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The role of the environment in the distribution and composition of Trichoptera assemblages in streams

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With 3 figures, 3 tables and 1 appendix

Abstract: We investigated how diversity and distribution of more than 11000 Trichoptera larvae in streams of central Portugal were related to chemical and physical environmental variables. Sixty-six sites were sampled in 2001, by kicknet, in 55 streams of the Mondego River basin, the largest entirely Portuguese river. Simultaneously 43 environmental variables were evaluated for each site. We identified 18 families and 70 species and genera of caddisflies, representing ≈ 20 % of all trichopteran taxa recorded for the Iberian Peninsula. The species *Calamoceras marsupus, Cheumatopsyche lepida, Hydropsyche bulbifera, Hydropsyche siltalai, Hydroptila* sp., *Lepidostoma hirtum, Mystacides azurea, Chimarra marginata, Polycentropus flavomaculatus, Tinodes waeneri, Rhyacophila adjuncta* and *Sericostoma* sp. were found to be the most important taxa in the Mondego River basin. Stream order, substrate quality, altitude, current velocity and alkalinity explained 41 % of the taxa distribution suggesting that at a wider (catchment) scale physical and hydrological parameters are more important for the distribution of Trichoptera assemblages than chemical parameters. Six significantly different groups of sites with similar Trichoptera assemblages were identified in the catchment and related to altitudinal, hydrological and substrate gradients. Our findings allow us to predict Trichoptera assemblages in streams of the Mondego catchment based on few environmental characteristics.

Key words: Trichoptera, Structuring environmental variables, Mondego catchment.

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Introduction

Trichoptera are one of the most abundant and diverse groups of invertebrates in streams. According to MORSE (1997), around 9500 living species have been described, classified into 600 genera and 45 families. Trichoptera are present in a very wide range of freshwater habitats, and can be classified in all functional feeding groups. Caddisflies are good indicators of environmental conditions because taxa have different tolerances to water pollution (DOHET 2002, ROSENBERG & RESH 1993).

Environmental conditions are the major responsible factor for macroinvertebrate community organization in streams (TOWSEND et al. 1997, BOYERO 2003). The parameters stream size, altitude, slope and water velocity were related to the distribution of caddisflies of unimpaired sites in the Fraser river catchment, Canada (RESH et al. 2002). In Denmark, WIBERG-LARSEN et al. (2000) found that species richness and assemblages of Trichoptera were primarily correlated with stream order, width and slope while temperature was of minor importance. SCHMERA & ERÕS (2004) concluded that season, stream order, riverbed morphology and interaction of these factors had a significant effect on the assemblage organisation of caddisflies of natural headwater systems in Hungary. BASAGUREN & ORIVE (1990 a) verified the disappearance of Trichoptera from low oxygen sites and the relatively insensitivity of species to conductivity, in the Vasc Country, North of Spain.

The studies on the Trichoptera of the Iberian Peninsula have been taxonomical (MCLACHLAN 1884, NAVÁS 1908, TERRA 1972, 1981, 1994, GONZÁLEZ et al. 1992, VIEIRA-LANERO 2000), focused on the distribution of particular groups such as the Hydropsychidae (GARCÍA DE JALÓN 1986), on temporal and spatial distributions (PUIG et al. 1981, MOLLES & TERRA 1987, CORTES 1992) and relationship with water quality (BASAGUREN & ORIVE 1990 a, b). A total of 329 Trichoptera species have so far been reported for the Iberian Peninsula (GONZÁLEZ et al. 1992, VIEIRA-LANERO 2000) and 167 species for Portugal (TERRA 1994).

Information on the distribution of caddisflies in central Portugal is mainly based on adults collected by light traps (MOLLES & TERRA 1987, GONZALEZ et al. 1992, TERRA 1972, 1981, 1994) and some river surveys (e. g. GRAÇA et al. 1989, CORTES 1992). Studying immature stages may, however, be a useful tool to overcome the weaknesses in classifications of habitats based on adults only (WIGGINS 1981). Therefore, the main objective of the current study is to provide new information of the Trichoptera species and their distribution patterns in this region, based on larvae collected in a large number of sites of a catchment in central Portugal. We investigated the composition of aquatic caddisfly assemblages in the Mondego River basin and aimed to determine the important environmental variables contributing to species distribution, and therefore, to be able to predict the assemblages composition from environmental data.

Methods

Study area

Mondego is the largest entirely Portuguese river. Its basin (Fig. 1) is located in the centre of Portugal between 39° 46′ and 40° 48′ N and 7° 14′ and 8° 52′ W. The river flows from NE to SW for 227 km, between Serra da Estrela and the Atlantic Ocean at Figueira da Foz. The greatest altitude in the drainage basin is 2000 m in the Serra da Estrela, with an average altitude of 375 m. The mean annual precipitation in the basin is 1130 mm and the mean annual temperature 13 °C, with smaller amplitudes nearer the coast than inland. The watershed covers around 6670 km^2 with a high variability of land use (Lima & Lima 2002, Marques et al. 2002).

The Upper Mondego region consists of mountainous areas with glacial valleys and includes the headwaters of Mondego River (Serra da Estrela, 1547 m) and the tributaries Dão and Alva. Here, human activities include scattered distribution of cattle and logging. The Middle Mondego region includes mountainous areas (Caramulo, Lousã

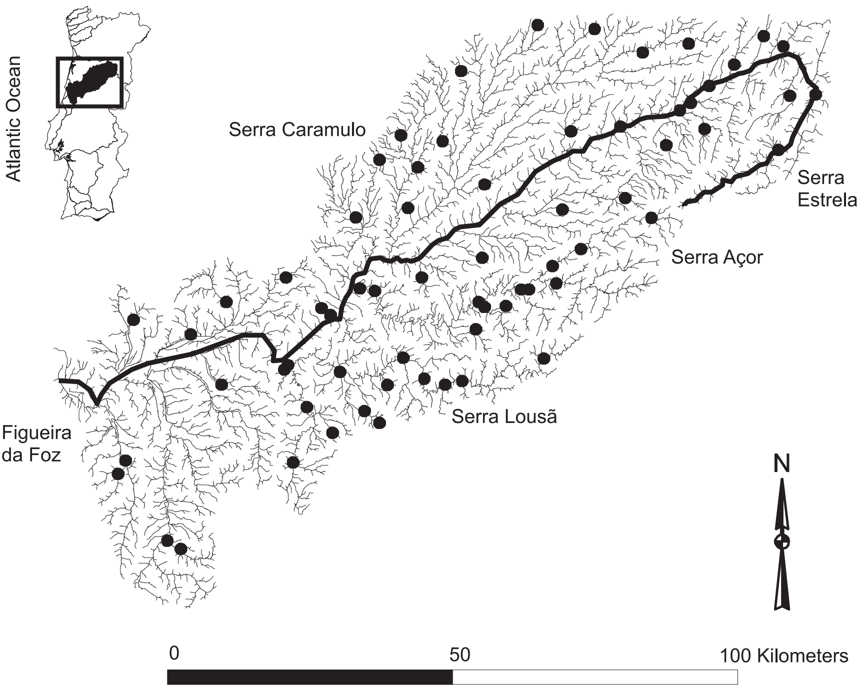

Fig. 1. Location of the Mondego River basin in Portugal and sampling sites distribution in the basin.

and Açor) but is more densely urbanized and has small to medium sized industries (mainly food and drinks, textiles, wood, cork, rubber and plastic). The main agricultural area is the vast alluvial plain of the Lower Mondego region. Both human and industrial densities are higher than in the previous mentioned areas (164 vs. 34 habitants km^{-2}; Marques et al. 2002, Lima & Lima 2002).

Sampling sites

We selected 66 sampling sites, distributed throughout the entire basin and covering altitudinal, geological and river order gradients of wadeable water courses (Fig. 1). Urban areas, impoundments and pollution sources were avoided as well as river sites showing recent disturbances in the natural stream bed such as sand extraction, damming and construction of roads or bridges. Samples were taken in summer 2001, between July and September since this is the period of low water level when almost all streams are accessible and kick-sampling is possible.

Field and laboratory work

Invertebrates, including Trichoptera larvae were collected with a hand net (0.3×0.3 m opening and 0.5 mm mesh size). The samples were taken in one transect across the stream that covered all micro-habitats present at the site (e. g. stones, sand, aquatic vegetations, riffles and pools). Each sample was a composite of either 3 or 6 sub-samples, if the stream was < 3 or > 3 m wide, respectively. Each sub-sample was obtained by kicking the substrate upstream from the net (in an area of approximately 1 m long × net width) for 0.5 minutes. Therefore, the total sampling time was 3×0.5 min (= 1.5 min) in small streams and 6×0.5 min in large streams (> 3 m). All counts were converted to animals $minute^{-1}$. Samples were fixed in 10 % formalin, sorted and stored in 70 % alcohol. Individuals were identified to species level (Vieira-Lanero 2000) except for very young larvae which were identified to genus level. For identifications and information about species biology and ecology we followed mainly Vieira-Lanero (2000), but also Hickin (1967), Pitsch (1993), Edington & Hildrew (1995), Wallace et al. 1995 and Waringer & Graf (1997).

The environment was characterized by variables describing geographic location, land use in the floodplain and catchment area, site morphology, atmospheric conditions, stream morphology and hydrology, riparian vegetation, water chemistry, characteristics of the aquatic habitat and human impacts on the stream and floodplain. For each site, 43 environmental parameters were obtained through field measurements, laboratory analysis of collected material (e. g. water and periphyton) or in bibliographic sources such as cartographic material (see Table 1). Chemical variables such as conductivity, water temperature, dissolved oxygen and total dissolved solids were measured once (during the time of macroinvertebrates collection) in the middle of stream. Water for chemical analysis was collected concurrently. Current velocity was measured 6 times along a transept and a mean value was obtained. Eighteen average stones were also selected along a representative transept in the stream reach and measured in their wider dimension to obtain a mean stone size.

Table 1. Environmental parameters obtained for each sampling site and sources. Variables selected by BVSTEP are in bold.

Environmental Variables	Description and Source
Stream Name	Military maps 1 : 50 000 or 1 : 250 000 (Instituto Geográfico do Exército), converted to numerical values (e. g. river Ceira = 1, river Alva = 2, river Mondego = 3, …)
Stream Order	Military maps 1 : 250 000 (Strahler system) (Instituto Geográfico do Exército)
Distance to Source (km)	Digital military maps (1 : 25 000; DRAOT-Centro)
Decimal Latitude and Decimal Longitude	GPS (GARMIN) and digital military maps (1 : 25 000)
Altitude (m)	idem
Valley Form	Field observations; Categories: 1 for V shapes; 2 for U shape, meander and plain floodplain
Mean Annual Temperature (°C)	Instituto do Ambiente (2003)
Mean Annual Total Precipitation (mm)	Idem
Mean Annual Precipitation (days $year^{-1}$)	Idem
Mean Stream Width (m)	Field measurements (6 measurements each transect)
Mean Stream Depth (m)	Idem
Current Velocity ($m\ s^{-1}$)	6 field measurements (VALEPORT 15277)
Discharge ($m^3\ s^{-1}$)	Stream width × Stream Depth X Current Velocity (n = 6)
Water Temperature (°C)	Field measurement (WTW OXI 92)
pH	Field measurement (JENWAY 3310)
Conductivity ($\mu S\ m^{-1}$)	Field measurement (WTW LF 330)
O_2 ($mg\ l^{-1}$) and **O_2 (%)**	Field measurement (WTW OXI 92)
Total Dissolved Solids ($mg\ l^{-1}$) (TDS)	Field measurement (WTW LF 330)
Chloride ($mg\ l^{-1}$)	Ion Chromatograph Dionex DX-120
Nitrate (NO_3^{2-}, $mg\ l^{-1}$)	Idem
Nitrite (NO_2^-, $mg\ l^{-1}$)	Idem
Sulphate ($mg\ l^{-1}$)	Idem
P-Phosphate ($mg\ l^{-1}$)	Idem
N-Ammonia ($mg\ l^{-1}$)	Idem
Alkalinity ($mg\ l^{-1}$)	Titration to an end pH of 4.5 (A. P. H. A., 1995)
Fine Particulate Organic Matter (FPOM) > 0.05 and < 1 mm (AFDM, g)	Collected in benthos samples, dried and burned to ashes (500 °C, 2 h) AFDM = Dry mass – Ashes mass
Coarse Particulate Organic Matter (CPOM) > 1 mm (AFDM, g)	Idem
Chlorophyll in Periphyton (mg/m)	Collection by stone scraping; washed with 300 ml of water and kept in WHATMAN GFC fibre-glass filters. Analysis according to A. P. H. A., 1995
Biomass of Periphyton (g/l)	Same collection procedure. Biomass = (dry mass filters + periphyton) – dry mass of filters
Substrate Quality	Field observation. Categories: 1: poor; 2: marginal; 3: sub-optimal; 4: optimal. Based in Barbour et al. 1999
Mean Stone Size (mm)	Field measurements of 18 average stones.
Habitat Complexity	Field observation. Categories: 1: poor; 2: marginal; 3: sub-óptimal; 4: optimal. Based in Barbour et al. 1999
Pool Quality	Field observation. Categories: 1: poor; 2: marginal; 3: sub-óptimal; 4: optimal. Based in Barbour et al. 1999
Lithology	Instituto do Ambiente (2003) Categories: 1 = sedimentary; 2 = sedimentary + metamorphic; 3 = plutonic rocks
Riparian Vegetation (total width; m)	Field measurement
Woody vegetation (%)	Field observation. Woody vegetation in the riparian corridor
Shading at zenith (%)	Field observation. Shading done by the riparian vegetation in the stream
Forest (%)	Measured in the area of a circle of 1 km radius marked around each sampling site. Data from Plano de Bacia Hidrográfica do Mondego (MAOT 2002)
Eucalyptus (%)	Idem
Industrial, urban and degraded areas (%)	Idem
Agriculture (%)	Idem

Data analysis

The data were analysed by multivariate statistical methods with Primer software (version 5.2.6, Primer-E Ltd), except when otherwise indicated. This software was used because in general, Primer procedures make few assumptions about the form of the data or the inter-relationship of the samples (non-metric ordination and permutation tests are fundamental to the approach) and concentrate on approaches that are straightforward to understand and explain. Moreover, the philosophy inherent to this software is to analyse the biotic data first ("letting the data tell its own story") and then ask how well the information on environmental variables match the community structure (Clarke & Warwick 2001).

Biotic data

When larvae were too young to be correctly identified to species level and if other individuals of the same genus appeared and could be identified to species level, they were all analyzed at the genus level to avoid considering the younger larvae as different taxa. A species was considered present in a site when more than one individual was sampled. Biotic data were transformed through double square root for multivariate analysis to reduce the weight of the very abundant species.

Dominant species in the Mondego basin were identified using the Biota-EnVironment matching with STEP algorithm (BVSTEP) (five restarts, 25 % randomly selected variables; Bray-Curtis similarity measure). This PRIMER routine operates sequentially and involves both forward and backward-stepping phases aiming to find the smallest subset of species with a higher or equal to 95 % correlation (Spearman rank correlation method) with the original matrix. The routine carries out the stepwise approach on the active Trichoptera matrix for a fixed similarity matrix (Clarke & Warwick 2001). Similarity relationships among Trichoptera assemblages of all sites were determined by the Bray-Curtis coefficient. Groups of similar sites in terms of Trichoptera composition, samples (sites) were identified using Non-Metric Multi-Dimensional Scaling (NMDS) mapped in two dimensions and classified by Cluster analysis (group average mode). A cross-check of the NMDS patterns with an alternative technique, such as the Cluster analysis, is recommended by Clarke & Warwick (2001) whenever the ordination of sites presents stress values near 0.2.

To investigate the groups consistency the SIMilarity PERcentages-species contributions (SIMPER) analysis was used to obtain differences between all pairs of groups and the contribution of each species for the groups. SIMPER examines the contribution of each species to the average Bray-Curtis dissimilarity between groups of samples and also determines the contribution to similarity within a group (Clarke & Warwick 2001). Analysis of similarity test (ANOSIM) was used to test if the groups, based on biotic data, were significantly distinct. This is a simple non-parametric permutation procedure, which is applied to the rank similarity matrix underlying the ordination or classification of samples. The null hypothesis is that there are no differences in community composition of the groups. The procedure computes a test statistic (R), which is close to unity if there is complete segregation between groups and close to zero if there is little or no segregation, and a significance level (t) (Clarke & Warwick 2001).

Biotic-Abiotic matching

Non-normal variables (Kolmogorov-Smirnov normality tests, Sygma Stat 2.03) or those showing a marked skewness across the samples (Draftsman plots, Pearson correlation coefficient) were transformed by log $(x + 1)$ to stabilize the variance. The following variables were used with no transformation: stream, decimal latitude and longitude, mean substrate size, pH, % of forest and % of agriculture.

The BIOta-ENVironment matching procedure (BIOENV) was used to find the best fitting combination of environmental variables that explain the community pattern in the river basin. BIOENV routine is based on the premise that if the measured habitat features were responsible for structuring the community then the ordination based on the abiotic information would group sites in the same way as for the biotic plot. It selects environmental variables that "best explain" the community pattern, by maximizing a rank correlation between their respective similarity matrices. To run this analysis, the original set of 43 environmental variables was first reduced to a smaller set of variables (BVSTEP routine – see above, Spearman rank correlation, Euclidian distance). Those variables were then checked for co-linearity (Draftsman plots; Pearson correlation coefficient). The BIOENV procedure (all permutations of trial variables, Spearman rank correlation) was run with those variables which mutual correlations averaged less than 0.95 (as recommended by CLARKE & WARWICK 2001).

To find the environmental variables that could be used to describe the a priori categorization of sites based on Trichoptera taxa (NMDS and Cluster analysis of biotic data) the MODEL-ENV procedure was used (CLARKE & WARWICK 2001). This procedure identified the variables that distinguished between biotic groups. For that purpose, the BVSTEP routine was followed again, with all the environmental variables, but using a model matrix as fixed similarity matrix. One model matrix was devised for each pair of groups of sites reflecting equally-spaced inter-sites distances. This way we produced dissimilarity matrices with zeros between samples belonging to the same group and ones between samples of different groups. Then, the BVSTEP picked out a subset of environmental variables that best separated the samples into those two groups from the reduced habitat matrix (with only the sites that belonged to the two groups considered). The procedure was repeated for each pair of groups. The raw values of those variables distinguishing each pair of groups of sites were then observed, along with geographic location of sites in the map. This resulted in an association between groups discriminating environmental variables and respective Trichoptera assemblages. For example, if current velocity was one variable distinguishing group 1 from groups 2, 3, 4, 5 and 6, group 1 would be characterized as having an especially high or low current velocity (confirmed with the data), that separated it from the rest of the sites. Therefore, its particular Trichoptera assemblages should be somehow adapted to that high or low current velocity.

Results

Biotic information

A total of 11062 caddisflies was captured in the Mondego River basin during this study, with an average of 48 individuals per sample unit. This corresponds to 18 families and 70 species/genera (Appendix 1). From those, 9 species and two genera were not used in the data analysis as they were represented by one individual only or by individuals too young to be safely identified to species level. The distribution of the 12 most dominant species (*Calamoceras marsupus, Cheumatopsyche lepida, Hydropsyche bulbifera, Hydropsyche siltalai, Hydroptila* sp., *Lepidostoma hirtum, Mystacides azurea, Chimarra marginata, Polycentropus flavomaculatus, Tinodes waeneri, Rhyacophila adjuncta* and *Sericostoma* sp.) over the Mondego River watershed was correlated with the original set of species in the caddisflies communities (BVSTEP; Spearman, r = 0.952).

Six groups with two outliers were found for the Mondego river basin based on the NMDS, CLUSTER and SIMPER analysis (Fig. 2 a, b and Table 2). The similarity within groups ranged from 29 % to 37 % and the mean dissimilarities between groups ranged from 81 % (groups 2 and 1) to 96 % (groups 4 and 6). These groups were significantly different from each other (ANOSIM, Global test: R = 0.742, p = 0.001, 999 permutations). The contribution of the species for each group is given in Table 2.

Matching environment with Trichopteran assemblages

The original set of 43 variables was reduced to 21 (BVSTEP, Table 1). Those variables, chosen to be matched with the biotic data, and respective ranges in the sampling sites were: stream order (1–4), decimal latitude (39.88–40.71), substrate quality (1–4), altitude (17–1040 m), depth (0.12–0.53 m), current velocity (0–1.7 m s^{-1}), discharge (0–8.9 m^3 s^{-1}), water temperature (14.8–23.7 °C), pH (5.96–8.24), O_2 (40–113 %), total dissolved solids (19–762 mg l^{-1}), chloride (1.3–63.7 mg l^{-1}), nitrate (0–20 mg l^{-1}), nitrite (0–2.58 mg l^{-1}), alkalinity (3–150 mg l^{-1}), Fine Particulate Organic Matter (FPOM) in the sediment (0–3.62 g), periphyton biomass (0–1.7 g l^{-1}), % of forest (3–98 %) and % of eucalyptus (0–61 %) in the area of one km radius surrounding the sampling site, mean annual air temperature (7.5–17 °C), and total rainfall (500–1200 mm). Among these 21 variables, BIOENV identified a set of five explaining 41 % of the distribution of Trichoptera species across the river basin. The variables were: stream order, substrate quality, altitude, current velocity and alkalinity.

The environmental variables, obtained by MODEL-ENV procedure, that best distinguish the site groups formed previously with the biological data are

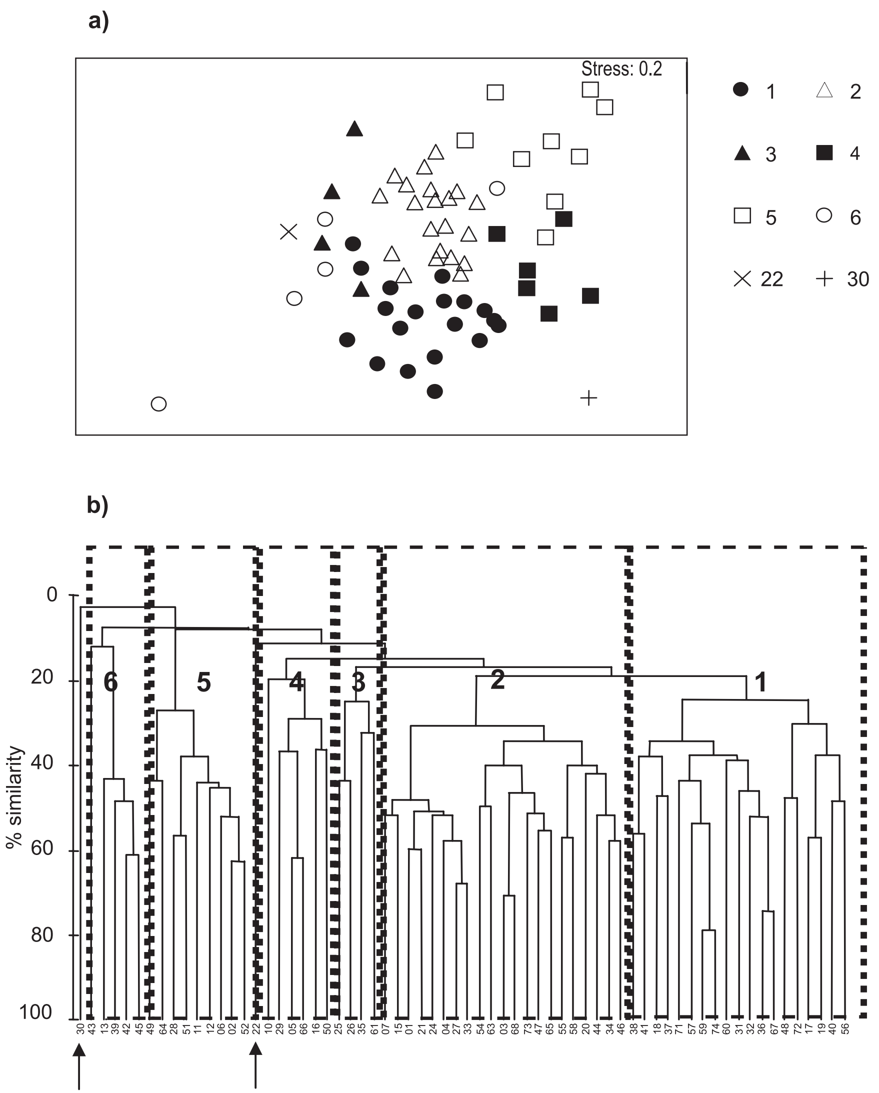

Fig. 2. Ordination by NMDS **(A)** and Cluster **(B)** of the sampling sites of the Mondego River basin. Outliers are indicated by two arrows (22 and 30).

shown in Table 3. Using those variables and the Trichoptera assemblages, the following associations resulted, for the Mondego river basin streams (Fig. 3):

1) Medium-large streams (mean width = 8.3 m) in low mountain areas (mean altitude = 337 m) with plutonic or metamorphic rocks, clean water and well developed riparian vegetation (16 m of mean total width and 68 % woody

Table 2. Most contributive species to the similarity within each group of sites (SIMPER, Primer 5.2.6).

Groups of sites	Number of sites	Most contributive species	Contribution to the group characterization
1	19	*Mystacides azurea, Calamoceras marsupus, Tinodes waeneri, Sericostoma* sp., *Polycentropus flavomaculatus*	94 % (*Mystacides azurea* contributed with 54 %)
2	21	*Sericostoma sp., Hydropsyche siltalai, Lepidostoma hirtum, Calamoceras marsupus, Polycentropus flavomaculatus, Larcasia partita, Rhyacophila relicta, Rhyacophila adjuncta*	91 %
3	4	*Hydropsyche siltalai, Rhyacophila adjuncta, Tinodes waeneri, Allogamus ligonifer, Rhyacophila relicta, Rhyacophila tristis*	93 %
4	6	*Calamoceras marsupus, Lepidostoma hirtum, Lype auripilis*	91 % (*Calamoceras marsupus* contributed with 72 %)
5	9	*Hydropsyche bulbifera*	93 %
6	5	*Cheumatopsyche lepida, Hydropsyche incognita, Chimarra marginata, Psychomyia pusilla*	93 %

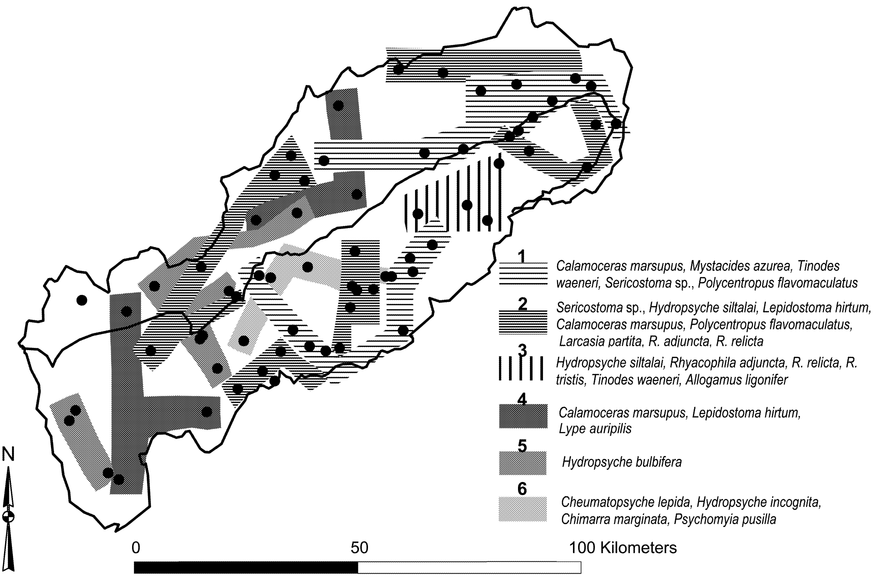

Fig. 3. Representation of the groups of sites based on Trichoptera distribution within the Mondego River basin with their most influential species.

vegetation) were associated with *Calamoceras marsupus, Mystacides azurea, Tinodes waeneri, Sericostoma* sp. and *Polycentropus flavomaculatus.*

2) Small (mean width = 3.2 m), near to source streams (mean distance to source = 9 km) in low mountain areas (mean altitude = 297 m) near plutonic and metamorphic rocks, in well developed forest areas (54 % of the land use) and clean water were associated with *Sericostoma* sp., *Hydropsyche siltalai, Lepidostoma hirtum, Calamoceras marsupus, Polycentropus flavomaculatus, Larcasia partita, Rhyacophila relicta* and *Rhyacophila adjuncta.*

3) Small streams (mean width = 2.2 m) located in highest regions (mean = 589 m), granitic areas with good stream bed substrate quality (mean category = 3, see Table 1) and well developed forest areas (43 % of the land use) were associated with the species: *Hydropsyche siltalai, Rhyacophila adjuncta, Rhyacophila relicta, Rhyacophila tristis, Tinodes waeneri* and *Allogamus ligonifer*.

4) Medium size streams (mean width = 4.2 m) in the Lower and Medium Mondego region with flat flood plains associated with sedimentary and metamorphic rocks, high alkalinities (mean = 72 mg l^{-1}), high concentration of dissolved solids (mean = 319 mg l^{-1}), low water oxygenation (mean = 79 %) and low level of water pollution (nutrients) were associated with *Calamoceras marsupus, Lepidostoma hirtum* and *Lype auripilis.*

5) Medium size (mean width 6.3 m; mean distance to source = 31 km), in the Lower Mondego region, with sedimentary to metamorphic areas, low discharge (1.0 m^3 s^{-1}), poor substrate quality (mean category = 2) and with high concentrations of chloride (mean = 23.1 mg l^{-1} and organic water pollution (e. g. mean nitrate = 9.2 mg l^{-1}; mean sulphate = 7.4 mg l^{-1}) were associated with *Hydropsyche bulbifera.*

6) Large streams (mean width = 16.4 m) in the Middle Mondego region, distant from the source (60 km), in sedimentary and metamorphic areas with good riparian corridors (15.8 m of mean total width) but with eucalyptus (25 % of the land use) and good water quality were associated with *Cheumatopsyche lepida, Hydropsyche incognita, Chimarra marginata*, and *Psychomyia pusilla.*

Discussion

Faunistics and taxonomy of Trichoptera in the Mondego River basin

The Iberian Peninsula has a distinct Trichoptera fauna; it has been calculated that almost half of the Trichoptera larvae are still not described and it is estimated that more than one third of the species are endemic (Vieira-Lanero 2000). The Mondego river basin has a high diversity of Trichoptera with 70 species, 40 genera and 18 families sampled in this study. The number of taxa found shows a comparatively high diversity compared to basins of the south and NW of the Iberian Peninsula where not more than 45 species were reported (Bonada 2003, Fernando-Alez et al. 2002). It corresponds to more than one fifth of the known species for the Iberian Peninsula, in spite of the fact that this study only included larvae captures. Therefore, the number of taxa reported here might be an underestimation, since Terra (1994) reported 126 species, 61 genera and 20 families for adults caught in the Mondego basin. This could be due to the sampling period, which was in summer, when many species have their adult phase. In fact, a study of seasonal changes in the Trichoptera of some streams in the Mondego catchment, indicates slightly higher species richness (based on larvae) during Autumn and Spring than in Summer (Feio 2004).

Among the species identified and listed in Appendix 1, the following eight specific cases deserve some attention. *Micrasema longulum, Metalype fragilis, Hydropsyche ambigua* and *Rhyacophilla occidentalis* were not reported in Portugal previously (Vieira-Lanero 2000). Larvae of *Synagapetus diversus, Catagapetus maclachlani, Agapetus incertulus* and *Chaetopteryx atlantica* have not been described yet in the literature. However, adults of these species were found by Terra (1994) in the area of the Mondego basin and are object of future publications.

Prediction of trichopteran assemblages in rivers

Even when a larger number of habitat variables is included in the analysis, the percentage of biotic variability explaining invertebrate assemblages might be low (Bonada 2003). In the present study, 41 % of the distribution of Trichoptera assemblages was explained by five variables out of 43. The other 59 % might be explained by potentially important environmental parameters which were not measured, such as the presence of competitors and predators, historical or stochastic events.

The most influential measured parameters in the overall distribution of the Trichoptera assemblages in the Mondego river basin are related to the stream morphology and hydrology (stream order, substrate quality, altitude, current velocity and alkalinity). These results agree with other studies showing that altitudinal changes take place in the diversity of macroinvertebrates communities dominated by insects (Wiberg-Larsen et al. 2000, Resh et al. 2002, Jacobsen 2003). Substrate type is also known to have an important influence on the distribution of benthic invertebrates on small and large spatial scales (see Jacobsen 1999). In the present study, our results were not surprising since the sampled streams included a wide range of substrate types, from sandy bottoms to great granite boulders, or mixtures of gravel and cobles.

The low importance of chemical variables indicators of water quality (such as nitrite, nitrate or other nutrients) may be a surprise. Trichoptera are known to be sensitive to organic pollution although some groups, such as the Hydropsychidae have only a fairly low sensitivity (e. g. Basaguren & Orive 1990 a,

Table 3. Best environmental variables discriminating the groups of trichopteran species and respective rank correlations between the habitat matrices and the fixed model matrix.

Biotic Groups	2	3	4	5	6
1	stream width riparian vegetation valley form **0.443**	% eucalyptus **0.581**	stream depth total rainfall valley form **0.582**	stream substrate quality altitude water temperature TDS % eucalyptus **0.629**	stream riparian vegetation rainfall lithology **0.464**
2		alkalinity % eucalyptus rainfall **0.675**	stream order width % eucalyptus air temperature total rainfall lithology **0.589**	stream distance to source % eucalyptus % industrial, urban and degraded areas **0.582**	stream distance to source pH alkalinity % eucalyptus **0.478**
3			stream chlorophyll % woody vegetation **0.761**	substrate quality chloride **0.581**	stream distance to source shading % agriculture **0.624**
4				stream nitrite **0.851**	stream altitude shading **0.752**
5					stream ammonium chlorophyll **0.828**

Resh 1993, Dohet 2002). Yet, we observed that the two geographically contiguous groups 4 and 5 (Fig. 3, Table 3) differ mainly in the concentration of nitrite in the water (higher in group 5). Also the mean concentration of sulphate was higher in group 5. Therefore, while at a wider scale (catchment) factors such as altitude and stream order seem to affect the general distribution of species, at a smaller range, where stream morphology and hydrology are more homogeneous, caddisfly assemblages are probably influenced by chemical water quality. In fact, while the most relevant species of group 4 (*Calamoceras marsupus, Lepidostoma hirtum* and *Lype auripilis*) are known to be sensitive to organic contamination (Vieira-Lanero 2000), in group 5, *Hydropsyche bulbifera* is considered tolerant to organic pollution (Basaguren & Orive 1990 a). Moreover, the assemblages found in group 5 could actually be the remnants of a community similar to group 4 since, in some sites of group 4, some individuals of *Hydropsyche bulbifera* were found and in three sites of group 5 one or two individuals of *Lepidostoma hirtum* or *Lype auripilis* were also present.

We identified six groups of sites based on environmental and biological data. In general, the most important species for each group belonged to different families. Often, the same family was present in different environments, but represented by different species. This suggests that species of the same family are spatially segregated, filling therefore different niches in terms of tolerance to the physical environment. It has been shown that families with a high number of species occupy a high diversity of niches while families with less species diversity are more consistent in the type of environment colonized (Bonada 2003, Hildrew & Edington 1979). This was also verified in our study for the families Hydropsychidae and Psychomyiidae, represented by 10 and 7 species, respectively, in 4 of the 6 biotic groups. For example, *Hydropsyche siltalai,* usually found in stony rivers of medium to high altitudes (in Vieira-Lanero 2000) was present in the Upper or Middle region of the basin but absent from the lower areas, while *Hydropsyche incognita* and *Cheumatopsyche lepida* were only found in the Middle basin and *Hydropsyche bulbifera* in the Lower region. This is in agreement with other studies on the distribution of Hydropsychidae species along the longitudinal axis of river basins (Hildrew & Edington 1979, Camargo 1992, Sieglstetter et al. 1997, Czachorowski 1989). Similarly, Psychomyiidae had three highly representative species distributed within four biotic groups. The species *Tinodes waeneri*, found in Galicia, Spain, in streams between 100 and 300 m with current velocities to 0.25 m s^{-1}, occurred in Mondego basin also in low mountain areas (around 300 m) with similar current velocities, while *Psychomyia pusilla*, typical of medium to low reaches occurred preferentially in the Middle Mondego basin and *Lype auripilis*, which lives in submerged woody debris was representative of the Lower Mondego region were low water currents permit a higher deposition of organic matter and woody debris (Vieira-Lanero 2000).

The diverse Rhyacophilidae family (nine species) had a restricted distribution in the Mondego basin with representative species distributed within only two biotic groups: *Rhyacophila relicta* and *R. adjuncta* in the higher areas (Upper Mondego region) and *R. relicta, R. adjuncta* and *R. tristis* in lower mountain regions. This is in accordance with the literature (Vieira-Lanero 2000) where *Rhyacophila relicta* is considered an ubiquitous species usually associated to *R. adjuncta*. Moreover, *R. adjuncta* and *R. tristis*, which are considered intolerant to organic contamination (Basaguren & Orive 1990 a, Vieira-Lanero 2000), appeared in two groups of clean waters. *Rhyacophila lusitanica* was represented by two individuals sampled in one site of the Lower Mondego region (Ança). According to the literature, larvae of this species colonize preferentially near-source locations with cold water, stony substrata and fast currents (Vieira-Lanero 2000). The presence of *R. lusitanica* in Ança could be explained by the relatively high current velocities of this sampling site (0.568 m s^{-1}).

In contrast, the family Calamoceratidae, represented by the only species on the Iberian Peninsula, (*Calamoceras marsupus*) was abundant (maximum captures of 333 larvae/sample unit), widespread in the area and present in three out of six taxa groups. According to the literature, the larvae of this species are adapted to a variety of substrates and live in rivers of moderate current velocity, basic or acid waters and no organic pollution (García de Jalón et al. 1987, Vieira-Lanero 2000), which is in accordance with our data.

In conclusion, the Trichoptera assemblages in streams and rivers of the Mondego basin seem to be very diverse and at a macro-scale, the distribution of species is controlled mainly by physical parameters. The best represented families appear to have their species distributed according to catchment gradients (altitude, current velocity, substrate) and individually, species distribution follows known characteristics including relative tolerance/intolerance to organic pollution. Since no comparative data are available for other Portuguese rivers it would be interesting to investigate in a broader scale how diversity is related to the gradients of productivity, evapotranspiration, variability of temperatures and flow, and land use. This work contributed to the knowledge of four new species in the Mondego River basin, patterns of co-occurrence and distribution of Trichoptera species and showed the importance of several environmental variables to caddisfly larvae, at different scales. Moreover, our findings permit the prediction of potential assemblages of Trichoptera for streams of the Mondego catchment.

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Appendix 1. List of 18 families and 70 taxa of the order Trichoptera found in Mondego River basin.

Family	Species
Beraeidae	*Beraea terrai* MALICKY, 1975
	Beraea malatebrera SCHMID, 1952
Brachycentridae	*Micrasema longulum* MCLACHLAN, 1876
Calamoceratidae	*Calamoceras marsupus* BRAUER, 1865
Glossosomatidae	*Agapetus delicatulus* MCLACHLAN, 1884
	Agapetus incertulus MCLACHLAN, 1884
	Agapetus fuscipes CURTIS, 1834
	Catagapetus maclachlani MALICKY, 1975
	Synagapetus marlierorum BOTSANEANU, 1980
	Synagapetus diversus (MCLACHLAN, 1884)
	Glossosoma privatum MCLACHLAN, 1884
Goeridae	*Larcasia partita* NAVÁS, 1917
Helicopsychidae	*Helicopsyche helicifex* (ALLAN, 1857)
Hydropsychidae	*Cheumatopsyche lepida* (PICTET, 1834)
	Diplectrona felix MCLACHLAN, 1878
	Hydropsyche sp.
	Hydropsyche bulbifera MCLACHLAN, 1878
	Hydropsyche ambigua SCHMID, 1952
	Hydropsyche siltalai DÖHLER 1963
	Hydropsyche incognita PITSCH, 1993
	Hydropsyche lobata MCLACHLAN, 1884
	Hydropsyche urgorrii GONZÁLEZ & MALICKY 1980
	Hydropsyche tibialis MCLACHLAN, 1884
Hydroptilidae	*Hydroptila* sp.
	Oxyethira sp.
	Ptilocolepus extensus MCLACHLAN, 1884
Lepidostomatidae	*Lepidostoma hirtum* (FABRICIUS, 1775)
Leptoceridae	*Adicella reducta* (MCLACHLAN, 1865)
	Adicella meridionalis MORTON, 1906
	Athripsodes sp.
	Triaenodes ochreellus MCLACHLAN, 1877
	Oecetis testacea (CURTIS, 1834)
	Mystacides azurea (LINNAEUS, 1761)
	Setodes argentipunctellus MCLACHLAN, 1877
Limnephilidae	*Allogamus laureatus* (NAVÁS, 1918)
	Allogamus ligonifer (MCLACHLAN, 1876)
	Limnephilus sp.
	Limnephilus guadarramicus SCHMID, 1955
	Halesus radiatus (CURTIS, 1834)
	Chaeptoteryx atlantica MALICKY, 1975
Odontoceridae	*Odontocerum lusitanicum* MALICKY, 1975
Philopotamidae	*Chimarra marginata* (LINNAEUS, 1767)
	Philopotamus perversus MCLACHLAN, 1884
	Wormaldia sp.

Appendix 1. Continued.

Family	Species
Polycentropodidae	*Cyrnus* sp.
	Cyrnus cintranus McLachlan, 1884
	Polycentropus sp.
	Polycentropus kingi McLachlan, 1881
	Polycentropus flavomaculatus (Pictet, 1834)
	Polycentropus corniger McLachlan, 1884
Psychomyiidae	*Lype auripilis* McLachlan, 1884
	Lype phaeopa Stephens, 1836
	Psychomyia fragilis (Pictet, 1834)
	Psychomyia pusilla (Fabricius, 1781)
	Tinodes waeneri (Linnaeus, 1758)
	Tinodes assimilis (McLachlan, 1865)
	Tinodes maculicornis (Pictet, 1834)
Rhyacophilidae	*Rhyacophila occidentalis* McLachlan, 1879
	Rhyacophila lusitanica McLachlan, 1884
	Rhyacophila munda McLachlan, 1862
	Rhyacophila relicta McLachlan, 1872
	Rhyacophila tristis Pictet, 1834
	Rhyacophila melpomene Malicky, 1976
	Rhyacophila obelix Malicky, 1979
	Rhyacophila pulchra Schmid, 1952
Sericostomatidae	*Sericostoma* sp.
	Schizopelex festiva (Rambur, 1842)
Uenoidae	*Thremma tellae* González, 1978
	Thremma gallicum McLachlan, 1880