

## Effects of human impacts on diversity and distribution of chironomids (Diptera: Chironomidae) in prealpine springs

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### ABSTRACT

Diversity and distribution of chironomid fauna (Diptera: Chironomidae) in 36 springs in the Italian Prealps (Veneto and Trentino NE-Italy, 46°N, 10-11°E) was studied in relation to altitude, spring type and grade of disturbance. The springs were located between 62 and 1710 m asl of altitude, in three calcareous mountain areas (Mt. Baldo, Mts. Lessini and Mt. Pasubio). They differed in conservation status (natural, moderately and highly disturbed) and belonged to five hydromorphological types (rheocrene, limnocrene, rheohelocrene, rheolimnocrene, rheohydropetric). Each spring was surveyed once, between early summer and autumn, within 50 m of the spring's source (eucrenal). A total of 4198 chironomid larvae and pupae were sorted from 111 macroinvertebrate samples collected, belonging to five subfamilies (Tanypodinae, Diamesinae, Prodiamesinae, Orthocladiinae and Chironominae), 41 genera and 60 species/groups of species, and three juveniles taxa. As expected, Orthocladiinae accounted for a large part of specimens (88%) and species (74%), with *Tvetenia calvescens/bavarica* as the most frequent and abundant taxon, shared by pristine and disturbed springs and by all spring types. Most taxa were found in few sites, and frequencies declined gradually for most wider distributed species. A high number (74%) of rare (=present in less than 10% of sites) taxa were found and from one to 23 taxa were identified per spring. Maximum richness occurred in moderately disturbed (Shannon-H=1.29±0.60) springs, located at medium-high altitude (385-1266 m asl), according to the intermediate disturbance hypothesis. Even the evenness (Equitability-J) was higher as average in these springs. A Cluster Analysis run on Bray-Curtis similarity index highlighted a high similarity i) between the chironomid assemblages of moderately disturbed and natural springs (44 species in each spring group, with 29 species in common), and ii) between rheocrene and rheohelocrene types, thus the springs with the highest microhabitat heterogeneity. High individuality of springs was revealed, and new information about non-biting midges autecology provided. The utility of chironomids as bioindicators of water quality and ecological state of springs was confirmed, with some species associated with high disturbance level (e.g., *Polypedilum nubeculosum* gr. to water intake works) and others with pristine conditions (e.g., *Pseudodiamesa branickii*).

**Key words:** Orthocladiinae; eucrenal; mosaic; tapped springs; spring types, Italian Prealps.

**Contributions:** EM, springs sampling; EM, CS, LL, collaboration on data processing; VL, statistical analyses performing, manuscript writing and revision; LL, study concept and design.

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### INTRODUCTION

Spring-dwelling fauna is typically dominated by chironomids (Diptera: Chironomidae) in terms of both individual and species number (Lindegaard, 1995; Płóciennik *et al.*, 2016). Chironomids are diverse and exhibit a wide variety of ecological preferences that provide potential in establishing reference conditions for bioassessment of freshwater ecosystems (Serra *et al.*, 2018). However, due to taxonomic difficulties and poor knowledge of traits, chironomids are often neglected in biomonitoring programmes (Lencioni *et al.*, 2012).

From the earliest studies on spring fauna that included chironomids (Bornhauser, 1912; Nadig, 1942), European

literature on chironomid fauna in alpine springs and in spring-fed brooks increased from the end of the 90s (e.g., Orendt, 2000a; Füreder *et al.*, 2001; Rossaro *et al.*, 2006; von Fumetti *et al.*, 2006; Lencioni *et al.*, 2011; Robinson *et al.*, 2016; von Fumetti and Blattner, 2017). These studies emphasised that springs and their biota are good tools for monitoring changes in groundwater quality caused by anthropogenic impact. Springs are in fact extremely sensitive to any type of disturbance because of their small dimensions (Orendt, 2000b; Cantonati *et al.*, 2006). Springs have been seen as clean and pristine environments of high biological integrity, especially in mountain regions, at high altitude, but this condition is becoming more and more unusual. In the Alps, springs

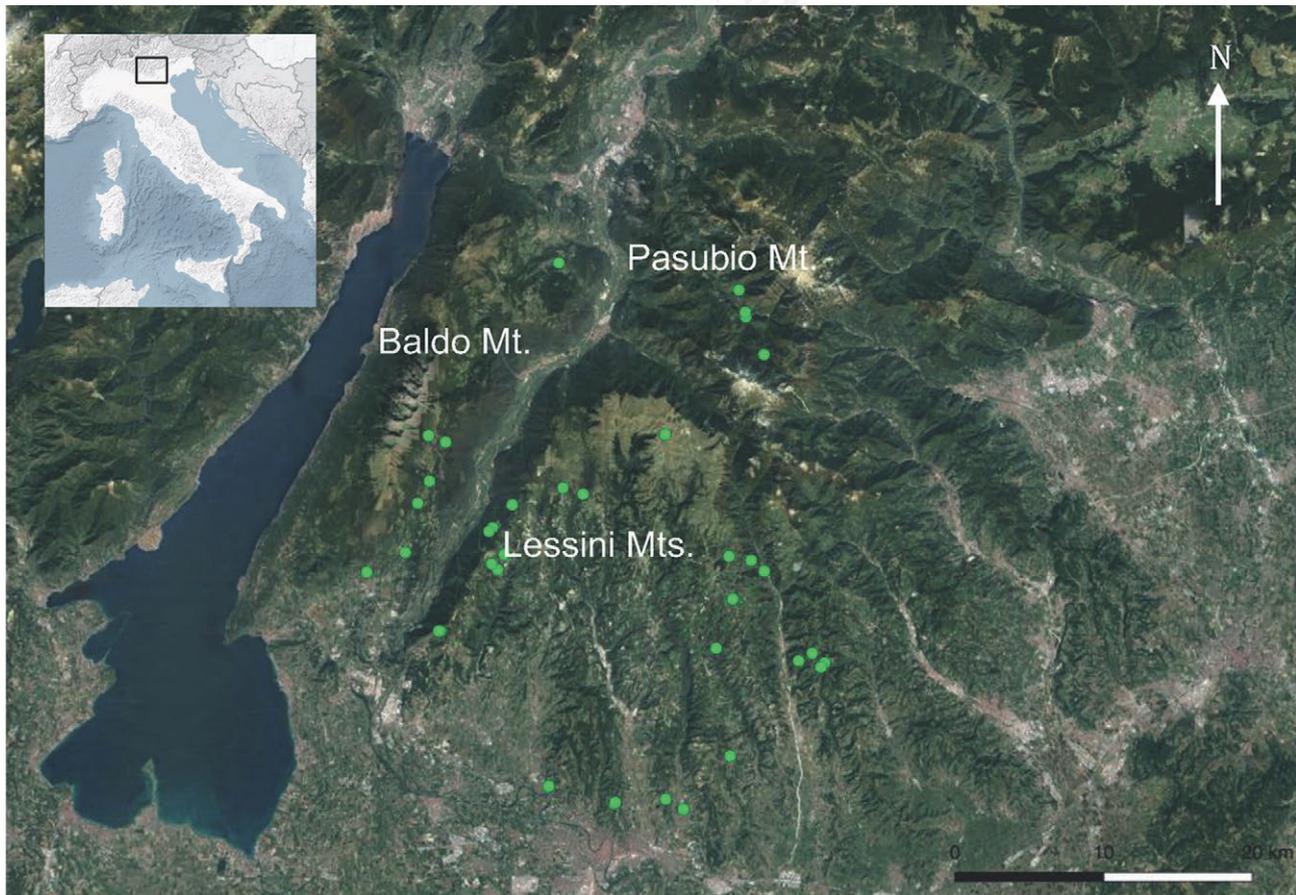
are frequently affected by water abstraction (that involves open or closed technical constructions for spring tapping and/or piping), forest management activities, agriculture, pasture sewage, trampling by cattle, road construction and drought due to global warming (Lencioni *et al.*, 2012; Reiss *et al.*, 2016). Nevertheless, no biotic indices have been developed to assess their ecological status. This is becoming an environmental emergency, in view of potential loss of ecosystem services provided by springs under global climate and environmental changes.

Although lists of chironomid species have been published on springs in Italian Prealps and Alps (*e.g.*, Crema *et al.*, 1996, Sambugar *et al.*, 2006; Lencioni and Sartori, 2012), the influence of disturbance on biodiversity has been still little investigated. The aim of this work was to analyse distribution and diversity of chironomid fauna in thirty-six Pre-alpine springs in relation to altitude and their state of conservation (from pristine to highly disturbed), in a calcareous region particularly rich in springs (Zorzin *et al.*, 2009).

## METHODS

### The springs

The thirty-six springs studied were located in three mountain areas (7 in Baldo Mt, 26 in Lessini Mts, 4 in Pasubio Mt) in the Italian Prealps (Veneto and Trentino NE-Italy, 46°N, 10-11°E) (Fig. 1), within 62 and 1710 m a.s.l., on limestone substrate (Latella *et al.*, 2012). The springs belonged to five hydromorphological types (17 rheocrenes-RHE, 7 limnocrenes-LIMNO, 5 rheohelocrenes-RHE-HEL, 6 rheolimnocrenes-RHE-LIMNO, 1 rheohygropetric-RHE-HYG) (Tab. 1). The level of disturbance was defined according to Lencioni *et al.* (2012), from 1=natural springs (14), 2=moderately disturbed springs (12) to 3=highly disturbed springs (10). Impacts were represented by open or closed technical constructions for water abstraction (spring tapping and/or piping), modified bed (generally associated to water abstraction), polluted inputs by agriculture and grazing.



**Fig. 1.** Study area: thirty-six springs located in three mountain areas (7 in Baldo Mt, 26 in Lessini Mts, 4 in Pasubio Mt) in the Italian Prealps (Veneto and Trentino NE-Italy, 46°N, 10-11°E), within 62 and 1710 m asl.

### Chironomid sampling

Each spring was surveyed once between early summer and early autumn 2001–2003 or 2007–2008. Chironomid larvae and pupae were collected in the eucrenal zone (=within 50 m of the spring's source) of each spring. One to three replicates were collected per spring by exploring

different substrate typologies according to Lencioni *et al.* (2011). A pond net (100 µm mesh size) was used for 30 seconds in coarse (>0.2 cm, from gravel to stones) and fine substrate (<0.2 cm, from sand to mud); submerged bryophytes were collected and washed in the laboratory to extract animals living within. The semi-quantitative samples

**Tab. 1.** List of thirty-seven springs, sorted by increasing altitude. Shannon Diversity is also reported for each spring.

Spring name	Province	Mt Group	Altitude (m asl)	Spring type	Conservation state	Year of sampling	Shannon_H
Squarà	Verona	Lessini	62	LIMNO	Highly disturbed	2008	0.45
S. Cristina	Verona	Lessini	75	RHE	Highly disturbed	2003	0.81
Balladoro	Verona	Lessini	125	RHE-LIMNO	Highly disturbed	2008	1.35
Torricelle	Verona	Lessini	220	RHE-LIMNO	Moderately disturbed	2008	1.68
Cava del Prete	Verona	Lessini	230	LIMNO	Moderately disturbed	2001	1.60
Gaon	Verona	Baldo	385	RHE-LIMNO	Moderately disturbed	2008	2.20
Pezza	Verona	Lessini	428	RHE-LIMNO	Highly disturbed	2007	0.30
Prealpa	Verona	Baldo	445	RHE-LIMNO	Moderately disturbed	2008	1.40
Vajo Fontane	Verona	Lessini	494	RHE-LIMNO	Natural	2003	0.86
Lodrone	Trento	Baldo	517	RHE	Natural	2007	0.79
Biasetti	Verona	Lessini	533	RHE-HEL	Natural	2008	2.20
Vajo del Croce	Verona	Lessini	580	LIMNO	Natural	2008	1.41
Veraghi	Verona	Lessini	622	RHE	Moderately disturbed	2007	0.00
Fonte Grande	Verona	Lessini	637	RHE-HYG	Moderately disturbed	2008	1.33
La Ferrara	Verona	Lessini	640	LIMNO	Highly disturbed	2007	1.49
Biuchele Speccheri	Trento	Pasubio	676	RHE	Natural	2007	1.39
Boschi	Verona	Lessini	690	LIMNO	Highly disturbed	2007	0.00
Cocher	Trento	Pasubio	690	RHE-HEL	Natural	2007	1.96
Bertani	Verona	Lessini	710	RHE	Highly disturbed	2001	0.00
Campopiano	Verona	Lessini	710	RHE	Natural	2001	1.23
Val di Canova	Trento	Pasubio	720	RHE	Highly disturbed	2007	0.29
Vaiara	Verona	Lessini	760	RHE-HEL	Natural	2001	1.23
Gorgusello2	Verona	Lessini	772	RHE	Moderately disturbed	2001	1.05
Gorgusello1	Verona	Lessini	802	RHE-LIMNO	Moderately disturbed	2001	1.15
Monte Catini	Verona	Baldo	840	LIMNO	Highly disturbed	2007	1.21
Covolo dell'acqua	Verona	Lessini	860	RHE	Natural	2003	0.43
Fenil dei Coltri	Verona	Baldo	894	LIMNO	Highly disturbed	2008	1.55
Druzzele	Verona	Lessini	923	RHE	Natural	2003	1.59
Fondo Comperlon	Trento	Pasubio	924	RHE	Natural	2007	1.48
Varalta	Verona	Lessini	950	RHE	Natural	2008	1.30
Gauli	Verona	Lessini	961	RHE-HEL	Natural	2003	1.13
Selvavecchia	Verona	Lessini	1030	RHE	Moderately disturbed	2003	1.28
Grola	Verona	Lessini	1129	RHE	Moderately disturbed	2003	1.92
Fontana della Teja	Verona	Baldo	1215	RHE	Moderately disturbed	2008	0.42
Malga Lonza	Verona	Baldo	1266	RHE-HEL	Moderately disturbed	2008	1.46
Fonte Chiarana	Verona	Lessini	1710	RHE	Natural	2003	0.70

RHE, rheocrene; LIMNO, limnocrene; RHE-LIMNO, rheolimnocrene; RHE-HEL, rheohelocrene; RHE-HYG, rheohydropetric.

were preserved in 75% ethanol. Chironomids were mounted on slides and identified to species/groups of species according to Ferrarese and Rossaro (1981), Rossaro (1982), Ferrarese (1983), Wiederholm (1983, 1986), Nocentini (1985), Schmid (1993), Janecek (1998) and Lencioni *et al.* (2007). Morphological voucher specimens are deposited in the MUSE's entomological collection (INV017).

In each spring, water temperature, pH and conductivity were measured with a field multiprobe (Hydrolab 20; HACH Hydromet, Loveland, CO, USA). 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, CA, USA).

### Statistical analyses

The mean abundance of each taxon per spring was considered, and relationships between frequency and abundance of taxa was investigated. Rare species, defined as present in less than 10% of springs (Lencioni *et al.* 2012), were included in the analysis, as recommended by Smith *et al.* (2001), resulting in a total of 63 taxa.

Species richness was calculated as sum of the number of species (count data=S) and diversity by the Shannon-Wiener Index (H) (Shannon and Weaver, 1949), Dominance (D)=1-Simpson index and Equitability (J) (known also as Pielou's Evenness Index, calculated as Shannon diversity divided by the logarithm of number of taxa, according to Hammer *et al.*, 2001). D ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the

community completely). J measures how evenly individuals are divided among the taxa present and assumes a value between 0 and 1 with 1 being complete evenness.

Significant differences in environmental features ( $P<0.05$ ) between sites were estimated by Kruskal-Wallis test. The effect of the environmental variables (explanatory variables) on the chironomid species richness and diversity (response variables) was investigated by Regression Analysis using the least squares method (Gotelli and Hellison, 2004; Hammer *et al.*, 2001). Two types of relationships were found, linear between altitude and water temperature (in the simple linear regression, the predictions of Y are plotted as a function of X forming a straight line:  $y=ax+b$ ) and quadratic (or 2nd order polynomial) between altitude and richness (in the quadratic regression, the predictions of Y are plotted as a function of a parabola:  $y=ax^2+bx+c$  with  $a \neq 0$ ).

Bray-Curtis similarities based on chironomid assemblages (abundance data were prior square root transformed) were calculated to compare springs grouped according to disturbance level (1, 2, 3) and to hydromorphological type (the rheohygropteric RHE-HYG type was excluded from the analysis because it was represented by only one spring). The similarity matrices were used to run a Cluster Analysis. Analyses were performed using PAST 3.18 (Hammer *et al.*, 2001).

## RESULTS

Temperature and conductivity increased while discharge decreased with increasing level of disturbance ( $P<0.05$ )

**Tab. 2.** Main physical and chemical features and biodiversity parameters of the thirty-six springs grouped according to their level of disturbance.

	Highly disturbed (10)	Moderately disturbed (12)	Natural (14)
Altitude (m asl)	518±322	729±370	806±309
Spring type (prevalent)	LIMNO	RHE-LIMNO	RHE, RHE-HEL
Substrate	Modified bed (cement); fine sediment (organic debris); scarce bryophytes	Modified bed; coarse substrate; some bryophytes	Cobbles, gravel, leaves; abundant bryophytes
Discharge (L s <sup>-1</sup> )	0.42±0.56	0.55±0.58	0.86±0.60
Temperature (°C)	11.2±2.6	9.7±2.1	8.6±1.5
Ph	7.6±0.4	7.6±0.2	7.6±0.3
Conductivity (µs cm <sup>-2</sup> )	447±120	390±134	384±135
Richness (S)	5±3	7±4	7±2
Shannon (H)	0.74±0.61	1.29±0.60	1.26±0.48
Dominance (D)	0.63±0.30	0.40±0.24	0.41±0.19
Equitability (J)	0.62±0.25	0.77±0.13	0.66±0.21

LIMNO, limnocrene; RHE-LIMNO, rheolimnocrene; RHE, rheocrene; RHE-HEL, rheohelocrene.

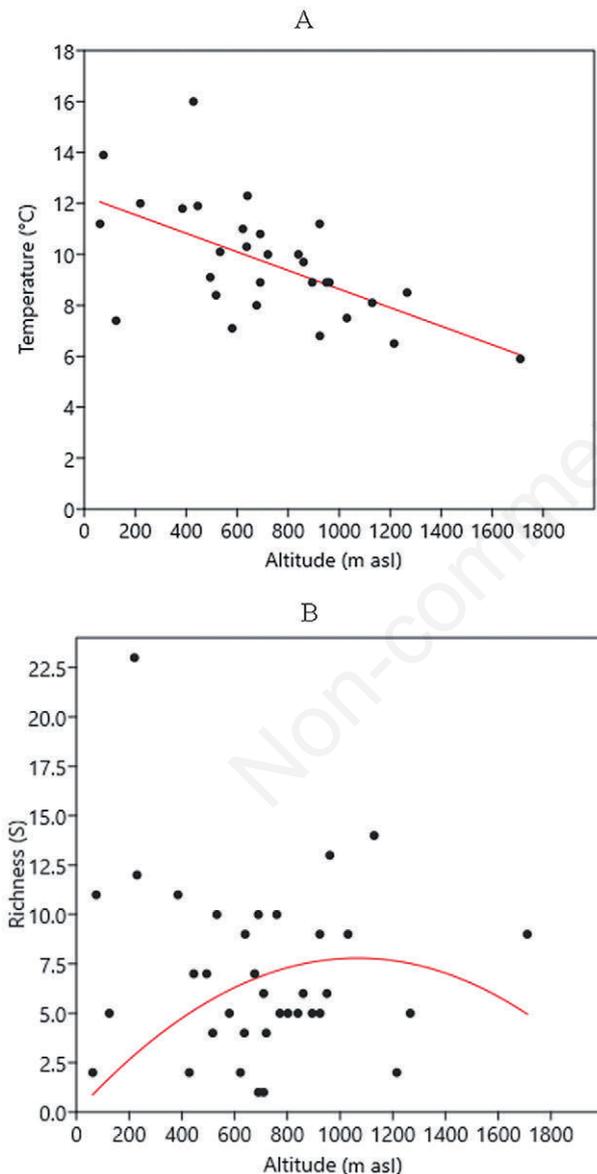
(Tab. 2). Natural springs, represented mainly by the rheocrene and rheohelocrene type, were on average the coldest ( $8.6 \pm 1.5^\circ\text{C}$ ;  $P=0.03$ ) They were found at the highest altitudes (494-1710 m asl;  $P=0.04$ ) and featured the highest discharge ( $0.86 \pm 0.60 \text{ L s}^{-1}$ ,  $P=0.04$ ) and the lowest conductivity ( $384 \pm 135 \mu\text{S cm}^{-2}$ ). Bryophytes were generally

abundant (Tab. 2). Highly impacted springs, represented mainly by the limnocrene type, were warmer ( $11.2 \pm 2.6^\circ\text{C}$ ), -flowing ( $0.42 \pm 0.56 \text{ L s}^{-1}$ ), and all located below 894 m asl. Moderately impacted springs exhibited intermediate values of the main abiotic parameters (Tab. 2).

Considering the environmental features of the thirty-six springs, a clear relationship was found only between altitude and water temperature, *i.e.* water temperature decreasing with increasing altitude (Fig. 2A,  $R^2=0.353$ ,  $P=0.0005$ ).

A total of 111 macroinvertebrate samples were collected, in which 4,168 chironomid larvae and pupae were counted, belonging to five subfamilies (Tanypodinae, Diamesinae, Prodiamesinae, Orthoclaudiinae and Chironominae), 41 genera and 60 species/groups of species (Tab. S1, Tab. S2). Three other taxa were identified as juveniles: Pentaneurini juv., Orthoclaudiinae juv. and Tanytarsini juv., for a total of 63 taxa. Orthoclaudiinae accounted for a large part of specimens (88%) and species (74%), followed by Chironominae Tanytarsini (6% of specimens and 9% of species), Tanypodinae (5% of specimens and 12% of species). Chironominae Chironomini, Diamesinae and Prodiamesinae contributed to less than 1% of the specimens and 5% altogether of the species. In total, twenty-three crenophilous taxa were collected (Table S1) among which *Krenopelopia binotata* (Wiedemann, 1817), *Trissopelopia longimana* (Stæger, 1839), *Chaetocladius dentiforceps* gr., *Chaetocladius piger* gr., *Corynoneura lobata* Edwards, 1924, *Synorthoclaadius semivirens* Kieffer, 1909, *Thienemannia gracilis* Kieffer, 1909, *Stempellinella flavidula* (Edwards, 1929). Most species were frequent also in rhithral (*e.g.*, *Eukiefferiella minorfitzkau*, *Tvetenia calvescens/bavarica* and *Rheocricotopus effuses* (Walker, 1856)) or lentic habitats (*e.g.* *Heterotrissoclaadius marcidus* (Walker, 1956), *Zavreliomyia* sp. and *Macropelopia* spp.).

Most taxa were found in few sites, and frequencies declined gradually for most widely distributed species. Many rare taxa were found (46=74%) with one to 23 taxa per spring. Of these, 27 (44%) occurred in only one spring (Fig. 3A). Among these all Diamesinae species, *Pseudokiefferiella parva* (Edwards, 1932), *Pseudodiamesa branickii* (Nowicki, 1873) and *Prodiamesa olivacea* (Meigen, 1818) were collected at >920 m a.s.l., in rheocrene and rheohelocrene springs. Widespread species had relatively higher abundance than those with restricted distributions, with local abundance of taxa significantly increasing with the number of sites ( $R^2=0.5750$ ;  $P<0.0001$ ) (Fig. 3B). Within exceptions, (species found in a low proportion of sites but abundant) *S. semivirens* accounted alone 21% of all specimens collected in the thirty-six springs. The most common taxa (=present in at least 40% of the sites) were: *T. calvescens/bavarica*, *Parametriocnemus stylatus* (Kieffer,



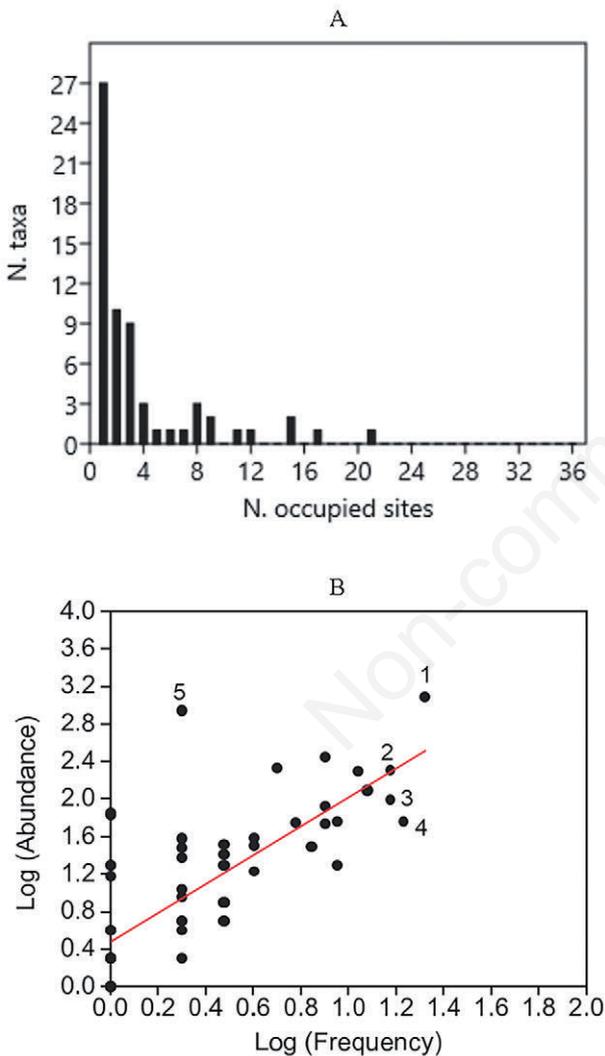
**Fig. 2.** A) Linear relationships between temperature ( $^\circ\text{C}$ ) and altitude (m asl) in the thirty-six springs (red line:  $R^2=0.351$ ,  $P=0.0005$ ). B) Local taxa richness of chironomids in the thirty-six springs as a function of Altitude. Red curve, quadratic fit 2<sup>nd</sup> order polynomial model ( $R^2=0.053$ ,  $P=0.406$ ).

1924), *Micropsectra* spp. and *R. effusus* (Fig. 3B). Among these, *T. calvescens/bavarica* was the most abundant, accounting 30% of the collected specimens.

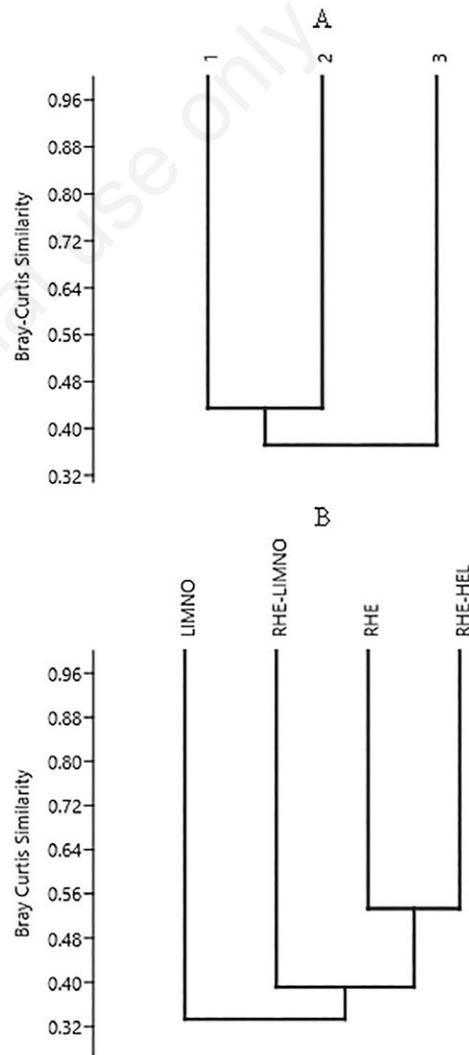
The highest richness (S) occurred at intermediate altitudes (Fig. 2B; quadratic fit 2<sup>nd</sup> order polynomial with a<, R<sup>2</sup>=0.053, P=0.406). It suggests that maximum richness is expected at intermediate altitude.

Maximum richness (S=7±4), diversity (Shannon-

H=1.29±0.60) and evenness (Equitability-J=0.77±0.13) were estimated in moderately disturbed springs located at medium-high altitude (385-1266 m asl) (Tab. 2). Richness and diversity were not significantly correlated with any physical and chemical variables (P>0.05) but diversity decreased with increasing level of disturbance (R<sup>2</sup>=0.112, P=0.046). Highly impacted springs were characterized by lowest richness (S=5±3) and diversity (H=0.74±0.61), and



**Fig. 3.** A) Number of taxa in relation to number of sites occupied. B) Abundance-occupancy relationships (R<sup>2</sup>=0.5750; P<0.0001). 1-4=taxa with a frequency >40%: 1. *Tvetenia calvescens/bavarica*, 2. *Micropsectra* spp., 3. *Rheocricotopus effusus*, 4. *Parametrioctenus stylatus*, 5. *Synorthocladius semivirens*: rare taxon (no. of occupied sites: 2) but abundant (accounting 21% of all specimens collected).



**Fig. 4.** Paired group (UPGMA) clustering based on the Bray-Curtis similarities (ranging from 0=total dissimilarity, to 1=complete similarity). Clustering constrained to preserve row order. Based on species composition (63 taxa). A) 1, natural; 2, moderately disturbed; 3, highly disturbed springs. B) 17 rheocrenes-RHE; 7 limnocrenes-LIMNO; 5 rheohelocrenes-RHE-HEL, 6 rheolimnocrène-RHE-LIMNO.

highest dominance ( $D=0.63\pm0.30$ ) due to the dominance of two species out of the 24 identified, *Paratrichocladius rufiventris/skirwithensis* and *Metriocnemus eurynotus* gr. accounting respectively 39% and 18% of the specimens collected in this group of springs. The rheohelocrene springs were the richest in taxa ( $S=10\pm3$ ), with the highest diversity ( $H=1.60\pm0.47$ ) and the lowest dominance ( $D=0.30\pm0.16$ ) (Tab. 3).

The Cluster Analysis highlighted a higher similarity between assemblages of moderately disturbed and natural springs (Fig. 4A). The index of similarity was 0.43 between groups 1 (=natural) and 2 (=moderately disturbed) and 0.31 between these two groups and group 3 (=highly disturbed). The three groups shared seventeen species. Group 1 and 2 contained forty-four species each, of which twenty-nine were in common, and included species, typically found in rheocene, rheohelocrene and rheolimnocene springs (e.g., *R. effusus*, *Corynoneura lobata* Edwards, 1924, *Orthocladius (Euorthocladius) rivicola* Kieffer, 1921). Highly disturbed springs (group 3), in agriculture areas and impacted by water abstraction, hosted species typical of eutrophic lentic habitats such as *Polypedilum nubeculosum* (Meigen, 1804).

Limnocene springs were separated from the other spring type by the Cluster Analysis (Fig. 4B) with Bray-Curtis similarity of 0.39 with RHE-LIMNO and 0.30 with RHE and RHE-HEL. The two types RHE and RHE-HEL were more similar to each other (Bray-Curtis Index=0.53), sharing twenty-two taxa. Species typical for lentic habitats found in LIMNO and in some RHE-LIMNO were *Macropelopia* spp., *Natarsia* sp., *Paramerina* sp., *Metriocnemus fuscipes* gr. In RHE and RHE-HEL rheophilous species were found (e.g. *E. minor/fittkawi*, *T. calvescens/bavarica* and *R. effusus*).

## DISCUSSION

This study highlighted that the level of disturbance influences richness and diversity in springs. Anthropogenic pressure led to a decrease in both taxon richness and diversity, as expected (Orendt, 2000b; Płóciennik *et al.*, 2016). The main disturbance was represented by water abstraction, associated with bed modification, building of fountains and artificial channels in cement, known to have the worst effect on chironomid biodiversity (Cantonati *et al.*, 2006). Due to such modifications many cold rheocene springs have been transformed in to warmer limnocene springs, with the detriment of rheophilous and cold stenothermal species (mainly within Diamesinae and Orthoclaudiinae) and for the benefit of species typical for lentic habitats, even semi-aquatic such as *Metriocnemus fuscipes* gr., and Tanytarsini.

In accordance with the “intermediate disturbance hypothesis” (Connell, 1978), we found that maximum diversity occurred at intermediate regimes of disturbance in the moderately impacted springs. Barquin and Death (2004) found support for this hypothesis in their work in springs in northwestern Spain. In moderately disturbed springs, not only was Shannon diversity higher but also a higher equitability and lower dominance characterized the chironomid assemblages in respect to the other springs. The mean richness was comparable in the pristine springs (they shared more than 50% of species with the moderately disturbed springs), but they hosted communities with low evenness because most of them were dominated by only one or two taxa. Among these were *T. calvescens/bavarica*, that prevailed in many pristine springs and can be considered ubiquitous (overall

**Tab. 3.** Main physical and chemical features and biodiversity parameters of the thirty-six springs grouped according to their hydromorphological type.

	LIMNO	RHE	RHE-HEL	RHE-HYG	RHE-LIMNO
Altitude (m asl)	562±308	819±359	842±282	637	419±236
Mean level of disturbance	2.6±0.8	1.7±0.8	1.2±0.4	2.0	2.2±0.8
Discharge (L s <sup>-1</sup> )	0.48±0.99	0.93±1.8	0.56±0.40	1.50	0.54±0.61
Temperature (°C)	10.1±1.8	9.1±2.3	9.1±0.7	10.3	11.3±3.3
pH	7.5±0.5	7.6±0.3	7.7±0.2	7.6	7.6±0.3
Conductivity (µS cm <sup>-2</sup> )	409±113	360±129	437±136	400	485±145
Richness (S)	6±4	7±4	10±3	4	8±7
Shannon (H)	1.10±0.62	0.92±0.58	1.60±0.47	1.33	1.28±0.60
Dominance (D)	0.46±0.29	0.57±0.26	0.30±0.16	0.28	0.40±0.24
Equitability (J)	0.76±0.13	0.59±0.21	0.75±0.20	1.0	0.73±0.20

LIMNO, limnocoenens (7); RHE, rheocoenens (17); RHE-HEL, rheohelocoenens (5); RHE-LIMNO, rheolimnocoenens (6); RHE-HYG, rheohydropetric (1).

this taxon was the most frequent and the most abundant in the study sites) and *S. semivirens*, one of the most abundant but rare species, occurring in only two sites (“Covolo dell’acqua”, rheocene, 860 m asl, with coarse substrate; “Gauli”, rheohelocene, at 961 m asl, with medium size substrate and abundant leave mats). This finding and the high number of rare species (74%) found in general, emphasized the highly individual nature of the springs, as stated for other alpine springs, *e.g.*, by Staudacher and Füreder (2007) for Carinthian springs in Austria. This can be explained by the “mosaic” and multiple ecotonal structure (between aquatic-terrestrial (micro)habitats, groundwater-surface water, and crenal-rhithral), of each spring to which faunal richness is related (Lindegaard, 1995; Bottazzi *et al.*, 2011). In fact, where the “mosaic” is more complex, as in mixed types where more microhabitats are present (*e.g.*, mosses, debris layers) (Cantonati *et al.*, 2006), richness is expected to be higher, while limnocene springs generally host fewer species (Smith and Wood, 2002; Smith *et al.*, 2003; von Fumetti *et al.*, 2006). Accordingly, a higher richness and diversity were estimated in the rheohelocene and rheolimnocene springs, as expected due to their highest microhabitat heterogeneity.

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## CONCLUSIONS

It is known that substrate lithology influences spring water chemistry, hydrologic regime, and substrate composition, affecting the faunal assemblages structure (van der Kamp, 1995). Having the same lithology, and all including more hydromorphological types and level of disturbance, no significant difference was found, on average, between the chemical features of the springs in the three mountain areas investigated. Furthermore, notwithstanding the isolation of the three areas that justify the occurrence of many endemics in the terrestrial entomofauna (Latella *et al.*, 2012), no endemics were found within chironomids (Lencioni *et al.*, 2011). The differences we detected are due to level of disturbance and spring type, and can be explained on the basis of chironomid autecology as reported in Eastern Alpine springs (Staudacher and Füreder, 2007) and in Dinaric Mountains springs (Pióciennik *et al.*, 2016), highlighting chemical factors (such as conductivity) and altitude having weak influence on spring invertebrate communities because invertebrates react to a set of natural and altered conditions according to their autecology.

The utility of chironomids as bioindicators for water quality was confirmed, highlighting the fact that some species are associated with high disturbance levels (*e.g.*, by water abstraction; *P. nubeculosum*) and others with pristine conditions (*P. branickii* and *S. semivirens*).

Specifically, *P. nubeculosum* was found in tapped springs with modified bed, with high conductivity due to agricultural sewage inputs as expected (this species is frequent in meso-eutrophic lakes and in depositional zones in streams characterized by high amounts of nutrients; Nocentini, 1985). Also the autecology of *P. branickii* was confirmed, being a cold stenothermal species known from pristine springs and spring brooks rich in bryophytes, even at high altitudes (Lencioni *et al.*, 2011). *S. semivirens* was described as a generalist taxon, a gatherer/collector and grazer living in both flowing and standing waters (Syrovátka and Brabec, 2008). Our results support its preference for coarse substrate and lotic habitats (it was absent in limnocene springs).

Overall these results suggest that each spring can be considered a unique habitat, spatially restricted, and prudent and conservative land management should assume that all springs sheltering such unique faunal assemblages need protection.

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