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge, UK.

LINDEGAARD, C., AND K. P. BRODERSEN. 1995. Distribution of Chironomidae (Diptera) in the River Continuum. Pages 257–271 *in* P. Cranston (editor). Chironomids: from genes to ecosystems. CSIRO Publications, Melbourne, Australia.

LODS-CROZET, B., V. LENCIONI, J. S. OLAFSSON, D. L. SNOOK, G. VELLE, J. E. BRITTAIN, E. CASTELLA, AND B. ROSSARO. 2001. Chironomid (Diptera: Chironomidae) communities in six European glacier-fed streams. Freshwater Biology 46: 1791–1809.

MCCUNE, B., AND M. J. MEFFORD. 1999. PC-ORD for Windows: multivariant analysis of ecological data. Version 4.20. MjM Software, Gleneden Beach, Oregon.

MAGALHAES, M. F., D. C. BATALHA, AND M. J. COLLARES-PEREIRA. 2002. Gradients in stream fish assemblages across a Mediterranean landscape: contributions of environmental factors and spatial structure. Freshwater Biology 47: 1015–1031.

MAIOLINI, B., AND V. LENCIONI. 2001. Longitudinal distribution of macroinvertebrate assemblages in a glacially influenced stream system in the Italian Alps. Freshwater Biology 46:1625–1639.

MENGE, B. A., AND A. M. OLSON. 1990. Role of scale and environmental-factors in regulation of community structure. Trends in Ecology and Evolution 5:52–57.

MEOT, A., P. LEGENDRE, AND D. BORCARD. 1998. Partialling out the spatial component of ecological variation: questions and propositions in the linear modelling framework. Environmental and Ecological Statistics 5:1–27.

MIMAM (MINISTERIO DE MEDIO AMBIENTE MADRID). 2000. Libro blanco del agua en España. Ministerio de Medio Ambiente, Madrid, Spain. (Available from: www.mma.es/secciones/acm/fondo_docu_descargas/libro_blanco/)

MINSHALL, G. W. 1988. Stream ecosystems: a global perspective. Journal of the North American Benthological Society 7:263–288.

MUNNÉ, A., N. PRAT, C. SOLÀ, N. BONADA, AND M. RIERADEVALL. 2003. A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. Aquatic Conservation: Marine and Freshwater Ecosystems 13:147–163.

MYKRA, H., J. HEINO, AND T. MUOTKA. 2007. Scale-related patterns in the spatial and environmental components of stream macroinvertebrate assemblage variation. Global Ecology and Biogeography 16:149–159.

NOCENTINI, A. 1985. Guide per il riconoscimento delle specie animali delle acque interne italiane. Chironomidi, 4. (Diptera: Chironomidae: Chironominae, larvae). AQ/1/233. Consiglio Nazionale delle Ricerche, Verona, Italy.

ORENDT, C. 1999. Chironomids as bioindicators in acidified streams: a contribution to the acidity tolerance of chironomid species with a classification in sensitivity classes. International Review of Hydrobiology 84:439–449.

PARDO, I., M. ALVÁREZ, J. CASAS, J. L. MORENO, S. VIVAS, N. BONADA, J. ALBA-TERCEDOR, P. JÁIMEZ-CUÉLLAR, G. MOYÀ, N. PRAT, S. ROBLES, M. L. SUÁREZ, M. TORO, AND M. R. VIDAL-ABARCA. 2004. El hábitat de los ríos mediterráneos. Diseño de un índice de diversidad de hábitat. Limnetica 21:115–133.

PINDER, L. C. V. 1983. Introduction. Pages 7–10 *in* T. Wiederholm (editor). The larvae of Chironomidae (Diptera) of the Holarctic Region. Entomologica Scandinavica Supplement 19.

PINDER, L. C. V. 1986. Biology of freshwater Chironomidae. Annual Review of Entomology 31:1–23.

POFF, N. L. 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society 16:391–409.

PRAT, N., X. MILLET, M. A. PUIG, AND G. GONZÁLEZ. 1983. Chironomid longitudinal distribution and macroinvertebrate diversity along the river Llobregat (NE Spain). Memoirs of the American Entomological Society 34:267–278.

PUNTÍ, T., M. RIERADEVALL, AND N. PRAT. 2007. Chironomidae assemblages in reference conditions from Mediterranean streams: seasonality, environmental factors and ecotypes. Fundamental and Applied Limnology 170:149–165.

RICHARDS, C., L. B. JOHNSON, AND G. E. HOST. 1996. Landscape-

scale influences on stream habitats and biota. Canadian Journal of Fisheries and Aquatic Sciences 53:295–311.

RIERADEVALL, M., AND S. J. BROOKS. 2001. An identification guide to subfossil Tanypodinae larvae (Insecta: Diptera: Chironomidae) based on cephalic setation. Journal of Paleolimnology 25:81–99.

ROBLES, S., M. TORO, C. NUÑO, J. ALBA-TERCEDOR, M. ÁLVAREZ, N. BONADA, J. CASAS, P. JÁIMEZ-CUÉLLAR, A. MELLADO, A. MUNNÉ, I. PARDO, N. PRAT, M. L. SUÁREZ, M. R. VIDAL-ABARCA, S. VIVAS, G. MOYÁ, AND G. RAMÓN. 2004. Descripción de las cuencas mediterráneas seleccionadas en el proyecto GUADALMED. Limnetica 21:35–61.

ROSSARO, B., V. LENCIONI, A. BOGGERO, AND L. MARZIALI. 2006. Chironomids from Southern Alpine running waters: ecology, biogeography. Hydrobiologia 562:231–246.

SAETHER, O. A. 1979. Chironomid communities as water quality indicators. Holarctic Ecology 2:65–74.

SÁNCHEZ MONTOYA, M. M., T. PUNTÍ, M. L. SUÁREZ, M. R. VIDAL-ABARCA, M. RIERADEVALL, J. M. POQUET, C. ZAMORA-MUÑOZ, S. ROBLES, M. ÁLVAREZ, J. ALBA-TERCEDOR, M. TORO, A. M. PUJANTE, T. MUNNÉ, AND N. PRAT. 2007. Concordance between ecotypes and macroinvertebrate assemblages in Mediterranean streams. Freshwater Biology 52:22–40.

SÁNCHEZ-MONTOYA, M. M., M. L. SUÁREZ, AND M. R. VIDAL-ABARCA. 2005. Propuesta de criterios para la selección de estaciones de referencia en ríos mediterráneos en el contexto de la Directiva Marco del Agua. Tecnología del Agua 266:42–52.

SANDIN, L., AND R. K. JOHNSON. 2000. Ecoregions and benthic macroinvertebrate assemblages of Swedish streams. Journal of the North American Benthological Society 19:462–474.

SANDIN, L., AND R. K. JOHNSON. 2004. Local, landscape and regional factors structuring benthic macroinvertebrate assemblages in Swedish streams. Landscape Ecology 19: 501–514.

SCHMID, P. E. 1993. A key to the larval Chironomidae and their instars from Austrian Danube region streams and rivers. Part 1: Diamesinae, Prodiamesinae and Orthocladiinae. Federal Institute for Water Quality, Vienna, Austria.

SCHNELL, O. A., M. RIERADEVALL, I. GRANADOS, AND O. HANSSEN. 1999. A chironomid taxa coding system for use in ecological and palaeoecological databases. Report No. 3710–97. Mountain Lake Research (MOLAR). Norwegian Institute for Water Research, Bergen, Norway.

SOININEN, J., R. PAAVOLA, AND T. MUOTKA. 2004. Benthic diatom communities in boreal streams: community structure in relation to environmental and spatial gradients. Ecography 27:330–342.

TOKESHI, M. 1999. Species coexistence: ecological and evolutionary perspectives. Blackwell Science, Oxford, UK.

URBAN, M. C., D. K. SKELLY, D. BURCHSTED, W. PRICE, AND S. LOWRY. 2006. Stream communities across a rural-urban landscape gradient. Diversity and Distributions 12:337–350.

VINSON, M. R., AND C. P. HAWKINS. 1998. Biodiversity of stream insects: variation at local, basin, and regional scales. Annual Review of Entomology 43:271–293.

WALKER, I. R. 2001. Tracking environmental change using lake sediments. Pages 67–81 *in* J. P. Smool, H. J. B. Birks, and W. Last (editors). Midges: Chironomidae and related Diptera. Kluwer Academic Publishers, Dordrecht, The Netherlands.

WARD, A., AND D. D. WILLIAMS. 1986. Longitudinal zonation and food of larval chironomids (Insecta: Diptera) along the course of a river in temperate Canada. Holartic Ecology 9:48–57.

WIEDERHOLM, T. 1983. Chironomidae of the Holartic Region. Keys and diagnosis. Part 1. Larvae. Entomologica Scandinavica, Supplement 19.

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APPENDIX 1. Main characteristics of catchments sampled in the Mediterranean region of Spain.

Catchments	Area (km^2)	Perimeter (km)	Discharge (m^3/s)	Maximum altitude (m)	Medium altitude (m)	Siliceous (%)	Carbonate (%)	Evaporite (%)	No. of sites
Northeastern									
Muga	795	740	4.8	1399	276	58.1	36.2	5.7	2
Fluvià	1039	745	9.1	1543	466	60.8	34.3	4.9	3
Ter	2994	2271	25.7	2825	720	73.3	21.8	4.9	7
Tordera	892	632	5.7	1633	341	76.4	17.3	6.3	3
Besòs	1038	762	4.1	1317	371	46.3	40.4	13.3	2
Llobregat	4995	2932	24.8	2435	636	19.2	57.9	22.9	11
Foix	315	281	0.8	987	381	17.3	66.6	16.1	1
Francolí	857	632	1.7	1157	457	24.8	65.6	9.5	1
Central Mediterranean coast									
Palancia	972	219	2.2	1607	662	2.4	88.2	9.4	2
Mijares	4026	1884	9.7	1998	943	4.1	89.0	6.9	2
Turia	6245	2551	11.6	1987	1016	5.8	83.4	10.8	2
Júcar	18,136	7063	52	1826	819	9.7	77.9	12.4	2
Segura									
Segura	14,657	4518	23	2031	696	14.6	75.9	9.5	5
Sierra Nevada									
Adra	743	148	1.8	2737	1075	60.9	37.3	1.9	2
Guadalfeo	1300	966	6.0	3435	1263	53.0	45.6	1.3	3
Genil	8198	3998	28.4	3304	708	16.6	72.8	10.6	3
Chillar	54	69	0.2	1761	748	1.9	98.1	0.0	1
Southwestern									
Verde	157	62	2.0	1862	665	82.4	16.8	0.8	1
Jara	58	40	0.6	772	246	0.4	73.8	25.8	1
Guadalhorce	3147	1689	13.4	1781	515	20.6	66.5	12.9	1
Guadina menor	6532	2691	14.7	3108	1089	21.3	66.4	12.3	3
Guadiaro	1416	747	20.4	1747	538	13.7	70.7	15.6	5

APPENDIX 2. Environmental variables measured at 63 sites in the Mediterranean region of Spain in spring 2003. Redundancy analysis was used to explore the relationship between environmental variables and Chironomidae assemblage composition. Variables were categorized by spatial scale (geographical, regional, and local) for further analyses. Geographical variables were calculated by including all terms of a cubic trend surface regression (i.e., x, y, x^2, xy, y^2, x^3, x^2y, xy^2, and y^3) with x (latitude) and y (longitude) (Borcard et al. (1992). $\lambda 1$ = marginal effect of environmental variable, n.s. = nonsignificant ($p > 0.05$), *$p < 0.05$, **$p <$ 0.0001.

Group (scale)	Variable	Mean ± SD	Range	Transformation	λ1	p
Geographical	Latitude	39.83 ± 2.3	36.10–42.43		0.033	*
	Longitude	–0.49 ± 2.89	−5.63–23.02		0.034	*
	Latitude^2	1594.10 ± 182.22	1303.46–1800.52		0.033	*
	LatitudeLongitude	–12.31 ± 112.57	−203.33 to −128.31		0.034	*
	Longitude^2	2573,819.82 ± 570,798.59	1,699,001.14–3,241,885.8		0.033	*
	Latitude^3	63,955.22 ± 10,807.98	47,059.28–76,400.87		0.033	*
	Latitude^{2}Longitude	–240.04 ± 4384.85	−7356.92–5443		0.034	*
	LatitudeLongitude^2	103888,696.49 ± 28308,388.68	61,339,762.1–13,7561,574.9		0.033	*
	Longitude^3	–20.47 ± 52.32	−178.64–27.68		0.041	**
Regional	Catchment area (km^2)	315.49 ± 801.27	2–4290	Log_{10}	0.066	**
	% carbonate	54.25 ± 38.82	0–100		0.041	**
	% evaporite	6.25 ± 10.66	0–36.96		0.021	n.s.
	% siliceous	39.5 ± 42.02	0–100		0.045	**
	% forest and bushland	91.44 ± 10.81	50.93–100	arcsine$\sqrt{}$	0.026	**
	% cropland	7.53 ± 10.41	0–48.42	arcsine$\sqrt{}$	0.016	n.s.
	% pasture	0.85 ± 2.18	0–12.51	arcsine$\sqrt{}$	0.019	n.s.
	% other land uses	0.19 ± 0.37	0–1.83	arcsine$\sqrt{}$	0.046	*
Local	Alkalinity (meq/L)	3.11 ± 1.81	0.10–7.08		0.039	**
	Cl^- (mg/L)	67.28 ± 263.03	1.23–1850.99	Log_{10}	0.021	n.s.
	Conductivity (μS/cm)	674.92 ± 1359.22	15.8–10,500	Log_{10}	0.040	**
	Dissolved O_2 (mg/L)	10.35 ± 1.85	6.66–15.94	Log_{10}	0.032	*
	pH	8.00 ± 0.59	5.8–8.81		0.043	**
	SO_4^{2-} (mg/L)	191.07 ± 660.62	20–4033.7	Log_{10}	0.022	n.s.
	Water temperature (°C)	12.10 ± 4.44	4–23	Log_{10}	0.041	**
	Discharge (L/s)	1.50 ± 2.42	0–11.5	Log_{10}	0.043	**
	Altitude (m)	686.42 ± 461.65	12–1940	Log_{10}	0.041	**
	Stream order	1.66 ± 1.00	1–5	Log_{10}	0.052	**
	Heterogeneity elements	6.65 ± 1.99	2–10		0.031	*
	Embeddedness	8.41 ± 4.56	0–20		0.026	*
	Riffles vs .pools	8.98 ± 1.86	2–10		0.031	*
	Shade	7.59 ± 2.65	3–10		0.025	*
	Substrate habitat	14.89 ± 2.42	9–20		0.022	n.s.
	Flow and depth regimes	7.97 ± 1.59	4–10		0.023	*
	Temporality	0.56 ± 1.48	0–6		0.019	n.s.
	Dry period %	27.17 ± 29.51	0–97	arcsine$\sqrt{}$	0.013	n.s.
	Riparian quality	23.75 ± 2.95	10–25		0.011	n.s.
	Riparian cover	21.95 ± 5.24	0–25		0.014	n.s.
	Riparian structure	21.48 ± 4.24	10–25		0.018	n.s.
	Riparian naturality	23.13 ± 4.24	5–25		0.019	n.s.
	Channel width (m)	9.10 ± 7.51	1.03–43.33	Log_{10}	0.04	**
	Channel depth (m)	0.21± 0.15	0.02–0.8	Log_{10}	0.028	*

APPENDIX 3. Chironomid taxa, relative abundances (%), and number of sites where each taxon was present in streams in the Mediterranean region of Spain.

Taxon	Code	Relative abundance	No. of sites
Subfamily Podonominae			
Paraboreochlus minutissimus (Strobl, 1984)	Para min	0.019	1
Subfamily Tanypodinae			
Ablabesmyia longistyla Fittkau, 1962	Abla lon	0.809	13
Conchapelopia Fittkau, 1957	Concind	1.806	24
Krenopelopia Fittkau, 1962	Krenind	0.088	5
Larsia Fittkau, 1962	Larsind	0.487	10
Macropelopia Thienemann, 1916	Macrind	0.288	9
Nilotanypus dubius (Meigen, 1804)	Nilt dub	0.434	13
Paramerina Fittkau, 1962	Parmind	0.204	7
Procladius Skuse, 1889	Procind	1.245	8
Rheopelopia Fittkau, 1962	Rhepind	1.296	27
Thienemannimyia Fittkau, 1957	Thiyind	1.321	21
Trissopelopia Kieffer, 1923	Trisind	1.028	18
Zavrelimyia Fittkau, 1962	Zavyind	1.064	15
Subfamily Diamesinae			
Diamesa bertrami Edwards, 1935	Diam ber	0.253	1
Diamesa cf. sp. A sensu Schmid (1993)	Diam?indA	0.101	1
Diamesa hamaticornis Kieffer, 1924	Diam ham	0.292	7
Diamesa hamaticornis type	Diam?ham	0.145	4
Diamesa latitarsis group	Diamglati	0.012	1
Diamesa sp. A sensu Schmid (1993)	DiamindA	0.273	7
Diamesa zernyi–thienemanni group	Diamgzer	1.776	16
Potthastia gaedii group	Pottggae	2.883	24
Potthastia longimana (Kieffer, 1922)	Pott lon	0.076	4
Pseudodiamesa branickii (Nowicki, 1873)	Psed bra	0.019	2
Subfamily Prodiamesinae			
Prodiamesa olivacea (Meigen, 1818)	Prod oli	0.129	5
Subfamily Orthocladiinae			
? Chaetocladius	? Chae	0.05	2
? Eukiefferiella	? Euki	0.009	1
Brillia bifida Kieffer, 1909	Bril bif	1.705	6
Brillia longifurca Kieffer, 1921	Bril lon	0.059	24
Cardiocladius Kieffer, 1912	Cardind	0.921	9
Corynoneura coronata Edwards, 1924	Cory cor	0.223	7
Corynoneura Winnertz 1846	Coryind	0.536	9
Corynoneura lacustris Edwards, 1924	Cory lac	0.029	1
Corynoneura lobata Edwards, 1924	Cory lob	1.029	15
Corynoneura scutellata group	Corygscu	0.957	11
Cricotopus (*Cricotopus*) Van der Wulp 1874	Criccri	0.386	4
Cricotopus (*Cricotopus*) *trifascia* Edwards, 1929	Cric tri	0.213	6
Cricotopus (*Isocladius*) Kieffer 1909	Criciso	0.021	1
Cricotopus (*Isocladius*) *sylvestris* group	Cricgsyl	0.252	8
Cricotopus (*Isocladius*) *trifasciatus* (Meigen in Panzer 1813)	Cric trd	0.023	1
Epoicocladius flavens (Malloch, 1915)	Epi fla	0.207	4
Eukiefferiella brevicalcar (Kieffer, 1911)	Euki brv	3.405	23
Eukiefferiella cf. *lobifera* sensu Schmid (1993)	Euki?lob	0.029	2
Eukiefferiella claripennis (Lundbeck, 1898)	Euki cla	0.046	3
Eukiefferiella clypeata (Kieffer, 1923)	Euki cly	0.096	3
Eukiefferiella coerulescens (Kieffer in Zavrel 1926)	Euki coe	0.077	6
Eukiefferiella devonica (Edwards, 1929)	Euki dev	0.625	12
Eukiefferiella fuldensis Lehmann, 1972	Euki ful	0.031	3
Eukiefferiella gracei (Edwards, 1929)	Euki gra	1.164	13
Eukiefferiella ilkleyensis (Edwards, 1929)	Euki ilk	0.603	20
Eukiefferiella Thienemann 1926	Eukiind	0.127	4
Eukiefferiella lobifera Goetghebuer, 1934	Euki?lob	0.198	1
Eukiefferiella minor-fittkaui group	Euki mfi	1.193	23
Eukiefferiella similis Goetghebuer, 1939	Euki sim	0.04	2
Eukiefferiella tirolensis Goetghebuer, 1938	Euki tir	0.19	3
Heleniella ornaticollis (Edwards, 1929)	Hele orn	0.358	11

APPENDIX 3. Continued.

Taxon	Code	Relative abundance	No. of sites
Heleniella Gouin, 1943	Heleind1	0.016	1
Heterotrissocladius marcidus (Walker, 1856)	Hete mar	0.11	3
Krenosmittia camptophleps (Edwards, 1929)	Kren cam	0.17	1
Limnophyes Eaton, 1875	Limnind	0.08	4
Metriocnemus fuscipes group (Meigen 1981)	Metrgfus	0.003	1
Metriocnemus Van der Wulp, 1874	Metrind	0.019	2
Metriocnemus eurynotus group (Holmgren 1883)	Metr obs	0.069	2
Nanocladius bicolor (Zetterstedt, 1838)	Nano bic	0.004	1
Nanocladius rectinervis (Kieffer, 1911)	Nano rec	0.035	2
Orthocladiinae indet 1	sfortho1	0.01	1
Orthocladiinae indet 2	sfortho2	0.037	1
Orthocladiinae indet 3	sfortho3	0.01	1
Orthocladiinae unknown	sfortho	0.197	9
Orthocladius (*Euorthocladius*) Thienemann 1935	Ortheuo	0.113	5
Orthocladius (*Euorthocladius*) *rivulorum* Kieffer, 1909	Orth riv	0.645	14
Orthocladius–Cricotopus	OrthCric	16.924	55
Paracladius conversus (Walker, 1856)	Parl con	0.095	4
Paracricotopus niger (Kieffer, 1913)	Parr nib	0.594	9
Parakiefferiella cf. *coronata* sensu Schimd (1993)	Park?cor	0.054	2
Parakiefferiella cf. *gracillima* sensu Schimd (1993)	Park?gra	0.075	2
Parametriocnemus stylatus (Kieffer, 1924)	Pare sty	3.672	44
Paraphaenocladius pseudirritus Strenzke, 1950	Parh pse	0.097	4
Paratrichocladius Santos Abreu, 1918	Patrind	3.449	31
Paratrissocladius excerptus (Walker, 1856)	Pats exc	0.754	15
Psectrocladius (*Allopsectrocladius*) *obvius* (Walker, 1856)	Psec obv	0.123	4
Psectrocladius (*Psectrocladius*) *sordidellus* group (Zetterstedt, 1838)	Psecgsor	1.227	2
Pseudorthocladius Goetghebuer, 1932	Pseoindet	0.124	3
Pseudosmittia holsata Thienemann & Strenzke, 1940	Pses hol	0.016	1
Rheocricotopus chalybeatus group	Rheo cha	2.271	26
Rheocricotopus effusus (Walker, 1856)	Rheo eff	0.552	12
Rheocricotopus fuscipes (Kieffer, 1909)	Rheo fus	2.664	20
Rheocricotopus Thienemann & Harnish 1932	Rheoindet	0.03	1
Smittia Holmgren, 1869	Smitind	0.042	2
Symposiocladius lignicola (Kieffer in Potthast, 1915)	Symp lig	0.102	2
Synorthocladius semivirens (Kieffer, 1909)	Syno sem	0.312	17
Thienemannia Kieffer, 1909	Thieind	0.013	2
Thienemanniella acuticornis Kieffer, 1912	Thil acu	0.012	1
Thienemanniella clavicornis Kieffer, 1911	Thil cla	0.374	8
Thienemanniella flaviforceps group	Thilgfla	0.02	1
Thienemanniella Kieffer 1911	Thilindet	0.513	5
Thienemanniella majuscula (Edwards, 1924)	Thilmaj	0.018	1
Thienemanniella partita Schlee, 1968	Thil par	1.686	15
Thienemanniella sp. 1	Thilind1	0.093	4
Thienemanniella vittata (Edwards, 1924)	Thil vitt	1.674	21
Tvetenia bavarica–calvescens group	Tvet bca	5.605	44
Tvetenia discoloripes (Goetghebuer, in Thienemann1936)	Tvet dis	2.967	35
Tvetenia sp. A sensu Schimd (1993)	TvetindA	0.136	3
Subfamily Chironominae			
Tribe Chironomini			
Chironomus sp. 2	Chirind2	0.016	1
Chironomus sp. 6	Chirind6	0.509	5
Chironomus sp. 7	Chirind7	0.047	1
Cryptochironomus Kieffer, 1918	Crypind	0.172	5
Demicryptochironomus Lenz 1941	Demiind	0.114	1
Harnischia Kieffer, 1921	Harnind	0.099	3
Microtendipes pedellus group	Mictgped	0.439	13
Microtendipes rydalensis group	Mictgryd	0.275	7
Paracladopelma camptolabis group	Pardgcam	0.138	2
Paratendipes Kieffer, 1911	Patdind	0.128	5
Phaenopsectra Kieffer, 1921	Phaeind	1.387	11
Polypedilum albicorne (Meigen, 1838)	Poly alb	0.015	1

APPENDIX 3. Continued.

Taxon	Code	Relative abundance	No. of sites
Polypedilum pedestre group	Poly ped	0.171	4
Polypedilum cf. *cultellatum*	Poly?cul	1.029	11
Polypedilum cf. *breviantenatum* group sensu Nocentini, 1985	Poly?gbre	0.497	20
Polypedilum nubeculosum group	Polygnub	0.404	2
Polypedilum laetum group sp. 1	Polylae1	1.676	10
Polypedilum laetum group sp. 2	Polylae2	0.565	4
Saetheria Jackson 1977	Saetind	0.042	2
Tribe Tanytarsini			
Cladotanytarsus Kieffer, 1921	Clatind	0.248	6
Micropsectra sp. 1	Micrind1	1.018	10
Micropsectra sp. 2	Micrind2	0.849	17
Micropsectra sp. 3	Micrind3	0.003	1
Micropsectra sp. 4	Micrind4	1.223	18
Micropsectra sp. 5	Micrind5	0.103	4
Micropsectra sp. 6	Micrind6	0.041	3
Neozavrelia Goetghebuer, 1941	Neozind	0.12	4
Paratanytarsus Thienemann & Bause, 1913	Partind	0.368	9
Rheotanytarsus Thienemann & Bause, 1913	Rhetind	4.644	36
Stempellina bausei group	Stemgbau	0.063	1
Stempellina indet	Stemind	0.199	3
Stempellinella Brundin, 1947	Stepind	1.494	11
Tanytarsus chinyensis group	Tanygchi	0.572	6
Tanytarsus sp. 1	Tanyind1	0.119	5
Tanytarsus sp. 2	Tanyind2	0.348	7
Tanytarsus sp. 3	Tanyind3	0.636	14
Tanytarsus sp. 4	Tanyind4	0.087	4
Tanytarsus sp. 7	Tanyind7	0.074	1
Virgatanytarsus Pinder, 1982	Virgind	1.412	21