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Diversity and distribution of chironomids (Diptera, Chironomidae) in pristine Alpine and pre-Alpine springs (Northern Italy)

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ABSTRACT

*The diversity and distribution of chironomids (Diptera, Chironomidae) were studied in relation to environmental factors in 81 springs under pristine conditions in the Italian Prealps and Alps (Trentino and Veneto, NE-Italy, 46°N, 10-11°E). Each spring was surveyed once, between May and November, in 2005 or in 2007-2008, within 50 m of the spring's source (eucrenal). A total of 173 macroinvertebrate samples were collected, in which 26,871 chironomids (including larvae, pupae, pupal exuviae and adults) were counted. Five subfamilies (Tanypodinae, Diamesinae, Prodiamesinae, Orthocladiinae and Chironominae), 54 genera and 104 species/groups of species were identified. As expected, Orthocladiinae accounted for a large part of specimens (82%), followed by Diamesinae (10%), Chironominae Tanytarsini (6%) and Tanypodinae (2%). Together the Chironominae Chironomini and Prodiamesinae contributed less than 0.05% of the fauna. Larvae represented 97.5% of specimens, mostly juveniles (62.6%). Maximum richness and diversity occurred at intermediate altitudes (*ca *900-2100 m a.s.l.). Most taxa were found in a small proportion of sites, and frequencies declined gradually for more widely distributed species. A high number (67%) of rare (= present in less than 10% of sites) taxa were found. Three to 27 taxa were identified per spring. The rheocrene/rheo-helocrene springs were richest in taxa (generally >15 taxa), the mineral spring was poorest, with only three taxa. Most taxa were crenophilous, including lentic, rheobiontic and bryophilous taxa. A Principal Component Analysis (PCA) was performed including 98 taxa. Axes were interpreted calculating the correlation coefficients between site scores and 24 environmental factors. The species with the highest scores were* Pseudokiefferiella parva*,* Corynoneura *sp. A,* Metriocnemus eurynotus *gr.,* Paratrichocladius skirwithensis *and* Tvetenia calvescens*. Five clusters of sites were identified with K-means analysis on the basis of the first and second PCA axes and a Discriminant Analysis was used to detect environmental factors discriminating the clusters: altitude, canopy cover, hydrological regime, pH, and granulometry as percentage of cobbles and stones. The highly individual nature of springs was highlighted; within the same river basin, between springs and within a single spring. These results suggest that prudent and conservative land management should assume that all springs sheltering such unique faunal assemblages need protection.*

Key words: Orthocladiinae, biodiversity, eucrenal, spring types, south-eastern Alps

1. INTRODUCTION

As in other freshwater habitats, chironomids dominate many freshwater springs, in abundance and species number (Lindegaard 1995; Gerecke *et al.* 1998; Stur & Wiedenbrug 2006).

Nevertheless compared with other insects, chironomids from springs have been less intensively studied. This is mainly due to the difficulty in identifying larvae – in some genera even pupae and adults (Orendt 2000a), to species level. In fact, lists of midge species are rather uncommon in ecological studies and knowledge of the autecology, geonemy and phenology of spring-dwelling species is still fragmentary compared to other aquatic habitats (Orendt 2000b). The first works on spring fauna, focusing at least partly on chironomids, were the investigations by Bornhauser (1912), Nadig (1942), Zavřel & Pax (1951) and Thienemann (1954). Lindegaard (1995) gave a comprehensive review of the chironomid literature on springs listing 99 references, and discussed the main factors affecting midge distribution in springs. Over the last ten years, several papers focused on the invertebrate fauna of Alpine and pre-Alpine springs, and of spring-fed brooks (in Italy*): Crema *et al.* (1996)*, Bonettini & Cantonati (1998)*, Klein & Tockner (2000), Orendt (2000a), Rossaro *et al.* (2000)*, Füreder *et al.* (2001), Rossaro & Bettinetti (2001)*, Stur *et al.* (2002), Lencioni & Rossaro (2005)*, Sambugar *et al.* (2006)*, Stur & Wiedenbrug (2006), Lencioni (2007)* and Marziali *et al.* (2010)*.

Up to 200 chironomid species are reported from cold European springs, and 73 from Italian Alpine springs, representing about 20% of the species recorded in Europe and Italy, respectively (Lindegaard 1995; Crema *et al.* 2006; Ferrarese 2006; Lencioni 2007). Nevertheless most crenal systems remain unexplored and no biotic indexes have yet been developed to determine their ecological status (Cantonati *et al.* 2006; Marziali *et al.* 2010).

Springs and their organisms are good tools for monitoring changes in groundwater quality due to human impact. In particular, chironomids are the most useful indicators of the surface water quality, as well as the upper layer of groundwater, because the larvae are affected by organic content and heavy metal load in the sediments (Lafont & Durbec 1990). Within this context, two projects (CRENODAT and CESSPA) were recently financed by two Italian public administrations, the Autonomous Province of Trento and the Adige Basin Authority, both focused on springs and their fauna as tools for monitoring changes in groundwater quality due to human impact (water abstraction for potable or hydroelectric use, pesticide contamination, etc.). This work considers only a selected number of springs investigated within those frameworks, all natural and so considered pristine sites.

The aims of this work were to: i) analyse chironomid taxa assemblages in natural springs of alpine and pre-alpine regions; ii) test whether springs can be separated into distinct groups according to chironomid fauna; iii) determine the main environmental factors structuring chironomid taxa assemblages in mountain springs.

2. METHODS

2.1. Study area

A total number of 81 springs were investigated, located in the Italian Prealps (21) and Alps (60) (Autonomous Province of Trento and Veneto Region, NE-Italy, 46°N, 10-11°E). They lie in 5 siliceous and in 18 carbonate basins, within a wide altitudinal range (170-2792 m a.s.l.), and belong to 7 hydromorphological types: rheocrene, helocrene, limnocrene, hygropetric, rheo-helocrene, rheo-hygropetric, rheo-limnocrene (Tab. 1). One spring (PS1255 Fontane negre, Pale di San Martino) was mineral. Most springs were perennial, but 7 were intermittent (Cantonati *et al*. 2007): AD2314 Amola rock glacier, AD2739 Maroccaro rock glacier, AN430 Pozza 1 Lago Bagatol, CV1421 Tornante Slavarè, ML0580 Vajo del Croce, ML0950 Varalta, OC2792 Val di Pejo rock glacier.

2.2. Chironomid collection

Each spring was surveyed once, between May and November, in 2005 (CRENODAT project) and in 2007-2008 (CESSPA project). Chironomid larvae and pupae were collected in the eucrenal zone (= within 50 m of the spring's source) of each spring. From one to three replicates were collected per spring by exploring different substratum typologies, depending on the spring morphology: a) coarse substratum (>0.2 cm, from gravel to stones); b) fine substratum (<0.2 cm, from sand to mud); c) bryophytes. A pond net (100 µm mesh size) was used in a) and b); 50 mL of surface sediment were also taken with a syringe (50 g) (b). 10 g of bryophytes were collected and washed in the laboratory to extract animals living within (c). Extra samples of larvae, pupae, pupal exuviae and adults were taken using tweezers, drift and sweep nets. Samples were preserved in 75% ethyl alcohol. For more details (CRENODAT Project) see Gerecke *et al*. (2011).

Chironomids were mounted on slides and identified to species/groups of species according to Serra-Tosio (1971), Pinder (1978), Ferrarese & Rossaro (1981), Rossaro (1982), Ferrarese (1983), Wiederholm (1983, 1986, 1989), Nocentini (1985), Schmid (1993), Janecek (1998), Stur & Ekrem (2006), Lencioni *et al*. (2007a) and Rossaro *et al*. (2009).

2.3. Environmental variables

During each biological survey, environmental variables were recorded, including those used for the landscape classification. Altitude was measured by GPS with an instrument error of about 10-15 m. The percent grain-size composition of the substratum was evaluated visually as percentage of gravel, cobbles, sand, silt, stones and rocks. Water samples were collected in acid-cleaned graduated bottles for hydro-chemical analysis (conductivity, alkalinity, hardness, dissolved oxygen, % oxygen saturation, pH, nutrients, anions, cations, and metals). Analyses were performed using standard methods following the American Public Health Association (APHA, AWWA & WEF 2005). Water temperature was measured with a field multiprobe (Hydrolab). In addition, the canopy cover (shading) was visually estimated in five classes: 0%, 25%, 50%, 75%, and 100%. Discharge was measured using a graduated bucket, repeating the measurement in different areas of the spring. Average current velocity was measured with an OTT propeller-flow meter. Turbidity was recorded using a portable turbidimeter MicroTPI. More details are given in Cantonati *et al*. (2007).

2.4. Statistical analyses

Only semi-quantitative samples collected in the three main substratum typologies were considered in the statistical analyses (drift and sweep net samples were not included); the mean abundance of each taxon per spring was considered. Rare species were included in the analysis, as recommended by Smith *et al.* (2001), resulting in a total of 98 taxa.

Biological and environmental data were log (x+1) transformed, except for percentage values which were arcsine (square root) transformed. Shannon Diversity Index (Shannon & Weaver 1949) was calculated for each spring with the MVSP® 3.1 computer package.

Pearson correlations between biological and environmental variables were calculated with STATISTICA® 8.0 computer package (StatSoft Inc. 2007). Values with $p < 0.05$ were considered significant.

Ordination analysis was carried out by means of the CANOCO® 4.5 computer package (Ter Braak & Šmilauer 2002). A Detrended Correspondence Analysis (DCA) was first run to detect whether the data had a unimodal or a linear structure according to the gradient length of axes. A gradient length between 2.5 and 4.4 suggested a linear model (Principal Component Analysis, PCA) as more appropriate (Ter Braak & Šmilauer 2002), and four axes were calculated.

Tab. 1. General characteristics of the 81 springs sampled within the CRENODAT and CESSPA Projects. Spring code: letters = Mt. group, numbers = altitude as m a.s.l. R = Rheocrene, HE = Helocrene, HY = Hygropetric, L = Limnocrene, RHE = Rheo-Helocrene, RHY = Rheo-Hygropetric, RL = Rheo-Limnocrene. Altitude is given as m a.s.l.

Project	Spring code	Spring name	Region	Mt. Group	Province	Lithology	Altitude	Type
CRENODAT	AD0905	Vermongo bassa	Alps	Adamello	Trento	limestone	905	R
CRENODAT	AD1077	Frana edene	Alps	Adamello	Trento	limestone	1077	R
CRENODAT	AD1235	Ponte Prese	Alps	Adamello	Trento	siliceous granite	1235	R
CRENODAT	AD1300	Borzago	Alps	Adamello	Trento	siliceous granite	1300	R
CRENODAT	AD1665	Ponte delle Cambiali	Alps	Adamello	Trento	siliceous granite	1665	R
CRENODAT	AD1790	Lago di Nambino	Alps	Adamello	Trento	siliceous granite	1790	RHE
CRENODAT	AD2314	Amola rock glacier	Alps	Adamello	Trento	siliceous granite	2314	R
CRENODAT	AD2739	Maroccaro rock glacier	Alps	Adamello	Trento	siliceous granite	2739	R
CRENODAT	LD0584	Fontanone	Alps	Alpi di Ledro	Trento	limestone	586	R
CRENODAT	LD0720	Fiavè	Alps	Alpi di Ledro	Trento	limestone	720	RHE
CRENODAT	LD0928	Del Graì	Alps	Alpi di Ledro	Trento	limestone	928	R
CRENODAT	LD0930	Tof della glera alta	Alps	Alpi di Ledro	Trento	limestone	930	R
CRENODAT	LD1400	Cortelì	Alps	Alpi di Ledro	Trento	limestone	1400	R
CRENODAT	LD1502	Tormendos	Alps	Alpi di Ledro	Trento	limestone	1502	RHY
CRENODAT	AN0430	Pozza 1	Alps	Anauni	Trento	siliceous granite	430	R
CRENODAT	AN1000	Vergnana	Alps	Anauni	Trento	limestone	1000	R
CRENODAT	AN1474	Fondo	Alps	Anauni	Trento	limestone	1474	RHE
CRENODAT	AN1578	Palu Longià	Alps	Anauni	Trento	siliceous porphyry	1578	HE
CRENODAT	AN1950	Bordolona	Alps	Anauni	Trento	siliceous metamorphic	1950	RHE
CRENODAT	BR0470	Maso Gori	Alps	Brenta	Trento	limestone	470	R
CRENODAT	BR0658	Faè 2	Alps	Brenta	Trento	limestone	658	R
CRENODAT	BR0679	Tovare	Alps	Brenta	Trento	limestone	679	R
CRENODAT	BR0790	Sass Ross	Alps	Brenta	Trento	limestone	790	RHY
CRENODAT	BR0950	Acqua fredda	Alps	Brenta	Trento	limestone	950	R
CRENODAT	BR1315	Valagola	Alps	Brenta	Trento	limestone	1315	R
CRENODAT	BR1358	Nambi	Alps	Brenta	Trento	limestone	1358	R
CRENODAT	BR1379	Rislà 3	Alps	Brenta	Trento	limestone	1379	R
CRENODAT	BR1436	Scala di Brenta	Alps	Brenta	Trento	limestone	1436	HY
CRENODAT	BR1605	Igropetrica Vallesinella	Alps	Brenta	Trento	limestone	1605	RHY
CRENODAT	BR1765	Corna Rossa	Alps	Brenta	Trento	limestone	1765	R
CRENODAT	CS1350	Monzon	Alps	Catinaccio Sassolungo	Trento	limestone	1350	RHE
CRENODAT	CA1642	Teleferica Brusà	Alps	Cima d'Asta	Trento	siliceous metamorphic	1642	RHE
CRENODAT	CA2153	Bual del passetto	Alps	Cima d'Asta	Trento	siliceous metamorphic	2153	R
CRENODAT	VZ1178	Paul	Alps	Cime Bocche Viezzena	Trento	limestone	1178	RL
CRENODAT	MC1115	Poloni	Alps	Corno Mezzorona	Trento	limestone	1115	R
CRENODAT	PS1255	Fontane negre	Alps	Gruppo Pale	Trento	limestone	1255	RHY
CRENODAT	PS1880	Salto busa dei Laibi V. Venegia	Alps	Gruppo Pale	Trento	limestone	1880	R
CRENODAT	CV0250	Resenzuola palu'	Alps	Lagorai	Trento	limestone	250	R
CRENODAT	CV0854	Giardini bassa	Alps	Lagorai	Trento	limestone	854	RHE
CRENODAT	CV0962	Pian Gran	Alps	Lagorai	Trento	siliceous porphyry	962	R
CRENODAT	CV0992	Val tamburli	Alps	Lagorai	Trento	limestone	992	R
CRENODAT	CV1084	Pirga Roncegno	Alps	Lagorai	Trento	limestone	1084	R
CRENODAT	CV1200	Perengola	Alps	Lagorai	Trento	siliceous porphyry	1200	R
CRENODAT	CV1280	Val Calamento Telve	Alps	Lagorai	Trento	siliceous metamorphic	1280	R
CRENODAT	CV1421	Tornante Slavarè	Alps	Lagorai	Trento	siliceous granite	1421	R
CRENODAT	CV1433	Acq. minerale. leggera Vetriolo	Alps	Lagorai	Trento	limestone	1433	HY
CRENODAT	CV1435	Le mandre	Alps	Lagorai	Trento	siliceous porphyry	1435	R
CRENODAT	CV1575	Torbiera di Grugola bassa	Alps	Lagorai	Trento	siliceous granite	1575	HE
CRENODAT	CV1623	Valmaggiore	Alps	Lagorai	Trento	siliceous porphyry	1623	RHE
CRENODAT	CV1655	Busa delle rane	Alps	Lagorai	Trento	siliceous granite	1655	R
CRENODAT	CV1685	Campigol dei solai	Alps	Lagorai	Trento	siliceous porphyry	1685	R
CRENODAT	CV1855	Auzertol	Alps	Lagorai	Trento	siliceous porphyry	1855	R
CRENODAT	CV2182	Stellune	Alps	Lagorai	Trento	siliceous porphyry	2182	R
CRENODAT	LT1240	Daiano	Alps	Latemar	Trento	limestone	1240	R
CRENODAT	MD1670	I ciei Monzon	Alps	Marmolada	Trento	limestone	1670	R
CRENODAT	MD1871	Fedaia	Alps	Marmolada	Trento	limestone	1871	R
CRENODAT	OC2056	Belvedere	Alps	Ortles-Cevedale	Trento	siliceous metamorphic	2056	R
CRENODAT	OC2792	Val di Pejo rock glacier	Alps	Ortles-Cevedale	Trento	siliceous granite	2792	R
CRENODAT	PG0453	Trementina alta	Alps	Paganella Gazza	Trento	limestone	453	R
CRENODAT	SL1724	Antermont bassa	Alps	Sella	Trento	limestone	1724	L
CRENODAT	AT0972	Masere	Prealps	Altop Folgaria Tonezza	Trento	limestone	972	R
CRENODAT	MB0335	Diaol	Prealps	Baldo	Trento	limestone	335	R
CESSPA	MB0385	Gaon	Prealps	Baldo	Verona	limestone	385	R

(continued)

Tab. 1. Continuation.

Project	Spring code	Spring name	Region	Mt. Group	Province	Lithology	Altitude	Type
CESSPA	MB0445	Prealba	Prealps	Baldo	Verona	limestone	445	R
CESSPA	MB0517	Lodrone	Prealps	Baldo	Trento	limestone	517	R
CRENODAT	MB1440	Tolghe dx	Prealps	Baldo	Trento	limestone	1440	R
CRENODAT	BS0705	Coel	Prealps	Bondone Stivo	Trento	limestone	705	R
CRENODAT	BS1527	Viotte	Prealps	Bondone Stivo	Trento	limestone	1527	R
CRENODAT	BC0170	Lago Bagatol	Prealps	Brento Casale	Trento	limestone	170	R
CRENODAT	BC0503	Madonina Val Lomasona	Prealps	Brento Casale	Trento	limestone	503	L
CRENODAT	BC0565	Laurel	Prealps	Brento Casale	Trento	limestone	565	R
CESSPA	ML0533	Biasetti	Prealps	Lessini	Verona	limestone	533	RHE
CESSPA	ML0580	Vajo del Croce	Prealps	Lessini	Verona	limestone	580	L
CESSPA	ML0950	Varalta	Prealps	Lessini	Verona	limestone	950	R
CRENODAT	MP0656	Vallarsa	Prealps	Pasubio	Trento	limestone	656	R
CESSPA	MP0676	Biuchele Speccheri	Prealps	Pasubio	Trento	limestone	676	R
CESSPA	MP0690	Cocher	Prealps	Pasubio	Trento	limestone	690	RHE
CESSPA	MP0924	Fondo Comperlon	Prealps	Pasubio	Trento	limestone	924	R
CRENODAT	MP1566	Sette albi	Prealps	Pasubio	Trento	limestone	1566	HY
CRENODAT	SC0250	Ramon Freddo Fontanazzo	Prealps	Sette Comuni	Trento	limestone	250	R
CRENODAT	VF0745	Madonna del Sass Mezzano	Prealps	Vette Feltrine	Trento	limestone	745	HY

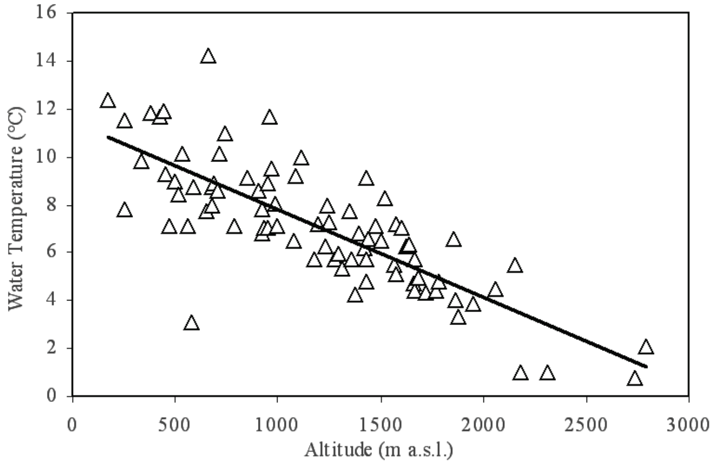

Fig. 1. Water temperature in relation to altitude.

The resulting ordination axis scores were interpreted against 24 environmental factors (Appendix 1) by calculating Pearson's product-moment correlation coefficients.

A K-means Cluster Analysis was carried out to cluster sites into similar groups based on chironomid taxon assemblages, considering site scores of the first two PCA axes as variables. K-means with five groups gave results of major ecological relevance, compared with K-means performed with 3, 4, 6 and 7 groups. A Discriminant Analysis based on the Wilk's lambda test was performed to detect the environmental factors separating the 5 K-means groups. Values with p <0.05 were considered significant.

3. RESULTS

3.1. Environmental features in the springs

In table 2 the main physico-chemical and hydromorphological features of the 81 springs are shown. The study sites are rather heterogeneous, with wide ranges for all parameters. For example, water temperature ranged from 0.8 to 14.2 °C, % oxygen saturation from 35 to 105%, pH from 6.3 to 8.3, conductivity from 16 to 2120 µS cm^{-1}, sulphate from 0.82 to 1368 mg L^{-1}, nitrate nitrogen from 20 to 2853 µg L^{-1}, total phosphorus from 1.8 to 73 µg L^{-1}, orthophosphate from 0.8 to 48 µg L^{-1} and silica from 0.6 to 13 mg L^{-1}. A strong correlation was found between altitude and water temperature (R^2 = 0.63, p <0.01) (Fig. 1), the latter decreasing with increasing altitude. Generally, the lowest values of water temperature, pH, conductivity and nutrients were recorded at the springs located at highest altitudes, in siliceous basins of the Mt. Groups Adamello, Ortles-Cevedale and Lagorai (Appendix 1). More details are given in Cantonati *et al.* (2007).

3.2. Chironomid diversity and distribution

A total of 173 replicates of macroinvertebrates were collected, in which 45% (= 26,871 specimens including larvae, pupae, pupal exuviae and adults) were chironomids.

Tab. 2. List of chironomid taxa in the 81 springs investigated. Substratum and spring type preferences are reported in bold, variables significantly ($p < 0.05$) correlated to a specific taxon. R= Rheocrene, HE= Helocrene, HY= Hygropetric, L= Limnocrene, RHE= Rheo-Helocrene, RHY= Rheo-Hygropetric, RL= Rheo-Limnocrene. *= species new to the Italian springs; ** = previously reported as Genus spp.; † species new to Italy. Empty cells when no association can be given (too low abundance or equal distribution of the taxon within microhabitats). ●: crenophilous-crenobiont taxa.

Subfamily	Species	Species code	Substratum preference	Spring type preference
Tanypodinae	*Nilotanypus dubius* (Meigen, 1804)*	*N_dubius*	sand	
	Apsectrotanypus sp.*	*Apsectrot*	**silt-mud**	**HE**
	Psectrotanypus sp.*	*Psectrot*	sand	**HY**
	● *Krenopelopia* sp.	*Krenopel*		
	● *Macropelopia fittkaui* Ferrarese & Ceretti, 1987	*M_fittkaui*	**silt-mud**	
	Macropelopia nebulosa (Meigen, 1804)*	*M_nebulosa*	**silt-mud**	**L, HE**
	● *Macropelopia notata* (Meigen, 1818)	*M_notata*	**cobbles/stones**	**RHY**
	Natarsia sp.*	*Natarsia*	**bryophytes**	**L**
	Thienemannimyia sp.*	*Thienema*	bryophytes	**RHY**
	● *Trissopelopia* sp.	*Trissope*	**sand**	**HE**
	● *Zavrelimyia* sp.	*Zavrelim*	silt-mud, sand	
Diamesinae	● *Diamesa aberrata* Lundbeck, 1889	*D_aberra*	cobbles/stones	**R**
	Diamesa cinerella Meigen, 1935*	*D_cinere*	bryophytes	
	● *Diamesa dampfi* gr.	*D_dampfi*	bryophytes	**RHE**
	● *Diamesa incallida* (Walker, 1856)	*D_incall*	**cobbles/stones**	**R**
	● *Diamesa insignipes* Kieffer, 1908	*D_insign*	**cobbles/stones**	**R**
	● *Diamesa latitarsis* gr.	*D_latita*	**cobbles/stones**	**R**
	Diamesa starmachi Kownacki & Kownacka, 1970*	*D_starma*	bryophytes	
	Diamesa steinboecki Goetghebuer, 1933*	*D_steinb*	**cobbles/stones**	**R**
	Diamesa tonsa (Walker, 1856)*	*D_tonsa*	**cobbles/stones**	
	Diamesa vaillanti Serra-Tosio, 1972*	*D_vailla*	**cobbles/stones**	**R**
	Potthastia gaedii (Meigen, 1838)*	*P_gaedii*	**cobbles/stones**	**R**
	● *Pseudodiamesa branickii* (Nowicki, 1873)	*P_branic*	**bryophytes**	**RHE**
	● *Pseudokiefferiella parva* (Edwards, 1932)	*P_ parva*	**cobbles/stones**	
Prodiamesinae	*Prodiamesa olivacea* (Meigen, 1818)	*P_olivac*	**silt-mud**	**RL, HE**
Orthocladiinae	*Acamptocladius reissi* Cranston & Sæther, 1981	*A_reissi*	bryophytes	
	● *Brillia bifida* (Kieffer, 1909)	*B_bifida*	gravel/cobbles	
	Brillia longifurca Kieffer, 1921*	*B_longif*		
	Bryophaenocladius spp.*	*Bryophae*	**bryophytes**	**RHE**
	● *Chaetocladius dentiforceps* (Edwards, 1929)**	*C_dentif*	**bryophytes**	**HY**
	Chaetocladius perennis (Meigen, 1830)**	*C_perenn*	**cobbles/stones**	
	● *Chaetocladius piger* gr.**	*C_piger*	**silt-mud**	**RHE**
	● *Chaetocladius vitellinus* gr.**	*C_vitell*		
	● *Corynoneura lobata* Edwards, 1924**	*C_lobata*	**cobbles/stones**	
	Corynoneura scutellata Winnertz, 1846**	*C_scutel*	**silt-mud**	**R**
	Corynoneura sp.A**	Cory_sp.A	**gravel**, bryophytes	**R**, RL, HE
	● *Cricotopus annulator* Goetghebuer, 1927*	*C_annula*	bryophytes	
	Cricotopus fuscus (Kieffer, 1909)	*C_fuscus*	bryophytes	
	Cricotopus tremulus (Linnaeus, 1756)*	*C_tremul*	**cobbles/stones**	
	Cricotopus trifascia Edwards, 1929*	*C_trifas*	**cobbles/stones**	**R**
	Diplocladius cultriger Kieffer, 1908*	*D_cultrig*	**bryophytes**	**RHE**
	● *Eukiefferiella brehmi* gr.*	*E_brehmi*	**silt-mud**	**R**
	Eukiefferiella brevicalcar (Kieffer, 1911)	*E_brevic*	bryophytes, cobbles/stones	**HY**
	● *Eukiefferiella claripennis* Lundbeck, 1898	*E_clarip*	**silt-mud**	**HY**
	● *Eukiefferiella coerulescens* (Kieffer, 1926)*	*E_coerul*	**cobbles/stones**	
	Eukiefferiella cyanea Thienemann, 1936*	*E_cyanea*	**cobbles/stones**	**HY**
	Eukiefferiella devonica gr.	*E_devonic*	cobbles/stones	**R**
	Eukiefferiella gracei gr.	*E_gracei*	sand	
	Eukiefferiella minor (Edwards, 1929)	*E_minor*	**stones/rock**, bryophytes	**RHY**, HY
	Eukiefferiella similis Goetghebuer, 1939*	*E_simili*	bryophytes	**RHE**
	Eukiefferiella tirolensis Goetghebuer, 1938*	*E_tirole*	bryophytes	
	● *Heleniella serratosioi* Ringe, 1976	*H_serrat*	sand, silt	
	● *Heterotanytarsus apicalis* (Kieffer, 1921)	*H_apical*	**silt-mud**	
	Heterotrissocladius marcidus (Walker, 1956)	*H_marcid*	**silt-mud**	**HE**, RL
	Hydrobaenus sp.*	*Hydrobae*		**R**
	● *Krenosmittia borealpina* (Goetghebuer, 1944)**	*K_boreal*	bryophytes	
	Limnophyes spp.	*Limnophy*	cobbles/stones	**L**
	Limnophyes asquamatus Søgaard Andersen, 1937*	*L_asquam*	**bryophytes**	**HY**
	Metriocnemus fuscipes gr.	*M_fuscip*	bryophytes	
	Metriocnemus eurynotus gr.	*M_euryno*	**bryophytes**	

(continued)

Tab. 2. Continuation.

Subfamily	Species	Species code	Substratum preference	Spring type preference
	Metriocnemus inopinatus Strenzke, 1950*	*M_inopina*	**bryophytes**	**R**
	Metriocnemus terrester Pagast, 1941*	*M_terres*	silt-mud, bryophytes	
	● *Orthocladius* spp.	*Orthocl*	**bryophytes**, **cobbles/stones**	**R**
	Orthocladius (*Euorthocladius*) *frigidus* (Zetterstedt, 1838)	*E_frigidu*	**cobbles/stones**, bryophytes	
	Orthocladius (Eudactylocladius) fuscimanus Kieffer, 1908*	*E_fuscim*	cobbles/stones	
	Orthocladius (*Euorthocladius*) *rivicola* Kieffer, 1921	*E_rivico*	**stones/rock**	**RHY**, **HY**
	Orthocladius (*Symposiocladius*) sp.*	*Symposio*	**cobbles/stones**	**R**
	Parachaetocladius sp.*	*Parachae*	**bryophytes**, **silt-mud**	**RHE**
	Paracricotopus niger (Kieffer, 1913)*	*Paracric*	**bryophytes**	**RHE**
	● *Parakiefferiella gracillima* (Kieffer, 1924)	*P_gracil*	**bryophytes**	**R**
	Parametriocnemus boreolapinus Gouin, 1942	*P_boreoa*	silt-mud	
	Parametriocnemus sp.A*	*Param_spA*		
	● *Parametriocnemus stylatus* (Kieffer, 1924)	*P_stylatus*	silt-mud	
	Paraphaenocladius impensus (Walker, 1856)*	*P_impens*	**silt-mud**	R
	Paratrichocladius rufiventris (Meigen, 1830)	*P_rufive*	**cobbles/stones**	RL, RHE
	● *Paratrichocladius skirwithensis* (Edwards, 1929)	*P_skirwi*	**bryophytes**, cobbles/stones	
	Paratrissocladius excerptus (Walker, 1856)*	*P_excerp*	**silt-mud**	
	Parorthocladius nudipennis (Kieffer, 1908)	*P_nudipe*	cobbles/stones	
	Pseudorthocladius sp.*	*Pseudort*	cobbles/stones	**R**
	Pseudosmittia sp.*	*Pseudosm*	**bryophytes**	**R**
	Rheocricotopus chalybeatus (Edwards, 1929)*	*R_chalyb*	**cobbles/stones**	**R**
	● *Rheocricotopus effusus* (Walker, 1856)	*R_effusu*	**silt-mud**	**RL**, HY
	● *Rheocricotopus fuscipes* Kieffer, 1909*	*R_fuscip*	bryophytes, cobbles/stones	**RL**
	● *Stilocladius montanus* Rossaro, 1979*	*S_montan*	bryophytes, cobbles/stones	**L**
	● *Synorthocladius semivirens* Kieffer, 1909*	*S_semivi*	**cobbles/stones**	
	● *Thienemannia gracilis* Kieffer, 1909**	*T_gracili*	**bryophytes**, silt-mud	**RL**
	Thienemanniella clavicornis (Kieffer, 1911)**	*T_ clavic*	bryophytes, cobbles/stones	
	Thienemanniella vittata (Edwards, 1924)**	*T_vittat*	**cobbles/stones**	
	● *Tvetenia bavarica* (Goetghebuer, 1934)	*T_bavari*	bryophytes	**RHY**
	● *Tvetenia calvescens* (Edwards, 1929)	*T_calves*	**cobbles/stones**, bryophytes	**R**, HY, RHY
	Tvetenia verralli (Edwards, 1929)*	*T_discol*	**bryophytes**	**RHE**
Chironominae	*Paracladopelma* sp.*	*Paraclad*	**bryophytes**, silt-mud	**RHE**
	● *Krenopsectra fallax* Reiss, 1969*	*Krenopse*	silt-mud	
	Micropsectra aristata Pinder, 1976*	*M_arista*	silt-mud, bryophytes	**HE**
	Micropsectra atrofasciata (Kieffer, 1911)*	*M_atrofa*	bryophytes, cobbles/stones	
	Micropsectra bavarica Stur & Ekrem, 2006*	*M_bavari*	**silt-mud**	**HY**
	● *Micropsectra schrankelae* Stur & Ekrem, 2006*	*M_schran*	**cobbles/stones**	**HY**
	● *Micropsectra seguyi* Casas & Laville, 1990*	*M_seguyi*	**silt-mud**	
	Micropsectra sofiae Stur & Ekrem, 2006*	*M_sofiae*	**silt-mud**	
	Micropsectra longicrista Stur & Ekrem, 2006** †	*M_longic*	bryophytes, cobbles/stones	
	Rheotanytarsus sp.	*Rheotany*	silt-mud	
	Stempellinella sp.	*Stempell*	sand	
	● *Tanytarsus heusdensis* Goetghebuer, 1923**	*T_heusde*	**cobbles/stones**	**RL**
	Tanytarsus pallidicornis (Walker, 1856)**	*T_pallid*	silt-mud	

Five subfamilies (Tanypodinae, Diamesinae, Prodiamesinae, Orthocladiinae and Chironominae), 54 genera and 104 species/groups of species were identified (Tab. 2). Orthocladiinae accounted for 82% of the total chironomid fauna, followed by Diamesinae (10%), Chironominae Tanytarsini (6%) and Tanypodinae (2%). Together Chironominae Chironomini and Prodiamesinae contributed less than 0.05% of specimens. Larvae represented 97.5% of the animals, most of which were juveniles (62.6%).

No significant correlation was found between taxon richness and Shannon Diversity Index with altitude, the highest values for both being associated with an intermediate altitudinal range (Figs 2, 3). Most taxa occurred at a small proportion of the sites, and frequencies declined gradually for more widely distributed species. A high number (68 = 67%) of rare (= present in less than 10% of sites) taxa were found (Fig. 4). Of these, 35 (30%) occurred in only one site. These included several species of the genera *Diamesa* and *Eukiefferiella*, such as *Diamesa cinerella* and *Diamesa tonsa* (both only in AD1665 Ponte delle Cambiali), *Diamesa incallida* and *Eukiefferiella tirolensis* (in BR1358 Nambi), *Diamesa insignipes* (in OC2056 Belvedere), *Diamesa latitarsis* gr. (only in AD905 Vermongo Bassa), *Diamesa starmachi* (in MD1871 Fedaia), *Eukiefferiella cyanea* (in BR1436 Scala di Brenta), *Eukiefferiella devonica* gr. (in MP0676 Biuchele Speccheri), *Eukiefferiella similis* (in ML0533 Biasetti). Other very rare taxa were *Paraphaenocladius impensus* (in CV0250 Resenzuola Palù), *Paratrissocladius excerptus* (in AD905 Vermongo Bassa), *Rheocricotopus chalybeatus* (in MB0385 Gaon), *Rheotanytarsus* sp. (in AD1665 Ponte delle Cambiali) and *Paracladopelma* sp. (in AN1474 Fondo).

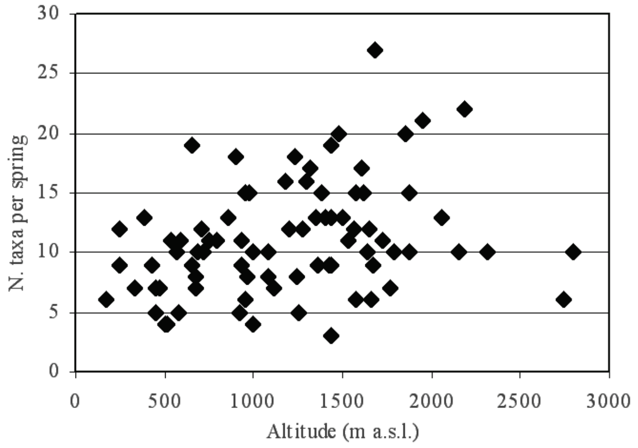

Fig. 2. Number of taxa per spring in relation to altitude (m a.s.l.).

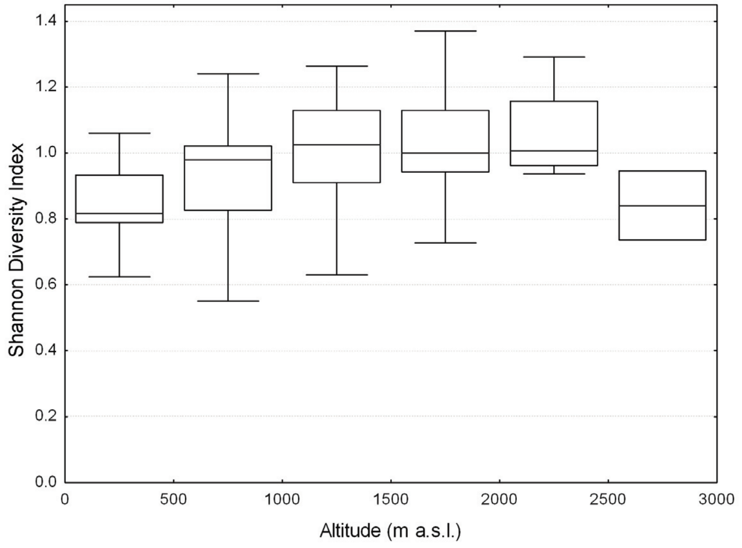

Fig. 3. Median, 25th, 75th percentile of Shannon Diversity Index in relation to altitude (m a.s.l.). Height of box (H)= 25th-75th percentile; whiskers = non-outlier values comprised within an interval of 1.5 x H.

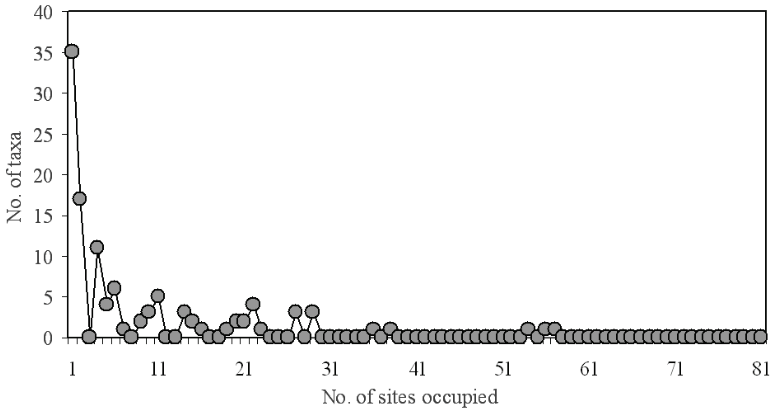

Fig. 4. Number of taxa in relation to number of sites occupied.

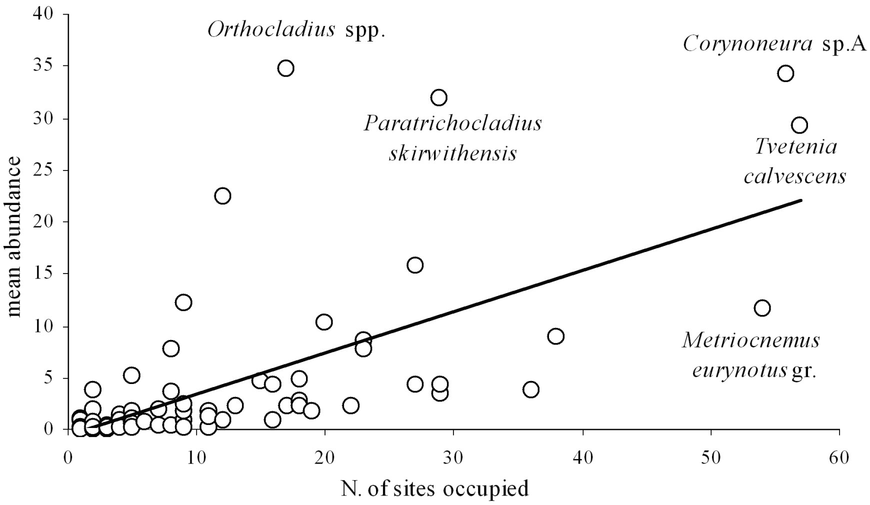

Fig. 5. Abundance-occupancy relationships.

Three taxa were present in at least 50% of the sites (= common taxa): *Tvetenia calvescens*, *Corynoneura* sp. A and *Metriocnemus eurynotus* (= *hygropetricus*) gr. (Fig. 5). No taxon was present in all 81 sites. Widespread species generally tended to have a relatively higher abundance than those with restricted distributions. The local abundance of taxa increased (but not significantly, $y = 0.396x - 0.541$, $R^2 = 0.48$) with the number of sites from which they were collected. The most frequent (= present in at least 50% of sites) and abundant (= mean density ≥29 ind./spring) taxa were *Tvetenia calvescens* and *Corynoneura* sp. A. Among the less common taxa, high abundance was observed for *Orthocladius* spp. (21% of the sites) and *Paratrichocladius skirwithensis* (36% of the sites) (Fig. 5). More than fifty taxa were new to Italian springs (Tab. 2) and one, *Micropsectra longicrista*, was new to Italy, found in the rheocrene Valagola (BR1315). In contrast, some previously recorded species (Ferrarese 2006) were not found, such as the ubiquist Chironominae *Chironomus lacunarius* (Wülker 1973), and the crenophilous Podonominae *Paraboreochlus minutissimus* (Strobl 1894).

From 3 to 27 taxa were identified per spring. Only five springs hosted more than 20 taxa and more than 130 individuals. Three of these, all rheocrenes and at altitudes >1470 m a.s.l., are located in the Lagorai Mt. Group, in siliceous basins (porphyry) (CV1685 Campigol dei Solai, CV2182 Stellune, CV1855 Auzertol). The other two, rheo-helocrenes, are both located in the Anauni Mt. Group, one in a siliceous metamorphic basin (AN1950 Bordolona) and one on limestone (AN1474 Fondo). Three to 5 taxa were counted in 8 springs, distributed over seven different Mt. Groups, of different hydro-morphological types and located mainly at <1000 m a.s.l. (PS1255 Fontane Negre, rheo-hygropetric; PG0453 Trementina alta, rheocrene; ML0580 Vajo del Croce, limnocrene; MP0924 Fondo Comperlon, rheocrene; CV0992 Val Tamburli, rheocrene; BC0503 Madonnina Val Lomasona, limnocrene; MB0517 Lodrone, rheocrene; CV1433 Acqua minerale Vetriolo, hygropetric). The last was the only mineral (sulphurous) spring (SO_4^{2-} = 409 mg L^{-1}, conductivity = 1239 µS cm^{-1}), in which the lowest richness was recorded (*Limnophyes asquamatus*, *Bryophaenocladius* spp. and *Paratrichocladius skirwithensis*).

Diversity was higher in mixed-type springs, such as rheo-helocrenes, rheo-hygropetric and rheo-limnocrenes, but lowest in the limnocrenes. The widest range of diversity values was recorded in the rheocrenes (Fig. 6).

44% of specimens were found on the coarse substratum (>0.2 cm), 38% in submerged bryophytes and 18% in the finer sediment. Diversity was highest in coarse substratum (1.89), followed by fine substrate (1.82) and bryophytes (1.78). Thirty-eight taxa were common to all three substrate types. Sixteen taxa were exclusive to coarse substrata (*Chaetocladius perennis*, *Corynoneura lobata*, *Cricotopus tremulus*, *Cricotopus trifascia*, *Diamesa incallida*, *Diamesa insignipes*, *Diamesa latitarsis* gr., *Diamesa tonsa*, *Diamesa vaillanti*, *Eukiefferiella coerulescens*, *Eukiefferiella cyanea*, *Macropelopia notata*, *Potthastia gaedii*, *Rheocricotopus chalybeatus*, *Orthocladius* (*Symposiocladius*) sp. and *Tanytarsus heusdensis*). Thirteen taxa were exclusive to fine substrata: *Corynoneura scutellata*, *Eukiefferiella brehmi* gr., *Eukiefferiella claripennis* gr., *Heterotanytarsus apicalis*, *Macropelopia fittkaui*, *Micropsectra bavarica*, *Parachaetocladius* sp., *Paraphaenocladius impensus*, *Paratrissocladius excerptus*, *Prodiamesa olivacea*, *Paracladopelma* sp., *Tanytarsus pallidicornis*. Nine taxa were found only in bryophytes (*Chaetocladius dentiforceps*, *Diplocladius cultriger*, *Eukiefferiella similis*, *Limnophyes asquamatus*, *Metriocnemus inopinatus*, *Natarsia* sp., *Parakiefferiella gracillima*, *Paracricotopus niger*, *Pseudosmittia* sp.).

No taxa were exclusive to helocrenes, limnocrenes and rheo-limnocrenes. Three taxa were captured only in

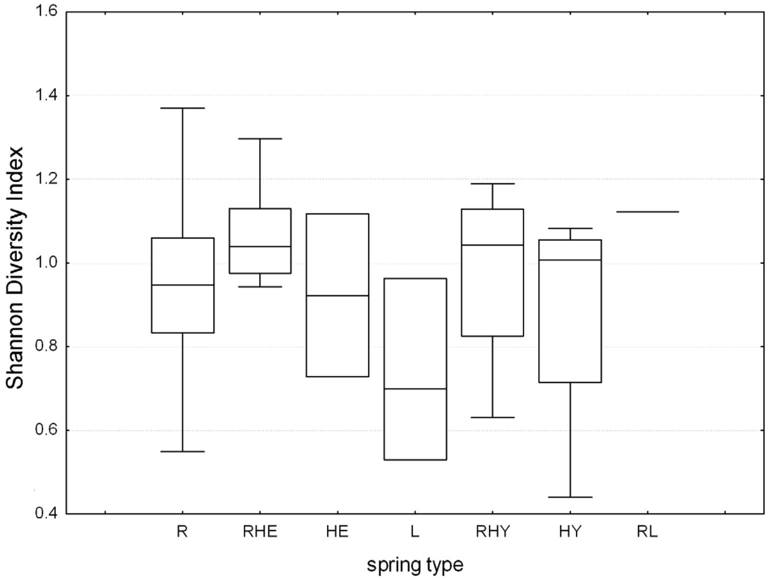

Fig. 6. Median, 25th, 75th percentile of Shannon Diversity Index in relation to spring types. Height of box (H)= 25th-75th percentile; whiskers = non-outlier values comprised within an interval of 1.5 x H. R= Rheocrene, RHE= Rheo-Helocrene, HE= Helocrene, L= Limnocrene, RHY= Rheo-Hygropetric, HY= Hygropetric, RL= Rheo-Limnocrene.

hygropetric springs (*Micropsectra bavarica*, *Eukiefferiella cyanea*, *Limnophyes asquamatus*), nineteen in rheocrenes (*Corynoneura scutellata*, *Cricotopus trifascia*, *Diamesa aberrata*, *D. incallida*, *D. insignipes*, *D. latitarsis* gr., *D. steinboecki*, *D. vaillanti*, *Eukiefferiella brehmi* gr., *E. devonica* gr., *Hydrobaenus* sp., *Metriocnemus inopinatus*, *Potthastia gaedii*, *Parakiefferiella gracillima*, *Paraphaenocladius impensus*, *Pseudorthocladius* sp., *Pseudosmittia* sp., *Rheocricotopus chalybeatus*, *Symposiocladius* sp.), three in rheo-helocrenes (*Eukiefferiella similis*, *Paracricotopus niger*, *Paracladopelma* sp.), and one in the rheo-hygropetric type (*Macropelopia notata*).

Cold-stenothermal taxa (such as all *Diamesa* species) were restricted to stations located in siliceous basins at the highest altitudes (>1400 m a.s.l.), where the lowest temperatures, pH, conductivity and canopy cover (<50%) were recorded.

3.3. Chironomid community in relation to environmental factors

Results of PCA, K-means Cluster Analysis and of Discriminant Analysis are given in tables 3, 4 and in figures 7, 8, 9. Four eigenvalues were selected by the Principal Component Analysis: 0.157, 0.085, 0.072 and 0.06 accounting for 37.4% of the total variance.

Five clusters were identified on the basis of the chironomid taxon assemblages in the 81 springs (K-mean analysis) (Tab. 3, Fig. 7). The Discriminant Analysis selected 6 environmental variables as best associated with the observed chironomid assemblages (Tab. 4).

A water temperature - altitude gradient was found along the first PCA axis (Fig. 8). Water temperature was positively correlated to conductivity, pH, high level of nutrients, canopy cover and limestone substratum, whereas altitude was correlated with current velocity, discharge, oxygen content, presence of bryophytes, coarse substratum. A canopy cover-lithology gradient was found along the second axis. Species-environment relationships accounted for 48.6% of total variance.

Rheocrene and hygropetric springs grouped in the first (cluster D, E) and fourth (clusters A, E) quadrants, whereas most of helocrenes and rheo-helocrenes grouped in the second (cluster B) and third (cluster C) quadrants (Fig. 7). Limnocrene springs occurred in different clusters (one in cluster B, two in cluster C, and one rheo-limnocrene in cluster E). Sites richest in nutrient and organic debris were grouped in cluster B, with higher canopy cover that ensures shading and more allochtonous food. All the intermittent springs grouped in cluster C, and cold, high-altitude springs with less nutrients in cluster A. The taxa best associated with the site clusters were *Paratrichocladius* skirwithensis and *Pseudokiefferiella parva* (cluster A), *Metriocnemus eurynotus* (cluster D), *Tvetenia calvescens* and *Corynoneura* sp. A (cluster E) (Fig. 9).

4. DISCUSSION

The percentage distribution of taxa within chironomid subfamilies was in accordance with previous studies (Lindegaard 1995; Stur *et al.* 2005; Stur & Wiedenbrug 2006), with Orthocladiinae as the most frequent, taxon-richest and abundant subfamily. Many cold stenothermal and rheophilous taxa were found, based on the prevalence of cold and rheocrene springs. Most larvae were juveniles, highlighting the role of stable crenal habitats, as nurseries, even for non-crenophilous species, and as a refuge against water current and abrupt environmental changes.

Tab. 3. K-means clusters of sites according to chironomid community.

	PC Axis 1	PC Axis 2	K-means Cluster	Distance
AD1665	-0.0768	-0.6126	A	0.33
AN1950	0.9147	-0.5382	A	0.37
BR1765	0.4515	-0.5615	A	0.04
CA2153	0.4943	-0.7107	A	0.12
CV2182	0.5422	-0.7681	A	0.17
MD1871	0.5851	-0.5342	A	0.14
OC2056	0.2026	-0.3456	A	0.21
PS1880	0.0322	-0.5791	A	0.26
AD1790	-0.293	0.0321	B	0.12
AN1000	-0.3259	0.0569	B	0.13
AN1578	-0.1638	0.1931	B	0.03
AT0972	-0.2594	0.2323	B	0.07
BC0565	0.0281	0.1927	B	0.15
BR0470	-0.224	0.0314	B	0.10
BR0790	-0.1976	0.0943	B	0.05
BR0950	-0.1464	0.4368	B	0.20
BS0705	-0.1987	0.2395	B	0.06
BS1527	-0.1897	0.0191	B	0.10
CS1350	0.0272	0.2032	B	0.15
CV0250	-0.0112	0.1577	B	0.12
CV0854	-0.2703	0.2479	B	0.09
CV0962	-0.1998	0.4259	B	0.19
CV1084	0.093	0.2619	B	0.21
LD0584	0.0377	0.2265	B	0.16
LD0720	-0.3717	0.0826	B	0.14
LD0930	-0.2155	0.2118	B	0.04
LD1400	-0.1672	0.0811	B	0.06
LT1240	-0.1353	0.4194	B	0.18
MB0335	-0.2753	0.1508	B	0.07
MB1440	-0.348	0.1111	B	0.12
MC1115	-0.2976	0.0383	B	0.12
MP0656	-0.2433	0.2847	B	0.10
PG0453	-0.1798	0.0403	B	0.09
SC0250	-0.1561	0.0032	B	0.11
SL1724	-0.0725	-0.0603	B	0.18
VF0745	-0.3853	0.1361	B	0.14
AD1077	-0.1792	-0.1235	C	0.12
AD2314	-0.2365	-0.592	C	0.33
AD2739	-0.4391	-0.2722	C	0.11
AN0430	-0.3885	-0.0491	C	0.07
BC0170	-0.3314	-0.1147	C	0.02
BC0503	-0.4723	-0.0498	C	0.11
BR0679	-0.2583	-0.1505	C	0.07
CA1642	-0.3433	-0.1889	C	0.03
CV0992	-0.3437	-0.0568	C	0.06
CV1433	-0.445	-0.2752	C	0.12
CV1575	-0.086	-0.2014	C	0.19
MB0385	-0.2961	-0.1073	C	0.05
MB0445	-0.4692	-0.0699	C	0.10
MB0517	-0.5076	-0.0455	C	0.13
MD1670	-0.3156	-0.0656	C	0.06
ML0533	-0.4608	-0.0305	C	0.11
ML0580	-0.4812	-0.0699	C	0.10
ML0950	-0.4565	-0.0804	C	0.09
MP0676	-0.4097	-0.102	C	0.05
MP0690	-0.3527	-0.0205	C	0.09
MP0924	-0.4551	-0.1187	C	0.07
OC2792	-0.0599	-0.3018	C	0.24
PS1255	-0.3197	-0.1605	C	0.03
AD1235	0.7271	0.868	D	0.39
AN1474	0.1475	0.5622	D	0.21
BR0658	0.5351	0.3398	D	0.11
BR1605	0.6548	0.3387	D	0.19
CV1280	0.227	0.3419	D	0.14
CV1623	0.2383	0.2728	D	0.16
CV1855	0.2769	0.2692	D	0.14
LD1502	0.5142	0.2618	D	0.14
MP1566	0.3253	0.5209	D	0.09
AD0905	0.3258	-0.014	E	0.12
AD1300	0.285	0.0712	E	0.17
BR1315	0.9106	0.2003	E	0.34
BR1358	0.2759	-0.2281	E	0.21
BR1379	0.5455	-0.1691	E	0.10
BR1436	0.238	0.0134	E	0.19
CV1200	0.5238	0.1428	E	0.13
CV1421	0.536	-0.0281	E	0.03
CV1435	0.5177	-0.0904	E	0.04
CV1655	0.6644	-0.0601	E	0.12
CV1685	1.2396	0.0051	E	0.53
LD0928	0.1845	-0.1644	E	0.24
VZ1178	0.2105	-0.1078	E	0.21

Tab. 4. Discriminant Analysis results. Wilks' Lambda: 0.066 approx. *F* (96,21)=2.18, *p* <0.001.

Environ. variable	Wilks'	Partial	*F*-remove	*p*-level	Toler.	1-Toler.
Altitude	0.086	0.772	3.907	0.007	0.229	0.771
pH	0.087	0.756	4.284	0.004	0.173	0.827
% cobbles	0082	0.806	3.195	0.020	0.334	0.666
% stones	0.087	0.763	4.112	0.006	0.385	0.615
Canopy cover	0.086	0.771	3.939	0.007	0.444	0.556
Regime	0.081	0.811	3.081	0.024	0.363	0.637

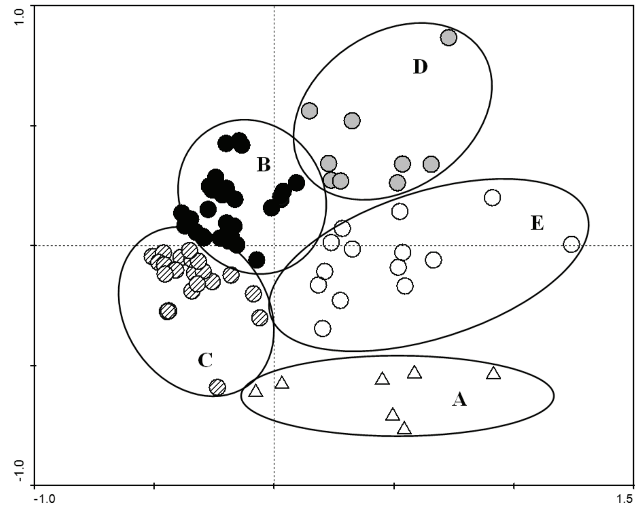

Fig. 7. Plot of springs in the plane of the first two PCA axes. Different colours and icons indicate different K-means clusters (A-E) of sites.

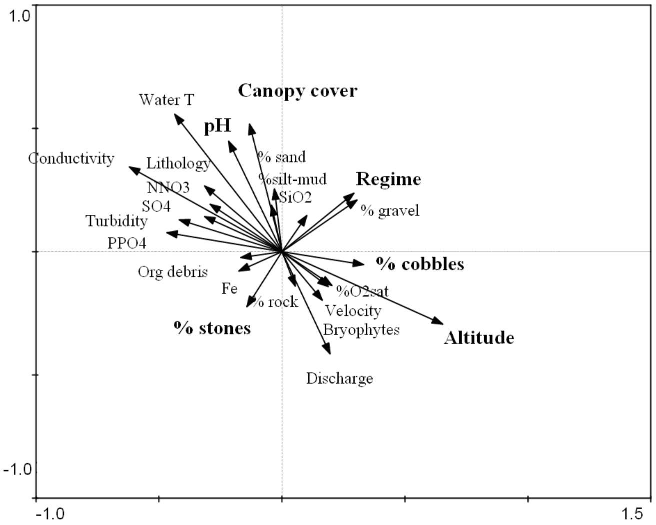

Fig. 8. Plot of environmental variables best correlated to site scores (axes 1 and 2) of PCA based on the chironomid communities living in the 81 sampled springs. In bold, the six variables selected by the Discriminant Analysis separating the 5 K-means clusters of sites.

This was also observed for the hyporheic zone of alpine streams, which are known to play a similar role for chironomids and other freshwater invertebrates (Lencioni *et al.* 2006).

Taxon richness and diversity had their maxima at intermediate altitudes (between about 900 and 2100 m a.s.l.), as noted by other authors (Orendt 2000a). As expected, the rheo-helocrene springs were the most species rich, being a mosaic of different niches (Lindegaard 1995; Cantonati *et al.* 2006; Sambugar *et al.* 2006). Many captured taxa were crenophilous, as defined by Thienemann (1954), Lindegaard (1995), Stur *et al.* (2005), Stur & Wiedenbrug (2006) and Novikmec *et al.* (2007) (Tab. 2). Lindegaard (1995) only defined one of these species, *Macropelopia fittkaui*, as a true crenobiont. However, according to Stur & Wiedenbrug (2006), due to the difficulty of recognising true crenobiont species within the chironomids, it is preferable to consider crenophilous taxa and crenobionts together, as a single category.

On the basis of our results, some species might be defined as lentic, rheobiontic (including madicolous-hygropetric) and bryophilous, based on their occurrence in specific habitats/spring types (see Tab. 2). Our findings generally confirmed what was already known for such taxa, albeit with some exceptions. For example, *Macropelopia notata* was found associated with the rheo-hygropetric spring type, but was previously reported as typical of moss-rich helocrenes (Stur *et al.* 2005); *Limnophyes asquamatus* and *Corynoneura scutellata* occurred as madicolous and rheobionts respectively, not as ubiquists (Lindegaard 1995); *Ortho-*

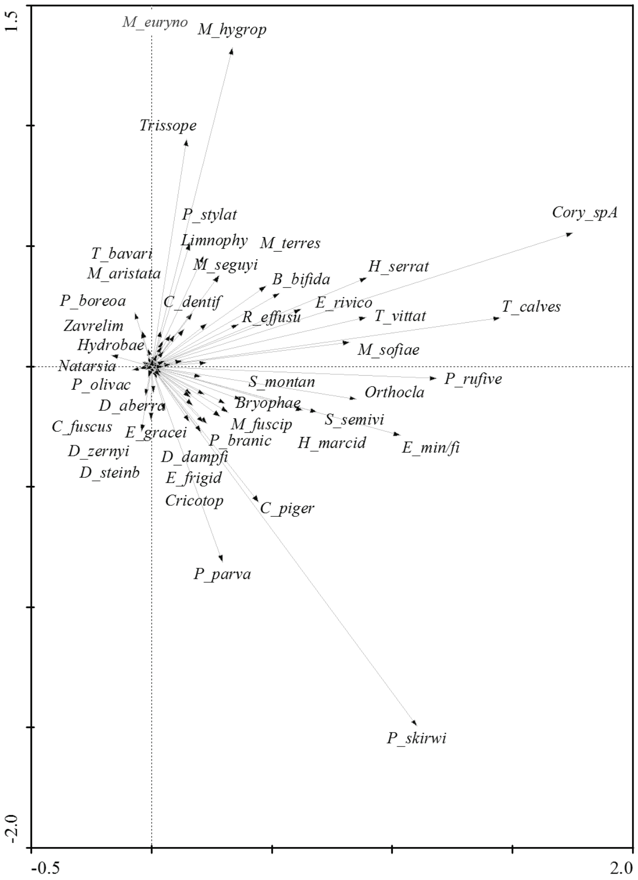

Fig. 9. Plot of species in the first two PCA axes. Only the names of the taxa accounting for the largest quote of variance are reported.

cladius (*Eudactylocladius*) *fuscipes* was not associated with bryophytes as previously reported (Lindegaard 1995; Stur & Wiedenbrug 2006); *Paraphaenocladius impensus* occurred as a rheobiont, but has been known as terrestrial/madicolous (Lindegaard 1995; Stur & Wiedenbrug 2006).

More than 50% of species are new for Italian springs and one is new to Italy, highlighting the current poor knowledge of the fauna in Italian springs (Lencioni 2007).

With respect to their the trophic role, Orthocladiinae (grazing organisms) were particularly common in bryophytes, Diamesinae were associated with coarse substrata, expected from their rheophilous habit. The predators or omnivores (Tanypodinae) were present in all microhabitat types, while the collectors (Tanytarsini, Prodiamesinae and Chironomini) were abundant in sediments.

The relationships observed between the distribution and abundance of common and rare species suggests that the chironomid fauna cannot be considered nested, even if some level of nestedness was highlighted and a few hotspots of chironomid biodiversity were found (e.g., the rheocrene spring CV1685 Campigol dei Solai accounted for 27 taxa, including 100% of the most common species and 13% of the rarest). Most taxa were found at a small proportion of the sites and frequency categories declined gradually for more widely distributed species. This distribution pattern has also been observed for blackflies and other aquatic insects from montane freshwater systems (Malmqvist *et al.* 1999; Lencioni *et al.* 2007b).

Species were distributed according to altitude, substratum composition, pH and canopy cover (and to those factors significantly correlated with them). These four factors were previously reported as determining the composition of macroinvertebrate assemblages (e.g., Glazier 1991; Smith *et al.* 2001; von Fumetti *et al.* 2006). However, as only 37.4% of the total variance was explained by the four principal canonical axes, other environmental factors may be important, such as competition and predation (Hahn 2000). No clear association with basin geology was highlighted, apart from the indirect link *via* positive correlations with pH and conductivity.

As observed by other authors (e.g., Smith *et al.* 2001), a transition from one spring type to another appears to be gradual, and there is almost a continuum between "traditional" types (most types were distributed in all K-means clusters).

Finally, as expected (Smith & Wood 2002; Smith *et al.* 2003; von Fumetti *et al.* 2006), intermittent springs, including three rock glaciers (AD2314 Amola, AD2739 Maroccaro, OC2792 Val di Pejo), hosted fewer taxa than the permanent ones, and none of the taxa was exclusive to such springs.

In conclusion, the highly individual nature of the springs was evident; within the same river basin, within a spring and between different springs. This suggests that prudent and conservative land management should assume that all springs need protection to conserve their faunal assemblages. Deeper knowledge of species autecology is needed to assess and monitor the ecological status of springs. Therefore, studying the distribution and dynamics of the characteristic spring fauna will help to identify the most appropriate measures to mitigate adverse man-made effects on springs, to which, due to their small spatial extent, they are extremely vulnerable.

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REFERENCES

APHA, AWWA & WEF. 2005. *Standard methods for the examination of water and wastewater*. 21th edition of the American Public Health Association 1015, 15th Street, NW, Washington D.C.: 1368 pp.

Bonettini, A.M. & M. Cantonati. 1998. Il macrozoobenthos. In: M. Cantonati (Ed.), *Le sorgenti del parco Adamello-Brenta. Ricerche idrobiologiche su fonti non captate*. Parco Documenti, 11: 125-143.

Bornhauser, K. 1912. Die Tierwelt der Quellen in der Umgebung Basels. *Int. Rev. Ges. Hydrobiol., Biologische Supplement*, 5: 1-90.

Cantonati, M., E. Bertuzzi & D. Spitale. 2007. *The spring habitat: biota and sampling methods*. Monografie del Museo Tridentino Scienze Naturali, 4: 1-350.

Cantonati, M., R. Gerecke & E. Bertuzzi. 2006. Springs of the Alps, sensitive ecosystems to environmental change: from biodiversity assessments to long-term studies. *Hydrobiologia*, 562: 59-96.

Crema, S., U. Ferrarese, D. Golo, P. Modena, B. Sambugar & R. Gerecke. 1996. *Ricerche sulla fauna bentonica ed interstiziale di ambienti sorgentizi in area alpina e prealpina*. Centro di Ecologia Alpina, Report N. 8: 1-104.

Ferrarese, U. 1983. Chironomidi, 3 (Diptera, Chironomidae: Tanypodinae). In: S. Ruffo (Ed.), *Guide per il riconoscimento delle specie animali delle acque interne italiane*, C.N.R. AQ/1/204, N. 26: 1-67.

Ferrarese, U. 2006. I Ditteri. In: B. Sambugar, G. Dessi, A. Sapelza, A. Stenico, B. Thaler & A. Veneri (Eds.), *Fauna sorgentizia in Alto Adige*. Provincia Autonoma di Bolzano - Alto Adige: 335-337.

Ferrarese, U. & B. Rossaro. 1981. Chironomidi, 1 (Diptera, Chironomidae: generalià, Diamesinae, Prodiamesinae). In: S. Ruffo (Ed.), *Guide per il riconoscimento delle specie animali delle acque interne italiane*, C.N.R. AQ/1/129, N. 12: 1-97.

Füreder, L., C. Schütz, M. Wallinger & R. Burger. 2001. Physico-chemistry and aquatic insects of a glacier-fed and a spring-fed alpine stream. *Freshwat. Biol.*, 46: 1673-1690.

Gerecke, R., M. Cantonati, D. Spitale, E. Stur & S. Wiedenbrug. 2011. The challenges of long-term ecological research in springs in the northern and southern Alps: indicator groups, habitat diversity, and medium-term change. In: M. Cantonati, R. Gerecke, I. Jüttner & E.J. Cox (Eds), *Springs: neglected key habitats for biodiversity conservation*. J. Limnol. 70(Suppl. 1): 168-187.

Gerecke, R., C. Meisch, F. Stoch, F. Acri & H. Franz. 1998. Eucrenon-hypocrenon ecotone and spring typology in the Alps of Berchtesgaden (upper Bavaria, Germany). A study of Microcrustacea (Crustacea: Copepoda, Ostracoda) and water mites (Acari: Halacaridae, Hydrachnellae). In: L. Botosaneanu (Ed.), *Studies in crenobiology. The biology of springs and springbrooks*. Backhuys Publishers, Leiden: 167-182.

Glazier, D.S. 1991. The fauna of north American temperate cold springs: patterns and hypothesis. *Freshwat. Biol.*, 26: 527-542.

Janecek, B.F.R. 1998. *Diptera: Chironomidae (Zuckmücken) – Larven. Fauna Aquatica Austriaca, Taxonomie und Ökologie aquatischer wirbelloser Organismen (Teil V)*. Universität für Bodenkultur, Abt. Hydrobiologie (Ed.), Wien: 1-128.

Hahn, H.J. 2000. Studies on classifying of undisturbed springs in Southwestern Germany by macrobenthic communities. *Limnologica*, 30: 247-259.

Klein, B. & K. Tockner. 2000. Biodiversity in springbrooks of glacial flood plain (Val Roseg, Switzerland). *Verh. int. Ver. Theor. Angew. Limnol.*, 27: 704-710.

Lafont, M. & A. Durbec. 1990. Essai de description biologique des interactions entre eau de surface et eau souterraine: vulnérabilité d'un aquifère a la pollution d'un fleuve. *Ann. Limnol.*, 26: 119-129.

Lencioni, V. 2007. Chironomids (Diptera, Chironomidae) in alpine and pre-alpine springs. In: E. Bertuzzi & M. Cantonati (Eds), *The spring habitat: biota and sampling methods*. Monografie del Museo Tridentino Scienze Naturali, 4: 247-264.

Lencioni, V. & B. Rossaro. 2005. Microdistribution of chironomids (Diptera: Chironomidae) in Alpine streams: an autecological perspective. *Hydrobiologia*, 533: 61-76.

Lencioni, V., L. Marziali & B. Rossaro. 2007a. I ditteri Chironomidi: morfologia, tassonomia, ecologia, fisiologia e zoogeografia. *Quaderni del Museo Tridentino di Scienze Naturali*, 1: 1- 172.

Lencioni, V., B. Rossaro & B. Maiolini. 2007b. Alpine chironomid distribution: a mere question of altitude? In: T. Andersen (Ed.), *Contributions to the Systematics and Ecology of Aquatic Diptera - A Tribute to Ole A. Sæther*, The Caddis Press, Ohio USA: 165-180.

Lencioni, V., B. Maiolini, R. Fochetti, M. Grasso, A. Boscaini & E. Dumnicka. 2006. Artificial substrate colonization by invertebrates in two high altitude Alpine streams. *Verh. int. Ver. Theor. Angew. Limnol.*, 29: 1866-1870.

Lindegaard, C. 1995. Chironomidae of European cold springs and factors influencing their distribution. *Journal of Kansas Entomological Society*, 68 (2): 108-131.

Malmqvist, B., Y. Zhang & P.H. Adler. 1999. Diversity, distribution and larval habitats of North Swedish blackflies (Diptera: Simuliidae). *Freshwat. Biol.*, 42: 301-314.

Marziali, L., V. Lencioni & B. Rossaro. 2010. The chironomids (Diptera: Chironomidae) from 108 Italian Alpine springs. *Verh. Internat. Ver. Limnol.*, 30: 1467-1470.

Nadig, A. 1942. Hydrobiologische Untersuchungen in Quellen des Schweizerischen Nationalparkes im Engadin (unter besonderer Berücksichtigung der Insektenfauna). *Ergebnisse der wissenschaftlichen Untersuchungen im Schweizerischen Nationalpark, Aarau (N.F.)*, 1: 270-432.

Nocentini, A. 1985. Chironomidi, 4 (Diptera, Chironomidae: Chironominae, larve). In: S. Ruffo (Ed.), *Guide per il riconoscimento delle specie animali delle acque interne italiane*, C.N.R. AQ/1/233, N. 29: 186 pp.

Novikmec, M., M. Svitok & P. Bitušík (Eds). 2007. *Limnology of streams in the Poloniny National Park (The East Carpathians, Slovakia)*. Technical University in Zvolen: 69 pp.

Orendt, C. 2000a. Chironomids of small Alpine water bodies (springs, spring brooks, pools, small lakes) of the northern Calcareous Alps. *Spixiana*, 23: 121-128.

Orendt, C. 2000b. The chironomid communities of woodland springs and spring brooks, severely endangered and impacted ecosystems in a lowland region of eastern Germany (Diptera: Chironomidae*). Journal of Insect Conservation*, 4: 79-91.

Pinder, C.L. 1978. *A key to adult males of British Chironomidae*. Freshwater Biology Association, Vol. 1: 169 pp.

Rossaro, B. 1982. Chironomidi, 2 (Diptera, Chironomidae: Orthocladiinae). In: S. Ruffo (Ed.), *Guide per il riconoscimento delle specie animali delle acque interne italiane*, C.N.R. AQ/1/171, N. 16: 80 pp.

Rossaro, B. & R. Bettinetti 2001. Chironomid distribution in north-western Italian glacial streams and cold springs. *Verh. int. Ver. Theor. Angew. Limnol.*, 27: 2388-2391.

Rossaro, B., V. Lencioni & L. Marziali. 2009. *Micropsectra sofiae* Stur & Ekrem, 2006 (Diptera, Chironomidae) redescribed. *Bollettino di Zoologia agraria e Bacchicoltura*, Ser. II, 41 (2): 129-139.

Rossaro, B., V. Lencioni & S. Mietto. 2000. Chironomids distribution in glacial streams and cold springs. In: O. Hoffrichter (Ed.), *Proceedings of "Late 20th Century Research on Chironomidae: an Anthology from the 13th International Symposium on Chironomidae"*. Shaker Verlag, Aachen: 393-403.

Sambugar, B., G. Dessì, A. Sapelza, A. Stenico, A. Thaler & A. Veneri. 2006. *Fauna sorgentizia in Alto Adige*. Provincia Autonoma di Bolzano - Alto Adige: 1-372.

Schmid, P.E. 1993. A key the larval Chironomidae and their instars from Austrian Danube region. Streams and rivers. *Wasser und Abwasser*, Supplement 3/93: 514 pp.

Serra-Tosio, B. 1971. *Contribution à l étude taxonomique, phylogénétique, biogéographique et écologique des Diamesini (Diptera, Chironomidae) d'Europe*. Doctoral thesis University of Grenoble, France, T-I:1-303, T-II: 304-462.

Shannon, C.E. & W. Weaver. 1949. *The mathematical theory of communication*. Univ. Illinois Press, Urbana.

Smith, H. & P.J. Wood. 2002. Flow permanence and macroinvertebrate community variability in limestone spring systems. *Hydrobiologia*, 487: 45-48.

Smith, H., P.J. Wood & J. Gunn. 2001. The macroinvertebrate communities of limestone springs in the Wye Valley, Derbyshire Peak District, UK. *Cave and Karst Sciences*, 28: 67-78.

Smith, H., P.J. Wood & J. Gunn. 2003. The influence of habitat structure and flow permanence on invertebrate communities in karst spring systems. *Hydrobiologia*, 510: 53-66.

StatSoft Inc. 2007. STATISTICA (data analysis software system), version 8.0. www.statsoft.com.

Stur, E. & T. Ekrem 2006. A revision of West Palaearctic species of the Micropsectra atrofasciata species group (Diptera: Chironomidae). *Zoological Journal of the Linnean Society*, 146: 165-225.

Stur, E. & S. Wiedenbrug. 2006. Familie Zuckmücken (Chironomidae). In: R. Gerecke & H. Franz (Eds), *Quellen im Nationalpark Berchtesgaden. Lebensgemeinschaften als Indikatoren des Klimawandels*. Nationalpark Berchtesgaden Forschungsbericht, 51: 183-194.

Stur, E., S. Wiedenbrug & H. Franz. 2002. Chironomid communities (Diptera, Chironomidae) in cold Alpine springs. Prospect for a long-term biomonitoring. In: Yeates (Ed.), *Abstracts 5th International Congress Dipterology*. Brisbane: 230.

Ter Braak, C.J.F. & P. Šmilauer. 2002. *CANOCO Reference manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5)*. Microcomputer Power, Ithaca, NY, USA.

Thienemann, A., 1954. *Chironomus*. Leben, Verbreitung und wirtschaftliche Bedeutung der Chironomiden. *Die Binnengewässer*, 20: 834 pp.

von Fumetti, S., P. Nagel, N. Scheifhacken & B. Baltes. 2006. Factors governing macrozoobenthic assemblages in perennial springs in north-western Switzerland. *Hydrobiologia*, 568: 467-475.

Wiederholm, T. 1983. *Chironomidae of the Holoarctic region. Keys and diagnoses. Part. 1. Larvae*. Entomologica Scandinavica, Supplement 19: 457 pp.

Wiederholm, T. 1986. *Chironomidae of the Holarctic region. Keys and diagnoses. Part. 2. Pupae*. Entomologica Scandinavica, Supplement 28: 482 pp.

Wiederholm, T. 1989. *Chironomidae of the Holarctic region. Keys and diagnoses. Part 3. Adult males*. Entomologica Scandinavica, Supplement 34: 532 pp.

Zavřel, J. & F. Pax. 1951. Die Chironomidenfauna mitteleuropäischer Quellen. *Arch. Hydrobiol. Supplement*, 18: 655-677.

APPENDIX

Appendix 1. Environmental variables included in the data analyses. The first five are dummy variables: Lithology= siliceous (0), limestone (1); Canopy cover= 0% (0), 0-25% (1), 25-50% (2), 50-75% (3), 75-100% (4); Bryophytes= 0, 1, 2….10 (quantitative); Organic debris= 0, 1, 2, 3, 4 (quantitative); Regime= permanent (1), intermittent (0). dl= detection level.

Spring code	Altitude (m a.s.l.)	Lithology	Canopy cover	Bryophytes	Organic debris	Regime	% gravel	% cobbles	% sand	% silt	% stones	% rock	Velocity (cm s^{-1})	Discharge (L s^{-1})	Turbidity (NTU)	Water Temp. (°C)	% O_2 saturation	pH	Cond. (µS cm^{-1})	SO_4^{2-} (mg L^{-1})	N-NO_3 (µg L^{-1})	P-PO_4 (µg L^{-1})	SiO_2 (mg L^{-1})	Fe (µg L^{-1})
AD0905	905	1	3	3	1	1	20	20	30	15	15	0	15	7.0	0.29	8.6	72	8	232	8	737	2	5.4	<dl
AD1077	1077	1	3	6	1	1	15	35	20	10	20	0	30	3.0	0.52	6.5	90	8	393	15	741	1	3.7	<dl
AD1235	1235	0	4	5	0	1	25	20	25	10	20	0	20	0.9	0.04	6.3	94	8	198	4	973	10	6.4	<dl
AD1300	1300	0	4	7	1	1	30	35	5	10	20	0	50	12.0	0.06	5.9	95	7	35	2	1224	3	6.1	6
AD1665	1665	0	1	6	0	1	15	30	10	5	35	5	80	95.0	0.07	5.7	97	7	29	1	354	1	4.6	<dl
AD1790	1790	0	2	6	2	1	30	0	40	30	0	0	10	0.7	0.40	4.8	96	7	28	2	297	3	8.0	<dl
AD2314	2314	0	2	7	2	0	10	30	15	15	30	0	30	0.5	0.15	1.0	95	6	19	1	1137	1	3.6	<dl
AD2739	2739	0	0	0	0	0	5	10	5	0	80	0	21	3.5	0.44	0.8	105	7	26	1	549	3	2.1	<dl
LD0584	586	1	4	3	2	1	15	45	15	10	15	0	30	4.5	0.24	8.7	88	8	313	6	1690	1	2.1	<dl
LD0720	720	1	5	9	0	1	10	0	30	50	0	0	5	0.1	2.95	10.1	85	8	392	10	768	3	7.1	<dl
LD0928	928	1	3	4	0	1	20	45	20	15	0	0	7	0.1	0.17	7.8	81	8	354	3	815	2	1.9	<dl
LD0930	930	1	5	5	0	1	30	10	5	0	5	50	40	0.5	0.09	7.0	79	8	229	2	597	2	4.1	<dl
LD1400	1400	1	4	0	1	1	25	40	10	10	15	0	5	0.1	0.28	6.8	76	8	271	7	1467	2	3.5	<dl
LD1502	1502	1	2	7	0	1	5	10	5	0	30	50	10	0.04	0.98	6.5	78	8	216	1	1117	2	1.9	<dl
AN0430	430	0	4	3	0	0	0	40	5	35	20	0	5	0.1	0.50	11.7	65	8	473	20	1890	48	6.3	<dl
AN1000	1000	1	2	0	0	1	20	20	10	5	20	25	40	0.6	0.56	7.1	90	8	271	7	772	1	5.9	<dl
AN1474	1474	1	3	9	0	1	20	5	55	20	0	0	10	0.5	0.53	7.1	83	8	345	5	562	1	2.3	<dl
AN1578	1578	0	1	5	4	1	30	0	30	40	0	0	10	0.2	0.46	7.2	70	7	49	3	55	2	5.1	<dl
AN1950	1950	0	2	9	0	1	5	50	10	10	25	0	20	4.0	0.23	3.9	89	6	24	4	327	2	4.3	<dl
BR0470	470	1	4	8	0	1	5	25	5	0	25	40	100	30.0	0.64	7.1	100	8	269	1	427	3	0.9	<dl
BR0658	658	1	4	4	0	1	30	10	50	10	0	0	13	0.5	1.47	14.2	81	8	362	2	159	2	6.2	3
BR0679	679	1	2	8	0	1	35	35	5	0	20	5	30	7.0	0.07	8.7	95	8	267	2	272	6	2.8	<dl
BR0790	790	1	4	9	0	1	20	10	10	30	0	30	20	1.0	0.12	7.1	92	8	368	3	318	6	3.3	<dl
BR0950	950	1	5	9	0	1	30	20	15	15	20	0	15	1.5	0.36	7.0	90	8	262	17	884	3	7.2	<dl
BR1315	1315	1	3	6	3	1	15	40	20	10	15	0	15	1.0	0.11	5.4	88	8	225	3	738	1	1.9	<dl
BR1358	1358	1	3	4	0	1	20	30	15	5	25	0	28	20.0	0.12	5.7	89	8	239	17	625	2	2.8	<dl
BR1379	1379	1	1	9	0	1	20	70	0	0	10	0	30	1.5	0.63	4.2	91	8	212	3	746	1	1.2	<dl
BR1436	1436	1	3	6	0	1	20	20	0	0	15	45	20	0.5	0.20	5.7	94	8	179	5	716	1	1.3	9
BR1605	1605	1	2	5	1	1	15	30	5	10	10	30	5	0.3	0.62	7.0	93	8	241	2	238	1	1.1	<dl
BR1765	1765	1	3	8	0	1	20	60	5	0	15	0	40	4.5	0.22	4.4	93	8	221	1	229	3	1.6	<dl
CS1350	1350	1	2	5	0	1	45	20	0	0	35	0	30	1.0	0.60	7.8	77	8	301	7	893	13	3.1	<dl
CA1642	1642	0	1	9	0	1	50	0	10	40	0	0	1	1.5	0.48	6.3	79	6	19	3	343	1	3.8	<dl
CA2153	2153	0	1	7	0	1	5	30	0	0	30	35	35	0.7	0.11	5.5	79	7	16	1	324	1	3.7	<dl
VZ1178	1178	1	4	7	3	1	25	20	5	15	30	5	15	2.0	0.03	5.7	72	8	230	29	365	1	6.6	<dl
MC1115	1115	1	4	9	0	1	20	5	20	10	45	0	10	0.3	0.16	10.0	65	8	120	4	92	6	5.5	<dl
PS1255	1255	1	4	7	0	1	20	0	30	50	0	0	6	0.3	0.14	7.3	35	8	2120	1368	112	1	5.8	<dl
PS1880	1880	1	1	1	0	1	0	80	0	0	15	5	35	7.0	0.02	3.4	86	8	207	6	247	5	2.6	<dl
CV0250	250	1	4	3	0	1	40	10	40	10	0	0	20	3.0	0.04	11.5	63	8	319	24	902	5	5.8	<dl
CV0854	854	1	4	9	0	1	0	45	0	0	5	50	5	0.01	6.90	9.1	74	8	477	27	2853	2	6.3	<dl
CV0962	962	0	5	3	0	1	5	30	5	10	50	0	40	0.5	0.49	11.7	65	7	44	3	108	2	12.5	<dl
CV0992	992	1	4	7	0	1	55	20	0	0	15	10	25	1.0	0.32	8.0	86	8	299	4	1175	13	3.9	<dl
CV1084	1084	1	5	0	0	1	10	60	10	15	5	0	5	0.1	0.99	9.2	88	8	166	37	621	3	7.6	<dl
CV1200	1200	0	3	3	0	1	10	45	5	5	10	25	35	3.0	0.40	7.2	91	7	64	2	361	7	10.7	<dl
CV1280	1280	0	5	6	0	1	55	20	10	10	0	0	15	4.0	0.34	5.7	78	8	157	25	504	1	6.6	<dl
CV1421	1421	0	4	6	0	0	40	30	0	0	25	5	30	1.0	0.06	6.2	85	7	29	4	250	1	3.1	<dl
CV1433	1433	1	1	9	0	1	0	0	0	0	0	100	5	3.5	25.6	9.1	89	6	1239	409	20	11	10.7	14210
CV1435	1435	0	3	8	0	1	15	25	50	10	0	0	25	5.0	0.06	4.8	93	7	35	2	717	6	5.1	<dl
CV1575	1575	0	2	7	0	1	20	0	40	40	0	0	1	1.0	0.25	5.1	81	7	28	3	293	2	5.7	<dl

(continued)

Appendix 1. Continuation.

Spring code	Altitude (m a.s.l.)	Lithology	Canopy cover	Bryophytes	Organic debris	Regime	%gravel	%cobbles	%sand	%silt	%stones	%rock	Velocity (cm s^{-1})	Discharge (L s^{-1})	Turbidity (NTU)	Water Temp. (°C)	% O_2 saturation	pH	Cond. (µS cm^{-1})	SO_4^{2-}(mg L^{-1})	N-NO_3 (µg L^{-1})	P-PO4 (µg L^{-1})	SiO2 (mg L^{-1})	Fe (µg L^{-1})
CV1623	1623	0	4	3	0	1	50	20	20	10	0	0	10	1.0	0.05	6.3	79	7	62	2	231	7	9.9	12
CV1655	1655	0	2	8	0	1	40	15	0	25	0	0	10	0.1	0.09	4.7	89	7	46	6	772	1	4.7	<dl
CV1685	1685	0	4	9	0	1	35	45	0	5	0	15	10	1.5	0.15	4.9	84	7	43	3	165	3	7.9	<dl
CV1855	1855	0	1	0	0	1	50	20	20	10	0	0	15	0.2	0.70	6.6	65	7	30	2	298	1	7.5	<dl
CV2182	2182	0	1	6	1	1	25	10	10	10	5	40	25	3.0	0.23	1.0	79	7	37	1	347	4	4.6	<dl
LT1240	1240	1	4	3	0	1	65	10	15	0	10	0	10	1.0	2.84	8.0	80	8	568	154	254	4	4.7	<dl
MD1670	1670	1	4	6	0	1	40	35	5	10	10	0	15	0.5	0.13	4.4	95	8	223	7	605	1	5.2	<dl
MD1871	1871	1	2	7	0	1	10	10	0	0	10	70	5	3.0	0.54	4.0	83	8	144	1	287	1	0.6	<dl
OC2056	2056	0	4	7	0	1	40	40	0	10	10	0	30	2.0	0.04	4.5	81	7	60	11	108	4	9.8	<dl
OC2792	2792	0	1	3	0	0	0	10	10	5	70	5	15	6.0	0.55	2.1	93	7	136	54	163	1	3.6	<dl
PG0453	453	1	5	0	0	1	35	30	5	5	5	20	20	2.0	0.19	9.3	83	8	240	2	654	1	2.0	<dl
SL1724	1724	1	4	9	0	1	5	65	5	10	15	0	8	3.0	0.34	4.3	80	8	207	7	543	3	1.6	54
AT0972	972	1	5	3	0	1	35	25	10	15	15	0	3	0.2	0.28	9.5	80	8	413	8	1230	4	4.7	<dl
MB0335	335	1	5	9	0	1	0	50	0	0	0	50	30	4.0	0.07	9.8	95	8	341	6	1271	14	4.0	<dl
MB0385	385	1	0	8	0	1	0	5	0	0	5	90	21	6.5	0.48	11.8	80	8	266	5	2257	11	4	100
MB0445	445	1	2	4	0	1	30	50	0	0	20	0	15	0.5	0.48	11.9	83	8	485	5	2257	12	3.7	30
MB0517	517	1	4	7	2	1	10	20	10	10	30	20	40	0.6	0.48	8.4	90	8	360	4	1129	12	3.7	<dl
MB1440	1440	1	2	4	0	1	40	20	10	0	30	0	7	0.2	0.88	6.6	87	8	261	4	1823	8	3.2	<dl
BS0705	705	1	5	7	0	1	0	45	30	5	0	10	10	0.3	0.28	8.6	67	8	393	7	304	1	5.2	<dl
BS1527	1527	1	2	7	0	1	25	25	10	0	40	0	10	0.5	0.15	8.3	77	7	354	2	265	4	4.6	<dl
BC0170	170	1	3	3	0	0	5	10	25	10	50	0	15	1.0	0.17	12.4	78	8	234	7	2094	15	3.6	<dl
BC0503	503	1	4	0	0	1	15	45	15	25	0	0	1	0.5	0.14	9.0	80	8	241	10	333	2	5.6	<dl
BC0565	565	1	2	5	0	1	35	25	15	10	15	0	15	0.8	1.66	7.1	83	8	204	5	803	7	3.7	<dl
ML0533	533	1	3	8	1	1	25	50	0	0	25	0	5	0.2	0.50	10.1	80	8	580	4	1257	7	2.8	<dl
ML0580	580	1	3	5	3	0	5	10	0	10	50	25	4	0.1	0.50	3.1	90	8	320	4	1257	7	2.9	<dl
ML0950	950	1	2	4	3	0	0	35	0	5	25	35	5	0.1	0.50	8.9	83	8	590	4	1257	7	2.9	<dl
MP0656	656	1	5	3	0	1	35	45	0	0	20	0	50	2.3	0.61	7.7	77	8	297	35	659	3	2.1	<dl
MP0676	676	1	2	8	1	1	50	0	30	20	0	0	40	1.0	0.69	8.0	80	8	300	25	677	3	2.1	<dl
MP0690	690	1	2	7	4	1	45	20	20	10	5	0	40	1.0	0.69	8.9	77	8	310	13	677	3	2.1	<dl
MP0924	924	1	3	7	2	1	10	55	5	0	30	0	16	0.7	0.69	6.8	75	8	480	86	903	3	2.1	<dl
MP1566	1566	1	1	3	1	1	15	0	5	5	0	70	15	0.2	0.76	5.5	77	8	202	13	916	1	1.7	<dl
SC0250	250	1	4	3	0	1	35	10	40	10	5	0	50	10.0	0.24	7.8	88	8	261	3	703	4	2.9	<dl
VF0745	745	1	4	5	0	1	10	20	0	0	30	40	25	0.3	0.01	11.0	87	8	340	4	915	1	1.9	<dl