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Chironomids as bioindicators of environmental quality in mountain springs

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Abstract. We analyzed responses of chironomid (Diptera:Chironomidae) communities to environmental factors in 124 natural, moderately, and highly disturbed springs in the Italian Prealps and Alps to investigate environmental factors influencing species distribution in springs and to evaluate chironomids as bioindicators of spring water quality. Self-Organizing Map analysis found differences among spring types and effects of anthropogenic pressures. Natural or little-disturbed springs at high altitude with low temperature, low conductivity, and high current velocity differed from lowland springs, including highly disturbed ones, with low current velocity and higher temperature, conductivity, and nutrient concentrations. Cold stenothermal intolerant species were clustered in the 1st group, tolerant and euriecious species in the 2nd group. Indicator value analysis detected species characterizing springs with different degrees of disturbance and of different types. Most species' distributions were related to water temperature and conductivity. Coinertia Analysis (CoA) detected relationships among species structure and environmental variables. CoA axis 1 represented a gradient of water temperature, altitude, alkalinity, and conductivity and separated cold stenothermal species (*Pseudokiefferiella parva*, *Pseudodiamesa branickii*, *Diamesa* spp.) from species tolerant of high temperatures (*Polypedilum nubeculosum*, *Phaenopsectra flavipes*, *Paratrissocladius excerptus*). Axis 2 represented a hydrologic (rheocrene–limnocrene) and anthropogenic disturbance (total disturbance, agriculture, organic debris) gradient and separated species by preference for water velocity and spring type (rheocrenes: *Eukiefferiella* spp.; limnocrenes: *Prodiamesa olivacea*, *Natarsia* sp.), and pollution tolerance (*P. nubeculosum, Macropelopia* spp.). Water temperature and chemical composition affected chironomid distribution. Some species were associated with degraded (*P. nubeculosum*) or pristine conditions (*Diamesa* spp., *Stilocladius montanus*).

Key words: spring type, anthropogenic pressure, Orthocladiinae, Italy, Prealps, Alps.

Springs and their biota are good tools for monitoring changes in groundwater quality caused by human disturbance. Springs are extremely sensitive to disturbances, such as trampling by cattle, water captation, sedimentation, removal of the surrounding vegetation, and nutrient inputs, because of their small dimensions and the importance of the fringing semiaquatic habitats (Cantonati et al. 2006). Springs traditionally have been seen as clean and pristine environments of high biological integrity. However, this condition is becoming more and more unusual. In the Prealps and Alps, springs frequently are affected directly by water captation and forest management activities and indirectly by deposition of nutrients or contaminants in the drainage basin (e.g., S and N oxides, NH_4^+, trace metals, and pesticides), global warming, and increased ultraviolet radiation (Cantonati et al. 2006). Nevertheless, biotic indices have not yet been developed to assess their ecological status.

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Each spring has a mosaic structure. Despite their generally small size, springs consist of several of microhabitats (e.g., mosses, debris). Furthermore, substrate lithology influences spring water chemistry, hydrologic regime, and substrate dimensions and characteristics (van der Kamp 1995). This within- and among-spring variability leads to a patchy environment with fluctuating conditions and gives rise to small, isolated populations as described in the patch dynamics concept (Townsend 1989). Thus, each spring can be considered a unique habitat, and a prudent land management plan should be based on the assumption that all springs need protection to conserve their special faunal assemblages (Zollhöfer et al. 2000, Lencioni et al. 2011).

Among macroinvertebrates, chironomids (Diptera: Chironomidae) are a candidate as the best bioindicators of spring water quality because they typically dominate spring fauna in terms of abundance and species number (Lindegaard 1995, Gerecke et al. 1998, Stur and Wiedenbrug 2006). Chironomids are the most useful indicators of the quality of surface water and the upper layer of groundwater because the larvae are affected by organic content and trace metal load in the sediments (Lafont and Durbec 1990). Nevertheless, knowledge of the autecology, geonemy, and phenology of spring-dwelling chironomid species is still fragmentary compared to knowledge for other aquatic habitats or of other insect orders (Orendt 2000a, b). The few faunistic investigations of springs and spring-fed brook ecosystems in Italian Prealps and Alps were mostly conducted over the last 15 y (Bonettini and Cantonati 1996, Crema et al. 1996, Rossaro et al. 2000, Rossaro and Bettinetti 2001, Sambugar et al. 2006, Lencioni 2007, Marziali et al. 2010). Lists of chironomid species were given but the influence of chemical, hydrological, and geomorphological factors on the fauna has been little investigated.

Chironomids and other macroinvertebrate assemblages in springs have been analyzed with multivariate statistical approaches, e.g., nonmetric multidimensional scaling (von Fumetti et al. 2006, Novikmec et al. 2007) or canonical correspondence analysis (Marziali et al. 2010) but not, to our knowledge, by means of Artificial Neural Networks (ANNs). Unsupervised and supervised ANNs have been used by several authors to pattern the structure of benthic communities from running waters and to understand communities with respect to environmental features in an attempt to develop predictive models (Céréghino et al. 2001, Schleiter et al. 2001, Park et al. 2003, Lencioni et al. 2007). In our study, relationships between chironomids and environmental variables (substrate composition, water temperature, nutrient concentration, and other physical, chemical, and hydrogeomorphological variables) were explored with Self-Organizing Map analysis (SOM; Kohonen 1982, Giraudel and Lek 2001), species Indicator Value (IndVal; Dufrêne and Legendre 1997), and Coinertia Analysis (CoA; Dolédec and Chessel 1994). The aim was to investigate which environmental factors influence species distribution in springs and to evaluate chironomids as bioindicators of spring water quality.

Methods

The springs

A total of 124 springs in the Italian Prealps (31) and Alps (93) (Trentino and Veneto regions, northeastern Italy; lat 46°N, long 10–11°E) were investigated. They lie in 5 siliceous and 18 carbonate basins over a wide altitudinal range (62–2792 m asl) and belong to 7 hydromorphological types: rheocrene (81), helocrene (3), limnocrene (11), hygropetric (4), rheohelocrene (12), rheohygropetric (6), and rheolimnocrene (7). Six springs are mineral and 15 intermittent. Eighty springs are natural sites (undisturbed), 30 are moderately disturbed (pasture or water captation), and 14 are highly disturbed (water captation, modified/concreted bed).

Environmental variables

Each spring was surveyed once between May and November 2005 (Alps) or 2007–2008 (Prealps), and environmental variables, including those used for landscape classification, were recorded. Altitude was measured by global positioning system (instrument error $\approx$ 10–15 m). Percent grain-size composition of the substrate was evaluated by visual assessment as % stones/rocks (>20 cm), cobbles (5 $\pm$ 20 cm), gravel (0.2 $\pm$ 5 cm), sand (0.01 $\pm$ 0.2 cm), and silt/mud (<0.01 cm).

Water samples were collected in acid-cleaned graduated bottles for chemical analysis (conductivity, alkalinity, hardness, dissolved O_2, % O_2 saturation, pH, nutrients, anions, cations, and metals) by standard methods (APHA 2005). Water temperature was measured with a field multiprobe (Hydrolab 20; HACH Hydromet, Loveland, Colorado). Discharge was measured using a graduated bucket, and measurements were repeated in different areas of the spring. Mean current velocity was measured with an acoustic Doppler velocimeter (FlowTracker Handheld-ADV; SonTek, San Diego, California). Turbidity was measured with a portable turbidimeter (Micro-TPI; HF®scientific, Fort Myers, Florida). Canopy cover (shading) was classified as a value ranging from 0 to

4: 0% (0), 1 to 25% (1), 26 to 50% (2), 51 to 75% (3), 76 to 100% (4). Other dummy variables were: bryophytes scaled from 0 to 9 (quantitative) and organic debris scaled from 0 to 4 (quantitative) with higher values meaning higher amounts; regime coded as permanent = 1 and intermittent = 0; mineral coded as 0 = no or 1 = yes; spring type coded as 0 = no or 1 = yes for each type; disturbance type (water captation, modified bed, pasture, agriculture, and roads), each scaled from 0 to 3 with higher values meaning higher disturbance; total disturbance (sum of disturbance types); and level of disturbance coded as 0 to 5: 1 = natural, 2 to 3 = moderately disturbed, and 4 to 5 = highly disturbed (Appendix 1, Table 1).

Chironomid sampling

Chironomid larvae were collected in the eucrenal zone (within 10 m of the spring source; Cantonati et al. 2006) of each spring. From 1 to 3 replicates/spring were collected from different substrate types, depending on spring morphology: 1) coarse substrate (>0.2 cm, gravel–stones), 2) fine substrate (<0.2 cm, sand–mud), and 3) submerged bryophytes. A pond net, constructed specifically for use in springs of very small size (10 × 10 cm = 0.01 m^2, 100-μm mesh size) was used for 30 s in coarse and fine substrate, and 50 mL of surface sediment were collected with a syringe (50 g). Animals living in submerged bryophytes were extracted from 50 g of bryophytes in the laboratory. Extra samples of pupae, pupal exuviae, and pharate adults were collected with tweezers and drift nets to confirm species identification. Samples were preserved in 75% ethyl alcohol. Chironomids were mounted on slides and identified to genus/species group/species with keys published by Ferrarese and Rossaro (1981), Rossaro (1982), Ferrarese (1983), Wiederholm (1983, 1986, 1989), Nocentini (1985), Schmid (1993), Janecek (1998), Langton and Visser (2003), and Langton and Pinder (2007).

Statistical analyses

Only larvae found in semiquantitative samples collected in the 3 main substrate types were considered for statistical analyses. 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 81 taxa.

Abundances were $\log(x + 1)$-transformed to reduce asymmetrical influence of the dominant taxa while ensuring that quantitative information was not lost. Forty-one environmental variables (of which 16 were dummy) were analyzed. Environmental variables, except pH and dummy variables, were $\log(x + 1)$-transformed. Percentage values were $\arcsin\sqrt{(x)}$-transformed for normalization. The Shannon Diversity Index was calculated (Shannon and Weaver 1949).

A SOM analysis (Kohonen 1982) was used to ordinate and classify sites according to chironomid community composition. This unsupervised neural network maps sites and species in 2 dimensions. Map size is critical. If it is too small, some important information can be lost, if too large, a detailed pattern of no ecological significance can appear. The optimum size is established by examining the quantization and the topographic error (Park et al. 2003). The resulting 2-dimensional map is composed by $r \times c$ cells, represented as hexagons in the map, and each cell is a cluster of sites. The number of sites included in a cell is bound to their faunal composition. Sites with a similar faunal composition are clustered in 1 cell. A k-mean clustering procedure is then done to cluster the sites into a smaller number of clusters (Trosset 2008). In the last step, environmental variables are included in the 2-dimensional maps (Park et al. 2003).

Indicator species analysis (IndVal; Dufrêne and Legendre 1997) was used to identify characteristic species for each spring type, different levels and types of disturbance, and different physicochemical features of springs. Four quantitative variables (water temperature, conductivity, NO_3–N, and current velocity) were coded in 5 classes from 1 to 5. Sixteen other variables were selected for use in IndVal as multistate variables (Table 1). Significance of the indicator value of each species was assessed by a randomization procedure (999 permutations).

Coinertia Analysis (CoA) allows mapping of sites in the space calculated from the environmental and faunal matrices (Dolédec and Chessel 1987, 1989, 1994, Dray et al. 2003, 2007, Chessel et al. 2004). The CoA factorial map explains the part of variability similar to each separate analysis. CoA was done to relate the 81 species with the 41 environmental variables and maximized the covariance between the 2 matrices prepared as sites × environmental variables and sites × chironomid species. The 2 coordinate systems were superimposed to demonstrate the relationship between the 2 matrices.

Various randomization procedures can be used to test the association between the environmental and the faunal matrices. In these procedures, the rows of a matrix are randomly permuted, a parameter measuring the association between the original and the permuted matrix is calculated, and the frequency distribution of the parameter plotted from simulated data is compared with the observed value of the parameter calculated from the original matrix. In the R^2 test, the parameter is the R^2 calculated between the

TABLE 1. List of environmental variables used in Coinertia and Indicator Value (IndVal) analyses. Temperature, current velocity, conductivity, and NO_3-N were coded into classes to be used in IndVal analysis. * indicates variable was used in IndVal analysis.

Variables	Value: Minimum	Value: Maximum	Class	Code
Coded variables				
Agriculture*	0	2		
Bryophyte*	0	9		
Water captation*	0	3		
Modified bed*	0	3		
Organic debris*	0	4		
Pasture*	0	2		
Roads*	0	1		
Shading*	0	4		
Total disturbance*	0	5		
Spring type				
Helocrene*	0	1		
Hygropetric*	0	1		
Limnocrene*	0	1		
Rheocrene*	0	1		
Rheohelocrene*	0	1		
Rheohygropetric*	0	1		
Rheolimnocrene*	0	1		
Sediment particle size				
% gravel	0	80		
% cobbles	0	85		
% sand	0	55		
% silt	0	70		
% stones	0	80		
% rock	0	100		
Quantitative variables				
Conductivity*	11	2120	10–50	1
			50–200	2
			200–300	3
			300–400	4
			400–2000	5
NO_3-N*	20	6885	2–250	1
			250–500	2
			500–750	3
			750–1100	4
			1100–7000	5
Temperature*	1	16	0.8–5	1
			5–6.5	2
			6.5–8	3
			8–10	4
			10–16	5
Velocity*	1	100	1–4.9	1
			5–10	2
			11–16	3
			17–30	4
			31–100	5
Alkalinity	0	8.67		
Altitude	62	2792		
Cl^-	0	23		
Discharge	0	120		
Dissolved organic C	0	9		
Fe	0	31		
Hardness	0	162		
SiO_2	1	16		
SO_4^{2-}	1	1368		
O_2	2	12		

TABLE 1. Continued.

Variables	Value		Class	Code
	Minimum	Maximum		
pH	3.07	8.34		
PO_4-P	0	47		
Total N	110	7400		
Total P	1	73		
Turbidity	0	26		

2 matrices (Chessel et al. 2004); in the Procrustean randomization test (PROtest; Jackson 1995), the parameter is the sum of the singular values of a Procrustean rotation of the faunistic and environmental matrix; and in the RV test (Heo and Gabriel 1998), the parameter is the sum of eigenvalues of a CoA.

Calculations were done in Matlab® (version R2011b; Matlab, Natick, Massachusetts) using Statistic Toolbox, Neural Network Toolbox, and SOM Toolbox (Vesanto et al. 2000) to run SOM analyses. The Eco-ANN tool developed by Park et al. (2003) was used to include environmental variables in SOM maps. Indicator values were calculated in R (version 2.14.0; R Development Core Team, Vienna, Austria), with the package *vegan* (version 1.15-2; Oksanen et al. 2009) and *labdsv* (version 1.3-1; Roberts 2007). The CoA analysis was done with *ADE*-4 package in the R environment.

Results

Environmental features of springs

The study sites were heterogeneous. Current velocity ranged from 1 to 100 m/s, discharge from 0.004 to 120 m^3/s, water temperature from 0.8 to 16°C, % O_2 saturation from 22 to 105%, pH from 3.1 to 8.3, conductivity from 11 to 2120 μS/cm, hardness from 0.4 to 162 mg/L $CaCO_3$, SO_4^{2-} from 0.82 to 1368 mg/L, NO_3–N from 20 to 6885 μg/L, total P from 1.4 to 73 μg/L, PO_4–P from 0.8 to 48 μg/L, and SiO_2 from 0.6 to 16 mg/L. A strong negative correlation was found between altitude and water temperature ($r = 0.79$, $p < 0.01$). The lowest values of water temperature (<4°C), pH (<7), conductivity (<60 μS/cm), and nutrients (NO_3–N < 500 μg/L) were recorded at springs at highest altitudes in siliceous basins mainly of the mountain groups Adamello, Ortles-Cevedale, and Lagorai. The highest values of water temperature (>10°C), pH (>7), conductivity (>300 μS/cm), and nutrients (NO_3–N > 1500 μg/L) were recorded at springs at the lowest altitudes in limestone basins mainly of the mountain groups Lessini and Baldo. These springs also included those highly affected by water captation and bed modification (e.g., ML640 La Ferrara and ML428 Pezza) (for other details, see Cantonati et al. 2007, Lencioni et al. 2011).

Chironomid fauna

A total of 37,116 larvae of chironomids were collected in 124 samples and identified. Eighty-one taxa belonging to 5 subfamilies (Tanypodinae, Diamesinae, Prodiamesinae, Orthocladiinae, and Chironominae) (Appendix 2) were identified to species level whenever possible. Orthocladiinae were represented by 54 species, followed by the Diamesinae (9 species), Chironominae Tanytarsini (6 species) and Tanypodinae (9 species). Chironominae Chironomini and Prodiamesinae were represented by only 2 and 1 species respectively. A significant correlation was found between the number of species and altitude ($r = 0.41$, $p < 0.01$), but the values peaked at altitudes between 1800 and 2000 m asl. A negative effect of anthropogenic pressures on species diversity was evident (Fig. 1A, B). The Shannon Diversity Index and total disturbance were negatively correlated ($r = -0.392$, $p < 0.001$). Most of the highly disturbed springs were intermittent or mineralized. Diversity was significantly higher ($p < 0.05$) in mixed-type springs (rheohelocrenes and rheohygropetric springs) and in the helocrenes. The lowest diversity was in the limnocrenes, whereas the highest number of species was recorded in the rheocrenes and rheocrene mixed types (Table 2).

Seventeen chironomid species were present in >30 springs, 42 species were present in <10 springs, and 9 species occurred in only 1 spring. From 1 to 32 species were identified per spring. Twenty springs had >30 species, and 15 springs had <5 species. Widespread species had higher abundance than species with restricted distributions. The most frequent species were *Tvetenia calvescens*, *Corynoneura scutellata*, *Metriocnemus eurynotus* gr., and *Micropsectra atrofasciata* gr., which were present in >60 springs. The most abundant species were *T. calvescens*, *C. scutellata*, *Paratrichocladius*

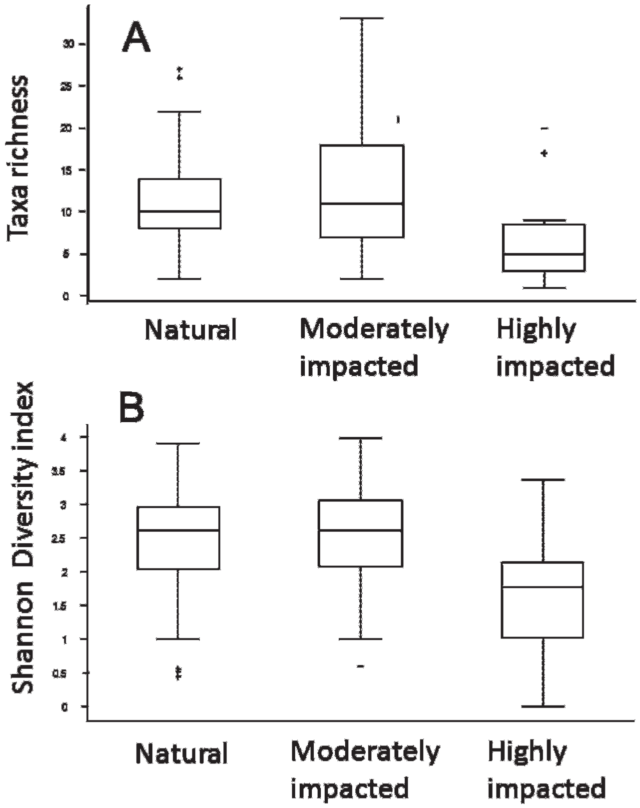

FIG. 1. Box-and-whisker plots for taxon richness (A) and Shannon Diversity Index (B) in natural and moderately or highly disturbed springs in the Italian Prealps and Alps. Lines in boxes are medians, box ends are quartiles, whiskers show ranges (non-outliers within an interval of 1.5 × height of the box), and + indicates outliers.

skirwithensis, *Orthocladius* spp., and *Paratrichocladius rufiventris.*

Relationships between fauna and environment

SOM.—SOM maps of different size were calculated. Map size 5 × 11 had the minimum quantization error (2.1838) and a low topographic error (<0.001), so this map size was selected for further analysis. Sites were arranged in the cells of the SOM map. Five clusters of sites resulted from *k*-mean clustering. They could be separated according to faunal composition and environmental variables (Fig. 2). The distribution maps of 12 environmental variables and of 12 taxa are given in Figs 3 and 4, respectively. The most evident separation was between natural or little-disturbed springs (including those affected by pasture) at high altitude and lowland springs. The 1st group of springs clustered in the lower right part of the map and were mostly rheocrenes in the Alps and had low temperature, high discharge, low conductivity, and coarse substrate (clusters 2, 4, 5 in Figs 2, 3). The 2nd group of

TABLE 2. Mean number of species in different conditions. See Appendix 1 for abbreviations.

Variable	Code or value	Number of species
Spring type	RHE	14
	R	11
	RHY	11
	RL	10
	HE	9
	HY	9
	L	6
Water captation	0	12
	1	10
	2	7
	3	4
Modified bed	0	12
	1	10
	2	11
	3	4
Pasture	0	10
	1	16
	2	20
Total disturbance	1	11
	2	10
	3	17
	4	11
	5	4

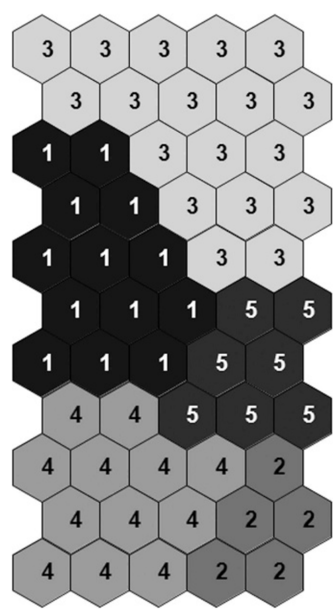

FIG. 2. Self-Organizing Map (SOM) of 124 sites mapped by environmental variables and faunal composition into 5 clusters. Each hexagon represents a single map unit. Cluster numbers correspond to clusters of hexagons aggregated with *k*-mean clustering (see text for explanation).

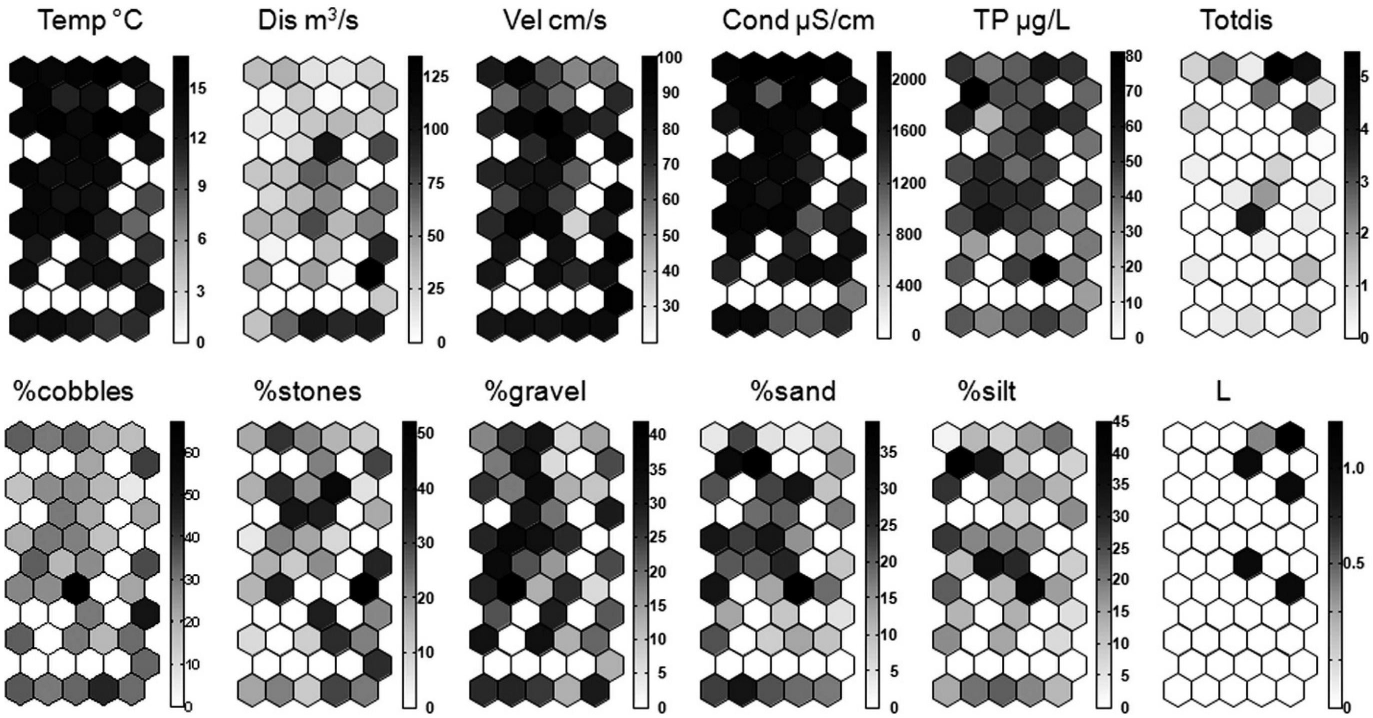

FIG. 3. Self-Organizing Map (SOM) of 12 of 41 environmental variables from 124 springs in the Prealps and Alps. Hexagons represent the same map units as in Fig. 2. The scale bars on the right side of each map indicate the value of each variable within a hexagon. For abbreviations and measurement units see Appendix 1.

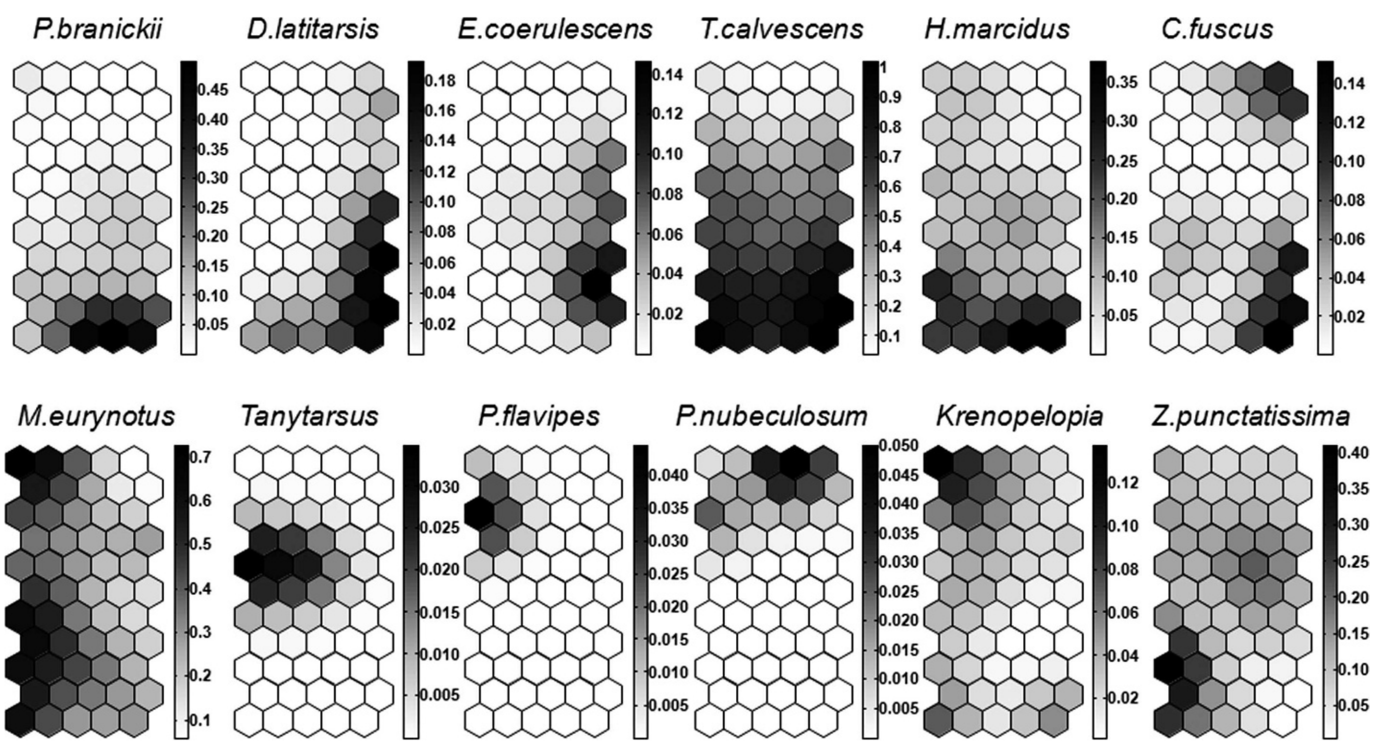

FIG. 4. Self-Organizing Map (SOM) of 12 of 81 species at 124 springs in the Prealps and Alps. Each hexagon represents the same map unit as in Fig. 2. The scale bars on the right side of each map show the coded abundance of each taxon. White indicates absence of species, and black indicates the highest coded value. See Appendix 2 for taxon codes.

TABLE 3. Results of Indicator Value (IndVal) analysis for species characteristic of spring types or environmental conditions. See Appendices 1, 2 and Table 1 for abbreviations and codes. Prob = probability of obtaining by chance as high an indicator value as observed.

Environmental variable	Code/value	Taxon	IndVal	Prob
Agriculture	3	P.nubecu	0.333	0.033
Bryophyte	4	C.lobata	0.372	0.019
	4	C.fuscus	0.368	0.012
Captation	3	Limnophy	0.665	0.002
	3	C.perenn	0.415	0.047
	3	Gymnomet	0.333	0.023
Conductivity	1	H.marcid	0.483	0.001
	1	M.atrofa	0.452	0.001
	1	Orthocla	0.397	0.004
	1	C.perenn	0.385	0.017
	1	P.rufive	0.346	0.004
	1	P.branic	0.339	0.002
	1	P.skirwi	0.297	0.004
	1	S.montan	0.272	0.008
	1	K.boreoa	0.217	0.005
	1	D.zernyi	0.194	0.003
	1	B.longif	0.172	0.014
	1	P.nudipe	0.152	0.025
	1	E.clarip	0.144	0.016
	1	D.dampfi	0.141	0.031
	1	D.latita	0.118	0.033
	2	T.calves	0.319	0.028
	2	M.euryno	0.311	0.017
Current velocity	2	Macropel	0.174	0.010
	2	P.olivac	0.147	0.018
	3	M.euryno	0.399	0.002
	4	P.parva	0.328	0.003
	4	P.skirwi	0.237	0.025
	4	P.branic	0.182	0.042
Helocrene	2	M.atrofa	0.884	0.012
	2	T.longim	0.855	0.003
	2	H.marcid	0.639	0.013
	2	M.hirtic	0.476	0.049
Hygropetric	2	C.dentif	0.451	0.034
	2	E.clarip	0.229	0.049
Limnocrene	1	C.scutel	0.633	0.014
	1	T.calves	0.594	0.048
	1	M.euryno	0.587	0.017
	2	Macropel	0.288	0.022
	2	Natarsia	0.171	0.022
Modified bed	3	C.dentif	0.474	0.010
	3	Limnophy	0.395	0.048
	3	Krenopel	0.331	0.019
	3	H.apical	0.188	0.039
NO_3-N	1	S.montan	0.246	0.030
	1	Bryophae	0.179	0.010
	1	D.incall	0.114	0.031
	4	P.stylat	0.319	0.029
Organic debris	3	K.boreoa	0.241	0.032
Pasture	2	P.rufive	0.473	0.050
	2	S.montan	0.452	0.032
	2	M.hirtic	0.441	0.041
	2	K.boreoa	0.296	0.040
	3	C.vitell	0.927	0.002
	3	Macropel	0.778	0.013
	3	Limnophy	0.725	0.033
	3	M.notesc	0.481	0.024
	3	D.aberra	0.306	0.043

TABLE 3. Continued.

Environmental variable	Code/value	Taxon	IndVal	Prob
Rheocrene	1	M.atrofa	0.461	0.009
	1	R.effusu	0.425	0.002
	1	H.marcid	0.273	0.002
	1	P.olivac	0.184	0.001
	1	Natarsia	0.070	0.033
	1	Psectrot	0.070	0.045
	2	T.vittat	0.326	0.005
Rheohelocrene	2	B.bifida	0.426	0.033
	2	C.dentif	0.331	0.039
	2	T.discol	0.162	0.028
	2	Parachae	0.161	0.015
	2	K.boreoa	0.161	0.048
Rheohygropetric	2	T.calves	0.690	0.031
	2	T.bavari	0.551	0.009
	2	E.rivico	0.367	0.016
	2	Thienema	0.189	0.044
Rheolimnocrene	1	T.calves	0.604	0.047
	2	R.effusu	0.655	0.003
	2	M.atrofa	0.533	0.037
	2	H.marcid	0.455	0.008
	2	P.olivac	0.342	0.007
Road	2	P.rufive	0.419	0.037
Shading	1	R.chalyb	0.250	0.026
	1	R.fuscip	0.244	0.005
	1	M.notesc	0.215	0.017
	2	P.skirwi	0.338	0.021
	2	M.atrofa	0.333	0.050
	2	S.montan	0.315	0.020
	2	H.marcid	0.300	0.043
	2	D.zernyi	0.208	0.040
	6	T.longim	0.306	0.038
Temperature	1	P.parva	0.532	0.001
	1	P.branic	0.223	0.013
	1	D.insign	0.083	0.039
	2	T.calves	0.427	0.002
	2	C.scutel	0.378	0.018
	2	E.devoni	0.363	0.001
	2	P.skirwi	0.352	0.002
	2	S.semivi	0.292	0.004
	2	M.hirtic	0.218	0.007
	2	D.dampfi	0.171	0.012
Total disturbance	3	M.fuscip	0.368	0.023
	3	Macropel	0.360	0.005
	3	P.olivac	0.317	0.014
	3	E.fuscim	0.221	0.037
	4	C.dentif	0.323	0.031

springs clustered in the upper left part of the map and were mainly limnocrenes in the Prealps and included highly disturbed springs. Springs in this group were characterized by higher temperature, conductivity, and nutrient (N and P) concentrations (clusters 1, 3 in Figs 2, 3). Cold stenothermal species (Diamesinae, many Orthocladiinae) were clustered in lower right part of the map (clusters 2, 4, and 5 in Figs 2, 4), whereas tolerant and euriecious species (especially Chironomini) were clustered in the upper left of the map (clusters 1, 3 in Figs 2, 4). Some species had a bimodal distribution; e.g., *Cricotopus fuscus* and *M. eurynotus* gr. These 2 species were prevalent in clusters 2 to 4, but had also peaks of abundance in other clusters.

Indicator values.—Indicator values emphasized the importance of different variables on different species (Table 3). For example, *Pseudokiefferiella parva* and *Eukiefferiella devonica* gr. were indicators of low temperature. *Heterotrissocladius marcidus*, *M. atrofasciata* gr.,

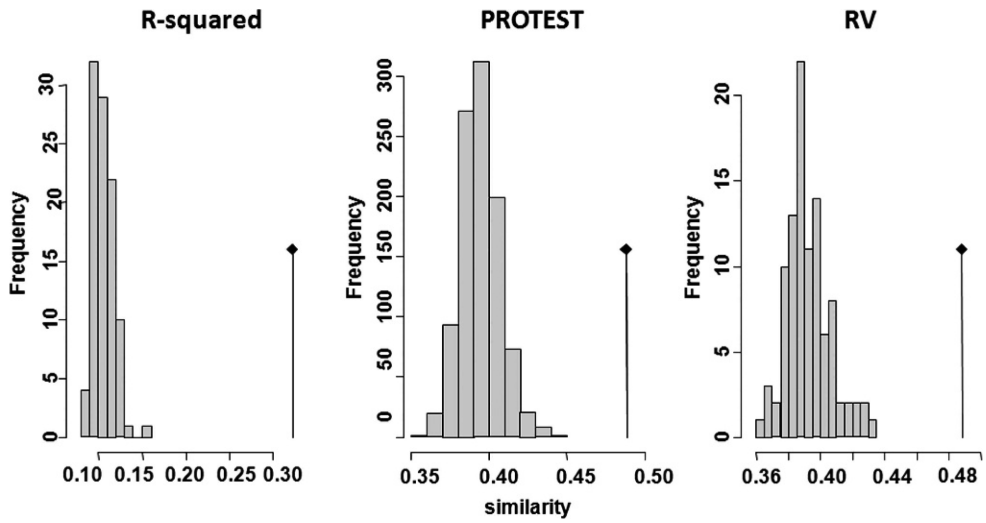

FIG. 5. Plot of R^2 (A), Procrustes randomization test (B), and RV test (C) results from the Coinertia Analysis showing histograms of simulated (Sim) values. The vertical line shows the observed value (see Methods for further explanation).

and *P. branickii* were indicators of low conductivity, whereas *P. parva, P. skirwithensis*, and *P. branickii* were indicators of high current velocity. Some species were significantly associated with a specific stressor. *Macropelopia* spp., *Diamesa aberrata*, *Chaetocladius vitellinus* gr., *Limnophyes* spp., and *Micropsectra notescens* gr. indicated pasture; *P. nubeculosum* indicated agriculture; *Chaetocladius perennis*, *Limnophyes* spp., and *Gymnometriocnemus* sp. indicated water captation; *Krenopelopia* sp., *Chaetocladius dentiforceps* gr., *Heterotrissocladius apicalis*, and *Limnophyes* spp. indicated bed modification. Other details can be examined in Table 3, where a high code/value means that a species is an indicator of high value of an environmental variable, and a low code/value means that the species is an indicator of low value of the environmental variable. The higher the indicator value (IndVal) and the lower the probability (Prob) value, the better the indicator.

CoA.—R^2, Procrustean, and RV tests showed that species structure and environmental variables were significantly related (Fig. 5A–C). CoA was carried out with 81 chironomid taxa and 41 environmental variables and ordered the 124 sites. The first 4 CoA axes explained ~57, 19, 15, and 9%, respectively, of total coinertia (Table 4). The correlation between the 2 data sets was 0.84 for axis 1 and 0.74 for axis 2. The inertia of each separate analysis and the projections of inertia on CoA axes are given in Table 5.

CoA showed the relations between the 41 environmental variables and the first 2 maximum covariance axes (Fig. 6) and between the 81 chironomid taxa and the same axes (Fig. 7; only the taxa with the highest scores were plotted). Water temperature and altitude had the highest loadings (0.308 and −0.302, respectively) on axis 1. Total disturbance and rheocrene had the highest loadings (0.296 and −0.277, respectively) on axis 2. CoA axis 1 represented a gradient of altitude, water temperature, alkalinity, and conductivity, whereas axis 2 represented a hydrological and geomorphological gradient and a gradient of anthropogenic disturbance (from natural to disturbed springs). Axis 2 separated rheocrene springs with high current velocity from severely disturbed limnocrene springs rich in organic debris. Diamesinae (*P. branickii*, *Diamesa* spp., *P. parva*) and cold stenothermal Orthocladiinae (*Eudactylocladius fuscimanus, Krenosmittia boreoalpina, Parorthocladius nudipennis*, *Stilocladius montanus, P. skirwithensis*) were plotted on the left part of the factorial 1 × 2 plane. Rheophilous

TABLE 4. Results of the Coinertia Analysis (CoA) of 2 data sets (environmental = E, faunistic = F) including eigenvalues (Eig), covariance (Covar), standard deviations (SdE, SdF) of the 2 sets of site scores on the CoA axes, correlations (corr) between the 2 sets of site scores, and % of variance (%var) accounted for by each axis (F1, F2, F3, F4).

Axis	Eig	Covar	SdE	SdF	Corr	%var
F1	1.474	1.214	0.546	2.640	0.842	57.010
F2	0.495	0.704	0.459	2.074	0.739	19.151
F3	0.390	0.625	0.464	1.786	0.754	15.107
F4	0.226	0.475	0.458	1.353	0.766	8.733

TABLE 5. Results of the Coinertia Analysis (CoA) of 2 data sets (environmental = E, faunistic = F) showing the comparison of the inertias of the accumulated projections of the environmental and faunistic data tables as projected in the coinertia analyses (CoinertiaE and CoinertiaF) with the maximum inertia of the axes of the separate ordinations (MaxE and MaxF). The ratio between these values (RatioE and RatioF) is a measure of the concordance between the 2 projections. Cumulative % of variance (%var) of the Coinertia axes also is given. The RV coefficient is the ratio of the total coinertia to the square root of the product of the squared inertias of the separate analyses. RV = 0.354.

Environmental table					Faunistic table				
Axes	CoinertiaE	MaxE	RatioE	%var	Axes	CoinertiaF	MaxF	RatioF	%var
E1	0.298	0.346	0.861	12.093	F1	6.969	7.260	0.960	14.222
E1 + E2	0.509	0.627	0.811	20.650	F1 + F2	11.269	11.930	0.945	22.999
E1 + E2 + E3	0.724	0.902	0.803	29.375	F1 + F2 + F3	14.459	15.099	0.958	29.508
E1 + E2 + E3 + E4	0.934	1.167	0.801	37.897	F1 + F2 + F3 + F4	16.289	17.975	0.906	33.243

species (*Eukiefferiella* spp., *Tvetenia* spp.) were separated from limnetic species (*P. nubeculosum*, *Natarsia* sp., *Phaenopsectra flavipes*) plotted on the right and top part of the factorial plane. *Macropelopia* spp. and *Prodiamesa olivacea* were associated with fine sediment. Species inhabiting limnocrene springs also were related to total disturbance (*Macropelopia* spp., *P. nubeculosum*). CoA showed good agreement between the 2 matrices prepared as sites × environmental variables and sites × chironomid species, demonstrating the potential of the chironomid fauna as an indicator of the environmental conditions of springs. The sites coded according to their spring type membership were plotted in the plane of the first 2 coinertia axes (Fig. 8).

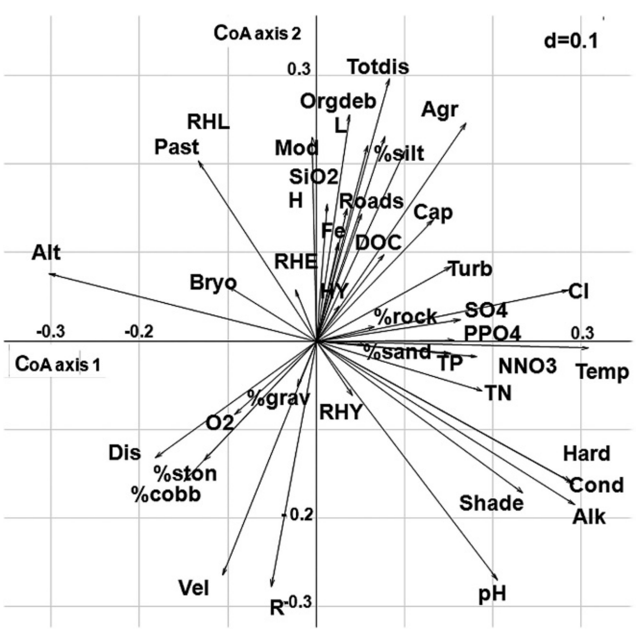

FIG. 6. Plot of abiotic variables on the first 2 Coinertia Analysis (CoA) axes. The direction and length of each arrow shows the strength of the correlation of that variable with the CoA axes. See Appendix 1 for variable codes.

Discussion

The alpine and prealpine springs were colonized by a substantial number of chironomid species, corresponding to ~20% of the species recorded in Italy. This richness could be related to the mosaic structure of each spring. The presence of different substrates and the broad food availability resulted in high biodiversity and population densities (Lindegaard 1995, Moog 2002, Lencioni and Rossaro 2005, Staudacher and Füreder 2007, Marziali et al. 2010). The highly individual nature of the springs was evident within the same basin, within a spring, and among springs. As expected, rheocrene springs were mosaics of different niches and were the most species rich (Lindegaard 1995, Cantonati et al. 2006, Sambugar et al. 2006). As found in previous studies (Smith and Wood 2002, Smith et al. 2003, von Fumetti et al. 2006), limnocrene and helocrene springs hosted fewer species.

Anthropogenic pressures led to a decrease in both taxon richness and diversity. Among disturbances, water captation associated with bed modification had the greatest effects on richness and diversity, whereas pasture seemed to have a positive effect on richness and diversity, possibly because it ensured a higher supply of nutrients in high-altitude springs typically poor in food (Cantonati et al. 2006). Comparably higher richness and diversity were recorded in moderately disturbed springs. This increase might be consistent with the intermediate disturbance hypothesis, which predicts a peak in biodiversity at an intermediate disturbance level (Connell 1978) and low diversity at a lower disturbance level. Barquín and Death (2004) found support for this hypothesis in their work in springs in northwestern Spain.

Ordination and classification analyses showed that chironomid assemblages responded to environmental variables. Water temperature, substrate composition, and anthropogenic disturbance were the main factors

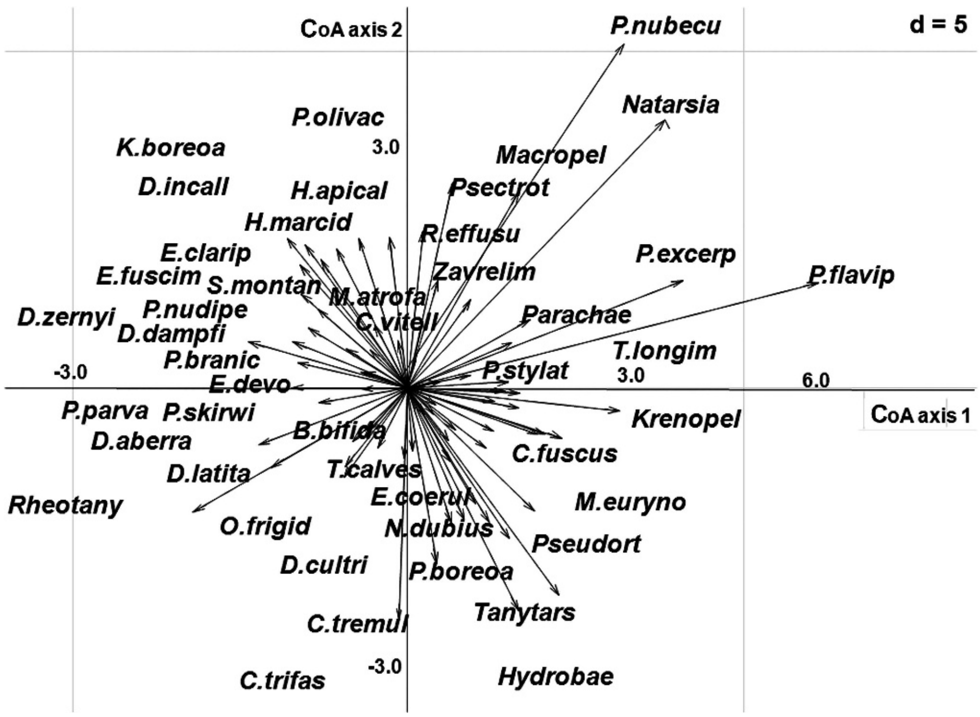

FIG. 7. Plot of taxon scores on the first 2 Coinertia Analysis (CoA) axes. The direction and length of each arrow shows the strength of the correlation of that taxon with the CoA axes. Only those species with the highest scores are shown. See Appendix 2 for taxon codes.

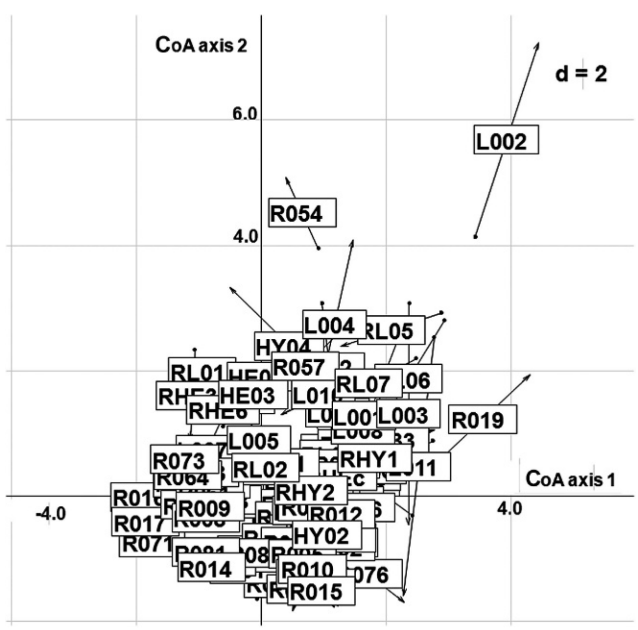

FIG. 8. Plot of sites based on site scores on the first 2 Coinertia Analysis (CoA) axes. The plot shows the position of the sites on the coinertia axes calculated using the environmental (origins of the arrows) and the species (arrowheads) weights. For abbreviations see Appendix 1.

determining chironomid distribution and abundance in alpine and prealpine springs. These variables also affect the composition of macroinvertebrate assemblages in springs (e.g., Glazier 1991, Smith et al. 2001, von Fumetti et al. 2006). *Diamesa* spp., *P. parva*, and *E. devonica* gr. were indicators of low temperature; *H. marcidus, M. atrofasciata* gr., and *P. branickii* of low conductivity; and *P. parva, P. skirwithensis*, and *P. branickii* of high current velocity (Ferrarese and Rossaro 1981, Rossaro 1991, Lindegaard 1995, Lencioni and Rossaro 2005). Many of the crenophilous species (Diamesinae, many Orthocladiinae, Tanytarsini) were cold stenothermal, as expected (Fischer 1996). These species clustered together and were well separated from tolerant and euriecious species (few Orthocladiinae, some Tanypodinae and Chironomini). Some species were associated with specific disturbance. Among these, *P. nubeculosum* was found in springs affected by water captation and bed modification in agricultural areas. This species is frequent in meso-eutrophic lakes and in depositional zones in streams characterized by high amounts of nutrients (Nocentini 1985).

Identification of sensitive species and assessment of complex anthropogenic disturbance were improved by using an unsupervised ANN. The SOM carried out on species data emphasized differences between different spring types and levels of anthropogenic pressures and highlighted gradients of spring types (from rheocrene to limnocrene) and of pollution. Taxonomic composition of assemblages changed gradually from one type to another. CoA results confirmed the SOM analysis, but SOM was able to detect bimodal distribution of some species (*C. fuscus, M. eurynotus* gr.) not detectable in CoA maps. IndVal analysis supported the SOM and CoA results and confirmed the importance of water temperature, current velocity, conductivity, and spring type in explaining species distribution. Nonetheless, anthropogenic disturbances interact with these factors making it difficult to separate the effects of single variables on species composition.

In conclusion, a complex of abiotic factors determine the distribution of chironomids in springs, with water temperature (related to altitude and anthropogenic disturbance via water captation and bed modification) and conductivity (related to lithology and anthropogenic disturbance via agriculture and pasture) as most important. The utility of chironomids as bioindicators of water quality was confirmed, highlighting the fact that some species are associated with high disturbance levels (e.g., by water captation or agriculture; *P. nubeculosum*) and others with pristine conditions (*Diamesa* spp., *Stilocladius montanus*).

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APPENDIX 1. List of 41 environmental variables used in the statistical analyses, abbreviations, and unit of measurement or coding (UMC). Variables are arranged alphabetically.

Environmental variable	Abbreviation	UMC
Alkalinity	Alk	meq/L
Altitude	Alt	m
Agriculture	Agr	0–2
Bryophytes	Bryo	0–9
Water captation	Cap	0–3
Cl^-	Cl	mg/L
Conductivity	Cond	µS/cm
Discharge	Dis	m^3/s
Dissolved organic C	DOC	mg/L
Fe	Fe	mg/L
Hardness	Hard	mg/L $CaCO_3$
Helocrene	H	0–1
Hygropetric	HY	0–1
Limnocrene	L	0–1
Modified bed	Mod	0–3
NO_3–N	NNO3	µg/L
Organic debris	Orgdeb	0–4
O_2	O2	mg/L
pH	pH	
Pasture	Past	0–2
% cobbles	%cobb	%
% gravel	%grav	%
% rock	%rock	%
% sand	%sand	%
% silt	%silt	%
% stones	%ston	%
PO_4–P	PPO_4	µg/L
Rheocrene	R	0–1
Rheohelocrene	RHE	0–1
Rheohygropetric	RHY	0–1
Rheolimnocrene	RHL/RL	0–1
Roads	Roads	0–1
Shading	Shade	0–4
SiO_2	SiO2	mg/L
SO_4^{2-}	SO4	mg/L
Total disturbance	Totdis	0–5
Total N	TN	µg/L
Total P	TP	µg/L
Turbidity	Turb	FTU
Velocity	Vel	m/s
Water temperature	Temp	°C

APPENDIX 2. List of 81 taxa used in the data analysis and their abbreviations.

Subfamily/tribe	Species	Abbreviation
Tanypodinae	*Apsectrotanypus* sp.	Apsectro
	Krenopelopia sp.	Krenopel
	Macropelopia spp.	Macropel
	Natarsia sp.	Natarsia
	Nilotanypus dubius	N.dubius
	Psectrotanypus sp.	Psectrot
	Thienemannimyia sp.	Thienema
	Trissopelopia longimana	T.longim
	Zavrelimyia sp.	Zavrelim
Diamesinae	*Diamesa aberrata*	D.aberra
	Diamesa dampfi gr.	D.dampfi
	Diamesa incallida	D.incall
	Diamesa insignipes	D.insign
	Diamesa latitarsis gr.	D.latita
	Diamesa zernyi gr.	D.zernyi
	Potthastia gaedii	P.gaedii
	Pseudodiamesa branickii	P.branic
	Pseudokiefferiella parva	P.parva
Prodiamesinae	*Prodiamesa olivacea*	P.olivac
Orthocladiinae	*Brillia bifida*	B.bifida
	Brillia longifurca	B.longif
	Bryophaenocladius spp.	Bryophae
	Chaetocladius dentiforceps	C.dentif
	Chaetocladius perennis	C.dentif
	Chaetocladius vitellinus gr.	C.vitell
	Corynoneura lobata	C.lobata
	Corynoneura scutellata	C.scutel
	Cricotopus fuscus	C.fuscus
	Cricotopus tremulus	C.tremul
	Cricotopus trifascia	C.trifas
	Diplocladius cultriger	D.cultri
	Eudactylocladius fuscimanus	E.fuscim
	Eukiefferiella brevicalcar	E.brevic
	Eukiefferiella claripennis gr.	E.clarip
	Eukiefferiella coerulescens	E.coerul
	Eukiefferiella devonica gr.	E.devoni
	Euorthocladius rivicola	E.rivico
	Euorthocladius frigidus	E.frigid
	Gymnometriocnemus sp.	Gymnomet
	Heleniella serratosioi	H.serrat
	Heterotanytarsus apicalis	H.apical
	Heterotrissocladius marcidus	H.marcid
	Hydrobaenus sp.	Hydrobae
	Krenosmittia borealpina	K.boreoa
	Limnophyes spp.	Limnophy
	Metriocnemus fuscipes gr.	M.fuscip
	Metriocnemus eurynotus gr.	M.euryno
	Metriocnemus hirticollis	M.hitic
	Orthocladius spp.	Orthocla
	Parachaetocladius sp.	Parachae
	Paratrichocladius skirwithensis	P.skirwi
	Parakiefferiella sp.	Parakief
	Parametriocnemus boreoalpinus	P.boreoa
	Parametriocnemus stylatus	P.stylat
	Paraphaenocladius sp.	Paraphae
	Paratrichocladius rufiventris	P.rufive
	Paratrichocladius skirwithensis	P.skirwi

APPENDIX 2. Continued.

Subfamily/tribe	Species	Abbreviation
	Paratrissocladius excerptus	P.excerp
	Parorthocladius nudipennis	P.nudipe
	Psectrocladius sordidellus gr.	P.sordid
	Pseudorthocladius sp.	Pseudort
	Paraphaenocladius sp.	Paraphae
	Rheocricotopus chalybeatus	R.chalyb
	Rheocricotopus effusus	R.effusu
	Rheocricotopus fuscipes	R.fuscip
	Stilocladius montanus	S.montan
	Symposiocladius sp.	Symposio
	Synorthocladius semivirens	S.semivi
	Thienemannia gracilis	T.gracil
	Thienemanniella vittata	T.vittat
	Tvetenia bavarica	T.bavari
	Tvetenia calvescens	T.calves
	Tvetenia discoloripes	T.discol
Chironominae/ Tanytarsini	*Micropsectra atrofasciata* gr.	M.atrofa
	Micropsectra attenuata gr.	M.attenu
	Micropsectra notescens gr.	M.notesc
	Rheotanytarsus sp.	Rheotany
	Stempellinella sp.	Stempell
	Tanytarsus sp.	Tanytars
Chironominae/ Chironomini	*Phaenopsectra flavipes*	P.flavip
	Polypedilum nubeculosum gr.	P.nubecu