

## Ephemeroptera, Plecoptera and Trichoptera in springs in Trentino (south-eastern Alps)

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### ABSTRACT

Within the CRENODAT project (Biodiversity assessment and integrity evaluation of springs of Trentino - Italian Alps - and long-term ecological research, 2004-2008) we studied a total of 90 springs in Trentino (south-eastern Alps, Italy), 75 of which were used for statistical analysis. The springs were grouped into seven different types and represented all the available lithologies in the study area. Macrozoobenthos (Ephemeroptera, Plecoptera and Trichoptera; EPT) was collected from stones, bryophytes and sediments. We investigated which physical, chemical or environmental features were important in determining EPT assemblage metrics at sites, by calculating the Shannon-Wiener diversity index, and applying a one-way ANOVA test, ANOSIM and SIMPER analyses. Statistical results highlighted the island character of the springs, each spring having its specific history and abiotic characteristics, which select for unique community patterns. For the faunistic analyses, we considered all springs where EPT taxa were recorded; a total of 88 taxa. Highest species richness was recorded among the Trichoptera, followed by the Plecoptera and Ephemeroptera, with 52, 30 and 6 taxa respectively. Fifteen Trichoptera and three Plecoptera species were recorded as new for the Autonomous Province of Trento. Our results confirm that, in the harsh environment of the Alps, the mild and stable ecological conditions that characterize spring-fed brooks contribute to maintaining and enhancing the regional biodiversity. Springs act as refuge areas for stream biota, providing more favourable conditions during spates or droughts (common in Alpine headwaters), or for particular stages of the insect life cycles. Springs also provide specific habitats for strictly crenobiontic species.

Key words: freshwater ecology, macroinvertebrates, benthos, crenon, headwaters

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### 1. INTRODUCTION

The specific ecological signature of springs is their constant physico-chemical characteristics (Glazier 1998; Williams & Williams 1998). Because springs are located at the interface between two distinct ecosystems (ground-water and surface-water) they create a mosaic of aquatic and semi-aquatic microhabitats, and can be considered as "hot spots" for aquatic biodiversity (Cantonati *et al.* 2006; Staudacher & Füreder 2007). The macroinvertebrate composition of springs can be influenced by various environmental, physical and chemical factors, such as water chemistry (Glazier 1991; Orendt 2000), water current velocity (Ilmonen & Paasivirta 2005; von Fumetti *et al.* 2006), substratum composition (Glazier & Gooch 1987; Hahn 2000) or altitude (Barquín & Death 2006).

In the Alps, springs represent a habitat that is less affected by natural environmental disturbance (floods, droughts, extreme temperatures) than headwater streams (Staudacher & Füreder 2007). Consequently they make an important contribution to the regional biodiversity of freshwater ecosystems (Ward & Tockner 2001; Ilmonen *et al.* 2009) and can be considered as patchy habitat islands (Myers & Resh 2002).

In Italy, faunistic studies have been conducted mainly in north-eastern Alpine and pre-Alpine springs

(Crema *et al.* 1996; Gerecke *et al.* 1998; Cantonati 1998; Stoch & Tomasin 2002; Mezzanotte & Sambugar 2004; Cantonati *et al.* 2006; Sambugar *et al.* 2006; Stoch *et al.* 2008) and in central and southern Apennine areas (Galassi 1997; Cianficconi *et al.* 1998; D'Ambrosio *et al.* 2003; Di Lorenzo *et al.* 2003; Di Sabatino *et al.* 2003; Bottazzi *et al.* 2008).

This study focused on the Ephemeroptera, Plecoptera and Trichoptera (EPT) communities of Alpine springs on the Italian north eastern Alps. The aim was to assess the faunal composition of selected springs, in particular (1) to analyse the distribution patterns of distinct EPT taxa; (2) to assess the EPT assemblages of the springs in the study area; and (3) to assess which physical, chemical or environmental factors are important in determining EPT assemblages and the species richness of each spring.

### 2. METHODS

#### 2.1. Study Area

The study area is located in Trentino (south-eastern Alps, Italy). A total of 108 springs were chosen as a representative subset of the area and sampled. The selected springs covered a wide altitudinal range (from 170 m a.s.l. to 2792 m a.s.l.) (Fig. 1) and their locations are representative of the various lithologies of Trentino: from limestones and dolomites to siliceous substrata.

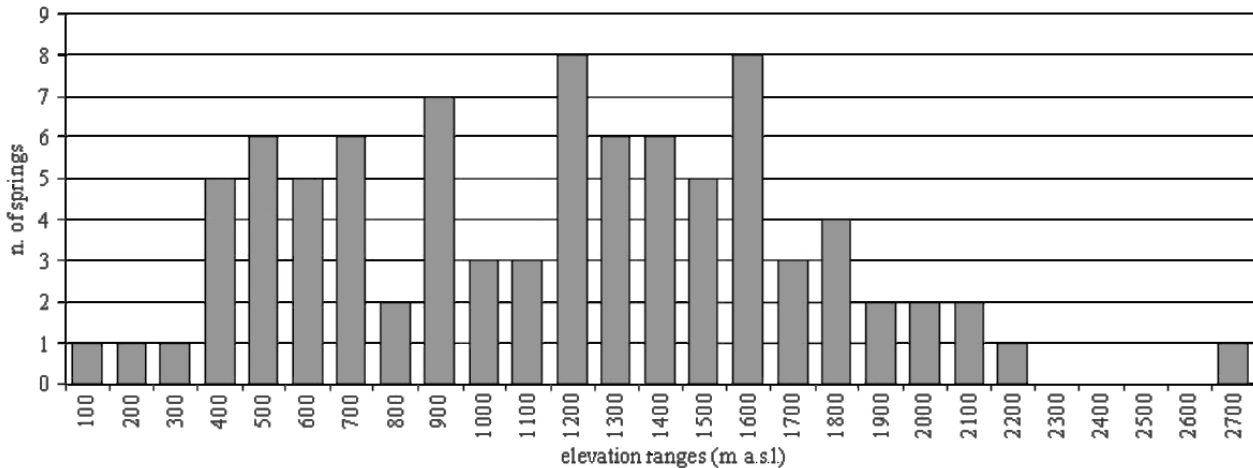


Fig. 1. Number of springs per elevation range, e.g. 100 = 100-199; 200 = 200-299 etc.

For a complete description of the study area and of the environmental dataset see Cantonati *et al.* (2005).

## 2.2. Data collection

EPT taxa were collected in 99 of the 108 springs, but specimens were identified with enough confidence only for the material from 88 sites (due to the presence of only juvenile stages in some samples) and from the two long-term study springs.

Macrozoobenthos was collected separately from stones, bryophytes and sediments in the eucrenal zone of each spring. Specimens living on stones were collected with a 100  $\mu\text{m}$  mesh-size net, disturbing a 0.06  $\text{m}^2$  area. About 10 g of bryophytes and 50 mL of surface sediments were collected and the samples were sorted in the laboratory. Organisms were expressed as number of individuals per  $\text{m}^2$ . Samples were collected for each spring once in the summer season of 2005 and 2006, with the exception of two springs which are part of a long term monitoring project.

A detailed description of the sampling methods is presented in Cantonati *et al.* (2007) Gerecke *et al.* (2011, this issue) and in Spitale *et al.* (2011, submitted).

Specimens were identified to species level according to Lechthaler & Stockinger (2005), Waringer & Graf (1997) and Moretti (1983) for Trichoptera, Fochetti & Tierno de Figueroa (2008) and Consiglio (1980) for Plecoptera, and Belfiore (1983) and Sartori & Landolt (1999) for Ephemeroptera. Adult specimens were identified when available.

## 2.3. Data processing and statistical analysis

In order to be able to compare with other results (Spitale *et al.* 2011, submitted), we ran the statistical analyses using only the 75 springs with identifiable EPT taxa. Non-hierarchical cluster analyses PAM (Partitioning Around Medoids) was applied to the environmental variables dataset to generate the spring types (Spitale *et*

*al.* 2011, submitted). Springs were thus divided into 7 groups: rheocrene springs at low altitude (RhLA), rheocrene springs at high altitude (RhHA), rheocrene springs on siliceous substratum (RhS), large rheocrene springs (RhL), hygropetric springs (Hyg), helocrene springs (Hel), and limnocrene springs (Lim). In particular, according to the analyses performed by Spitale *et al.* (2011, submitted), RhLA differed from RhHA in altitude (the latter were mainly located at higher elevations), in shading percentage (higher in RhLA), current velocity and discharge (higher in RhHA). Rheocrenes on siliceous substrata (RhS) differed from RhLA and RhHA in lower pH and lower degree of shading. RhS were also the group located at highest altitudes. Large rheocrene springs (RhL) were characterized by higher discharge and current velocity, whilst hygropetric rheocrenes (springs with a thin water film flowing over the bedrock, Hyg) presented low discharge but a relatively high velocity. Finally, helocrenes (Hel) and limnocrenes (Lim) differed from all other spring types in substratum composition (sand and clay in these two groups) and from each other by water velocity (Spitale *et al.* 2011, submitted). Lim differed from other spring types, except for RhL, also in higher discharge, while Hel showed the highest percentage of organic substratum.

This classification used a large number of environmental variables and was therefore used as a template to detect possible distributional patterns in Trentino EPT crenal communities.

All springs were sampled in 2005 (Tab. 1), four were also sampled in 2006 and long-term data were available for two, but we chose to perform the statistical analysis using only the data from 2005, considering one sample for each site, due to the lack of a complete dataset for 2006.

As a consequence, only 69 of the total 88 identified taxa were considered in the statistical analyses, while the whole species list was used for faunistic considerations.

**Tab. 1.** List of the 90 springs with EPT taxa, numbers represent species as listed in table 2. Sampling dates are presented for 2005. Besides the two long-term monitored springs, AD2153, LD0509, SC0250 (29-0/8) and BR1315 (6/5; 31/5 and 13/11) were also sampled in 2006. Elevation (m a.s.l.) is shown by the numeric part of the spring names. Lithology as in Cantonati *et al.* (2007).

Spring	EPT species assemblage	2005 sampling dates	Mountain Group	Lithology
AD0905	18;35;61;80;81	11/7	Adamello-Presanella	Ca-Cr
AD1077	2;9;10;30;38;47;70;76	11/7	Adamello-Presanella	Ca
AD1235	47;76	16/8	Adamello-Presanella	Ca
AD1300	1;2;3;4;5;10;13;14;15;16;17;19;23;25;27;29;30 40;41;42;47;56;57;58;67;68;72;73;76; 80;83	long-term monitoring	Adamello-Presanella	Cr
AD1353	23;42;51	11/10	Adamello-Presanella	Cr
AD1654	10;14;25;34	16/8	Adamello-Presanella	Ca
AD1790	51;69	26/10	Adamello-Presanella	Cr
AD1990	17;25;29;34;42;52;	1/9	Adamello-Presanella	Cr
AD2153	2;4;5;6;25;27;31;35;37;41;51;57;60	25/5; 24/10	Adamello-Presanella	Cr
AN0430	44;61	27/6	Anauni	Ca-Cr
AN0590	14;61;81	21/7	Anauni	Ca
AN1474	2;14;17;24;25;27;68;83	21/7	Anauni	Ca
AN1578	23;27	17/10	Anauni	Cr
AN1685	17;23;27;50;52;53;57	18/8	Anauni	Cr
AN1950	6;57;61	27/6;2/9	Anauni	Cr
AT0756	1;3;25;27;30;47	29/6	Vigolana	Ca
AT0972	3;11;14;25;47;61	15/7; 15/9	Vigolana	Ca
AT1052	41;58	11/8	Vigolana	Ca
BC0170	2;3;6;11;61	13/6	Brento-Casale	Ca
BC0503	44	9/7	Brento-Casale	Ca
BC0565	3;66;75;80;81	12/7	Brento-Casale	Ca
BR0470	1;2;4;5;31;35;74	13/7	Brenta	Ca
BR0510	3;7;14;23;25;27;28;44;47;66;88	22/6	Brenta	Ca
BR0658	3;7;27;47;61;66;77;81	1/8	Brenta	Ca-Cr
BR0679	1;12;25;35	3/8	Brenta	Ca
BR0686	35;36	29/11	Brenta	Ca
BR0735	14;18;81	2/8	Brenta	Ca
BR0790	1;2;14;27;47;77;81	12/7	Brenta	Ca
BR0804	14;24;27;41;82	22/7	Brenta	Cr
BR0950	25;27;57	12/7	Brenta	Ca-Cr
BR1315	3;23;25;28;45;56;57;65;76;83	31/5	Brenta	Ca
BR1358	1;3;4;9;10;12;14;15;17;20;21;25;29;31;35; 41;46;48;49;51;58;59;71;74;75;76;79;80	long-term monitoring	Brenta	Ca
BR1379	12;17;27;35;52;57;68	22/9; 4/11	Brenta	Ca
BR1436	24;27;78	17/8	Brenta	Ca
BR1605	14;22;23;25;76;79;86	17/8	Brenta	Ca
BR1765	27	26/10	Brenta	Ca
BR2240	24	10/10	Brenta	Ca
BS0420	27;39	29/7	Bondone-Stivo	Ca
BS0705	14;27;47;66;77	8/7	Bondone-Stivo	Ca
BS1527	6;24;66	24/8	Bondone-Stivo	Ca
CA1642	17;25	15/9	Lagorai-Cima d'Asta	Cr
CA2153	43;54;76	14/9	Lagorai-Cima d'Asta	Cr
CS1350	24;30;52;62	9/8	Catinaccio	Ca
CV0250	25;42	28/6	Lagorai-Cima d'Asta	Cr
CV0633	27;67;82	4/7	Lagorai-Cima d'Asta	Cr
CV0854	14;61;68	5/7	Lagorai-Cima d'Asta	Cr
CV0962	14;25;57;64	8/8	Lagorai-Cima d'Asta	Cr
CV0992	1;2;3;17;27;30;34;35;80	20/7	Lagorai-Cima d'Asta	Cr
CV1084	23;25;27;34;47	27/7	Lagorai-Cima d'Asta	Ca-Cr
CV1200	27;35;54;57;63;79	19/7	Lagorai-Cima d'Asta	Cr
CV1215	25;27;41;68	8/8	Lagorai-Cima d'Asta	Cr
CV1254	24;32;35;43	8/8	Lagorai-Cima d'Asta	Cr
CV1280	12;47;57;83	10/8	Lagorai-Cima d'Asta	Cr
CV1421	25;42;44;57;85	21/9	Lagorai-Cima d'Asta	Cr
CV1435	2;17;23;26;27;34;35;41;51;57;68	28/7	Lagorai-Cima d'Asta	Cr
CV1575	28	21/9	Lagorai-Cima d'Asta	Cr
CV1623	2;14;41	18/8	Lagorai-Cima d'Asta	Cr
CV1655	10;57;76	22/9	Lagorai-Cima d'Asta	Cr
CV1685	25;57;68	23/8	Lagorai-Cima d'Asta	Cr
CV1855	2;5;17;41;57	19/8	Lagorai-Cima d'Asta	Cr

(continued)

Tab. 1. Continuation.

Spring	EPT species assemblage	2005 sampling dates	Mountain Group	Lithology
CV2051	5;54	23/8	Lagorai-Cima d'Asta	Cr
CV2126	24;25	21/9	Lagorai-Cima d'Asta	Cr
LD0420	7;14;27;55	21/6	Alpi di Ledro	Ca
LD0509	14;27;28;44;45;47;81	6/7	Alpi di Ledro	Ca
LD0584	8;14;25;35;58;81	6/7	Alpi di Ledro	Ca
LD0720	14;17;27;81	7/9	Alpi di Ledro	Ca
LD0930	25;27;30;45;76;87	29/6	Alpi di Ledro	Ca
LD1160	10;24;25;27;76;84	26/7	Alpi di Ledro	Ca
LD1400	3;14;41	26/7	Alpi di Ledro	Ca
LD1502	23;41	17/7	Alpi di Ledro	Ca
LT1240	1;27;35;44;47;57;70;77	18/7	Latemar	Ca-Cr
MB0335	1	15/6	Monte Baldo	Ca
MB1440	57	2/11	Monte Baldo	Ca
MC1115	24;35;47;61	12/8	Monte Corona	Ca
MD1670	37	26/9	Marmolada	Ca-Cr
MD1871	24;27;35;43;77	26/9	Marmolada	Ca
MP0656	3;12;54;83;84	25/7	Pasubio	Ca
MP1566	27	2/11	Pasubio	Ca
OC0981	65	22/7	Ortles-Cevedale	Cr
OC2056	1;35;80	4/11	Ortles-Cevedale	Cr
OC2792	37;51;56;68	27/9	Ortles-Cevedale	Cr
PG0453	11;25;27;35;47;61	27/6	Paganella-Gazza	Ca
PG0474	2;3;34;42;61;81;83	14/6	Paganella-Gazza	Ca
PS1250	69	18/10	Pale di S.Martino	Ca
PS1255	69;81	20/9	Pale di S.Martino	Ca
PS1880	9;47;52;54;76	18/8	Pale di S.Martino	Ca-Cr
SC0250	14;23;24;25;28;61;83	28/6	Sette Comuni	Ca
SL1724	41	9/8	Sella	Ca
VF0745	16;30;69	20/9	Vette Feltrine	Ca
VZ1178	12;23;24;42;57;82;83	8/8	Viezzena	Ca

The two long-term survey springs were analyzed separately and were excluded from the statistical analysis.

All taxa were identified to species level, except for the *Rhithrogena* species-groups (*hybrida* and *lyolalae*) and *Ecdyonurus* gr. *helveticus*, because of poor specimen preservation, and *Rhyacophila sensu strictu* (Waringer & Graf 1997), due to the lack of adult specimens (taxonomic keys do not identify larval instars of this group to species level).

To test for significant differences among the seven spring types, we used one-way ANOVA on log (x+1) transformed abundance (N) and taxonomic richness (S) data, and on the Shannon-Wiener diversity index calculated from non-transformed data. Prior to analysis, we checked for normality using a Shapiro-Wilk Test, and for homoscedasticity (homogeneity of variance) using a Levene and Cochran Test. Subsequently, the Tukey post-hoc Test was used on those variables that were significantly different to find which pairs of spring types were significantly different. We performed a Canonical Correlation analysis (CANCOR) to identify any existing correlation between abundance, species richness and the environmental variables that mostly contribute to separate the spring group types (altitude, pH, shading, current velocity, discharge, substratum composition).

A NMDS (non-metric multidimensional scaling) analysis (Clarke & Gorley 2006) of the EPT taxa assemblages was performed to highlight possible patterns based on the spring typologies, after log (x+1)

transformation, using a Bray-Curtis similarity resemblance matrix. Outliers (6 springs) were removed from the ordination to reduce the stress (goodness of fit of the regression) of the analysis.

Differences in EPT assemblage patterns in the different spring typologies were tested by running an Analysis of Similarities (ANOSIM) based on the Bray-Curtis distance matrix (one-way ANOSIM, factor: spring typology). A SIMPER (Similarity Percentage) analysis was performed to detect how much two groups of springs differed, and which taxa contributed to the similarity within groups and dissimilarity between groups.

Analyses were performed using Primer 6 ver. 6.1.6 (Clarke & Gorley 2006), Statistica ver. 8.1 (StatSoft Inc. 2008).

### 3. RESULTS

#### 3.1. EPT taxa distribution

For the faunistic analyses we considered all 90 springs for which EPT taxa were identified, 88 taxa in total. Highest species richness was recorded among the Trichoptera (52 taxa), followed by the Plecoptera (39) and Ephemeroptera (6). All identified taxa are listed in table 2.

The EPT faunistic results from the CRENO DAT project were compared to the known distribution of the Italian fauna (Stoch 2000-2005).

**Tab. 2.** EPT species list of the 90 springs sampled in the CRENODAT project. n. = identification number of species, Ab. = abundance (ind m<sup>-2</sup>), Fr. = frequency in springs, Ca = relative frequency in carbonate springs, Ca-Cr = relative frequency in carbonate-crystalline springs, Cr = relative frequency in crystalline springs, elev. = relative frequency in springs above 1300 m a.s.l., min. elev. = minimum elevation of colonised spring, max. elev. = maximum elevation of colonized spring, \* = new for Trentino, according to the Italian check list (Stoch, 2000-2005).

n.	Species	Ab.	Fr.	Ca	Ca-Cr	Cr	elev.	min. elev.	max. elev.
<b>Ephemeroptera</b>									
1	<i>Baetis alpinus</i> (PICTET, 1843)	48	10	50	20	30	30	335	2056
2	<i>Baetis rhodani</i> (PICTET, 1843)	37	12	50		50	50	170	2153
3	<i>Ecdyonurus</i> gr. <i>helveticus</i>	121	13	77	8	15	31	170	1400
4	<i>Rhitrogena</i> gr. <i>hybrida</i>	9	4	50		50	75	470	2153
5	<i>Rhitrogena</i> gr. <i>loyolae</i>	17	6	33		67	83	470	2153
6	<i>Serratella ignita</i> (PODA, 1761)	10	4	50		50	75	170	2153
<b>Plecoptera</b>									
7	<i>Amphinemura sulcicollis</i> (STEPHENS, 1836)	15	3	67	33			420	658
8	<i>Capnia vidua</i> * Klapálek, 1904	1	1		100			586	586
9	<i>Dictyogenus alpinus</i> (PICTET, 1842)	32	3	67	33		67	1077	1880
10	<i>Dictyogenus fontium</i> (RIS, 1896)	18	6	67		33	67	950	2051
11	<i>Dinocras ferri</i> (PICTET, 1841)	26	3	100				170	972
12	<i>Isoperla rivulorum</i> (PICTET, 1842)	11	6	67	17	17	33	656	1379
13	<i>Leuctra alpina</i> KUEHNTREIBER, 1934	1	1			100	100	1300	1300
14	<i>Leuctra braueri</i> KEMPNY, 1898	241	21	71	5	24	33	250	2051
15	<i>Leuctra helvetica</i> AUBERT, 1956	6	2	50		50	100	1300	1358
16	<i>Leuctra inermis</i> KEMPNY, 1899	7	2	50		50	50	656	1300
17	<i>Leuctra major</i> BRINCK, 1949	14	11	36		64	82	720	1990
18	<i>Leuctra moselyi</i> MORTON, 1929	51	2	50	50			735	905
19	<i>Leuctra niveola</i> * SCHMID, 1947	1	1			100	100	1300	1300
20	<i>Leuctra rauscheri</i> AUBERT, 1957	3	1	100			100	1358	1358
21	<i>Leuctra rosinae</i> KEMPNY, 1900	1	1	100			100	1358	1358
22	<i>Leuctra teriolensis</i> KEMPNY, 1900	9	1	100			100	1605	1605
23	<i>Nemoura cinerea</i> (RETZIUS, 1783)	157	13	46	8	46	69	250	1853
24	<i>Nemoura flexuosa</i> AUBERT, 1949	38	13	77		23	54	250	2240
25	<i>Nemoura mortoni</i> (RIS, 1902)	178	25	46	15	39	48	250	2153
26	<i>Nemoura</i> (cf.) <i>obtusa</i> (RIS, 1902)	2	1		100		100	1435	1435
27	<i>Nemoura sinuata</i> (RIS, 1902)	176	30	55	13	32	37	420	2153
28	<i>Nemurella pictetii</i> Klapálek, 1900	37	6	67		33	50	250	2182
29	<i>Perlodes microcephalus</i> (PICTET, 1833)	4	3	33		67	100	1300	1990
30	<i>Protonemura auberti</i> ILLIES, 1954	16	7	71		29	29	745	2153
31	<i>Protonemura brevistyla</i> (RIS, 1902)	11	3	68		33	67	470	2153
32	<i>Protonemura caprai</i> * (AUBERT, 1954)	1	1			100		1254	1254
33	<i>Protonemura intricata</i> (RIS, 1902)	3	2	50		50	100	1358	2153
34	<i>Protonemura lateralis</i> (PICTET, 1836)	15	6	33	17	50	50	474	1990
35	<i>Protonemura nimborum</i> (RIS, 1902)	48	17	41	24	35	35	453	2153
36	<i>Protonemura</i> (cf.) <i>nitida</i> (PICTET, 1836)	1	1			100	100	686	686
<b>Trichoptera</b>									
37	<i>Acrophylax zerberus</i> * BRAUER, 1867	4	3		33	67	100	1670	2792
38	<i>Agapetus fuscipes</i> CURTIS, 1834	6	1		100			1077	1077
39	<i>Agapetus nimbulus</i> MCLACHLAN, 1879	1	1		100			420	420
40	<i>Allogamus hilaris</i> * (MCLACHLAN, 1876)	2	1			100	100	1300	1300
41	<i>Allogamus uncutus</i> (BRAUER, 1857)	44	12	42		58	75	804	2153
42	<i>Chaetopteryx fusca</i> BRAUER, 1857	12	7	29		71	57	250	1990
43	<i>Consorophylax consors</i> * (MCLACHLAN, 1880)	4	3	25		75	67	1215	2153
44	<i>Crunoecia irrorata</i> * (CURTIS, 1834)	10	6	50	33	17	17	430	1421
45	<i>Crunoecia</i> (cf.) <i>kempnyi</i> * MORTON, 1901	4	3	100			33	509	1315
46	<i>Cryptothrix nebulicola</i> MCLACHLAN, 1867	3	1	100			100	1358	1358
47	<i>Diplectrona</i> (cf.) <i>felix</i> * MCLACHLAN, 1878	79	16	63	25	13	13	453	1880
48	<i>Drusus annulatus</i> MCLACHLAN, 1878	27	1	100			100	1358	1358
49	<i>Drusus</i> (cf.) <i>biguttatus</i> (PICTET, 1834)	1	1	100			100	1358	1358
50	<i>Drusus chrysotus</i> (RAMBUR, 1842)	1	1			100	100	1685	1685
51	<i>Drusus destitutus</i> KOLENATI, 1848	38	6	17		83	100	1353	2792
52	<i>Drusus discolor</i> (RAMBUR, 1842)	70	5	40	20	40	100	1350	1990
53	<i>Drusus monticola</i> MCLACHLAN, 1876	1	1			100	100	1685	1685
54	<i>Ecclisopteryx</i> (cf.) <i>asterix</i> * MALICKY, 1979	16	5	20	20	60	60	656	2153
55	<i>Ernodes articularis</i> (PICTET, 1834)	1	1	100				420	420
56	<i>Halesus rubricollis</i> (PICTET, 1834)	18	4	33		67	100	1300	2792

(continued)



Tab. 2. Continuation.

n.	Species	Ab.	Fr.	Ca	Ca-Cr	Cr	elev.	min. elev.	max. elev.
57	<i>Lithax niger</i> (HAGEN, 1859)	42	17	22	11	67	65	950	2153
58	<i>Melampophylax</i> (cf.) <i>melampus</i> * (MCLACHLAN, 1876)	17	4	50	25	25	50	586	1358
59	<i>Metanoea rhaetica</i> SCHMID, 1955	8	1	100			100	1358	1358
60	<i>Micropterna sequax</i> MCLACHLAN, 1875	1	1			100	100	2153	2153
61	<i>Odontocerum albicorne</i> (SCOPOLI, 1769)	59	12	58	25	17	8	170	1950
62	<i>Parachiona picicornis</i> (PICTET, 1834)	1	1	100			100	1350	1350
63	<i>Philopotamus ludificatus</i> MCLACHLAN, 1878	7	1			100		1200	1200
64	<i>Plectrocnemia brevis</i> MCLACHAN, 1871	1	1			100		962	962
65	<i>Plectrocnemia conspersa</i> (CURTIS, 1834)	4	2	50		50	50	981	1944
66	<i>Plectrocnemia geniculata</i> MCLACHLAN, 1871	14	5	80	20		20	510	1527
67	<i>Potamophylax</i> (cf.) <i>cingulatus</i> (STEPHENS, 1837)	5	3	33		67	67	250	1665
68	<i>Pseudopsilopteryx zimmeri</i> * (MCLACHLAN, 1876)	15	8	25,0		75,0	75	854	2792
69	<i>Ptilocolopus granulatus</i> (PICTET, 1834)	5	4	75		25	25	745	1790
70	<i>Rhyacophila bonaparti</i> SCHMID, 1947	2	2	50	50			1077	1240
71	<i>Rhyacophila</i> (cf.) <i>dorsalis</i> (CURTIS, 1834)	5	1	100			100	1358	1944
72	<i>Rhyacophila glareosa</i> MCLACHLAN, 1867	1	1			100	100	1300	1300
73	<i>Rhyacophila hirticornis</i> MCLACHLAN, 1879	1	1			100	100	1300	1300
74	<i>Rhyacophila intermedia</i> MCLACHLAN, 1868	3	2	100			50	470	1358
75	<i>Rhyacophila</i> (cf.) <i>obliterata</i> * MCLACHLAN, 1863	4	3	100			67	565	1665
76	<i>Rhyacophila</i> (cf.) <i>producta</i> * MCLACHLAN, 1879	40	12	64	9	27	67	930	2792
77	<i>Rhyacophila pubescens</i> PICTET, 1834	19	5	60	40		20	658	1871
78	<i>Rhyacophila stigmatica</i> (KOLENATI, 1859)	1	1	100			100	1436	1436
79	<i>Rhyacophila tristis</i> PICTET, 1834	12	3	67		33	67	1200	1605
80	<i>Rhyacophila</i> (cf.) <i>vulgaris</i> PICTET, 1834	76	7	33	17	50	57	565	2056
81	<i>Sericostoma personatum</i> (KIRBY & SPENCE, 1876)	24	11	73	27			474	1255
82	<i>Silo nigricornis</i> (PICTET, 1834)	3	3	33		67		633	1178
83	<i>Synagapetus dubitans</i> MCLACHLAN, 1879	26	8	75		25	38	250	1474
84	<i>Synagapetus</i> (cf.) <i>krawanyi</i> * (ULMER, 1838)	4	2	100				656	1160
85	<i>Tinodes dives</i> (PICTET, 1834)	1	1			100	100	1421	1421
86	<i>Tinodes maclachlani</i> * KIMMINS, 1966	10	1	100			100	1605	1605
87	<i>Tinodes pallidulus</i> * MCLACHLAN, 1878	4	1	100			0	930	930
88	<i>Wormaldia occipitalis</i> * (PICTET, 1834)	1	1	100				510	510

Fifteen of the Trichoptera and three of Plecoptera species were new for the Trentino province, but known from other areas of the Italian Alps.

With respect to the altitudinal range, nine EPT species were found above their previously known altitudinal limit. Among these were the Trichoptera, *Ecclisopteryx* (cf.) *asterix* and *Dipletrona* (cf.) *felix*, and the Ephemeroptera, *Serratella ignita* and *Baetis rhodani*, which are rhithral species also recorded from springs (Bauernfeind *et al.* 2002). The Trichoptera *Acrophylax zerberus* was also recorded at 1670 m a.s.l., below its previously known altitudinal range in the Italian Alps (2000-2700 m a.s.l. (Stoch 2000-2005) (Tab. 2).

The most frequent EPT taxa encountered were the Plecoptera, *Nemoura sinuata*, *N. mortoni*, *Leuctra braueri* and *Protonemura nimborum*, recorded in 30, 25, 21 and 17 springs, respectively. *Lithax niger* and *Dipletrona* (cf.) *felix* were more widespread Trichoptera, found in 17 and 16 springs, respectively. The most frequent Ephemeroptera taxa were *Ecdyonurus* gr. *helveticus* (present in 13 springs), *Baetis rhodani* (in 12 springs) and *B. alpinus* (in 10 springs).

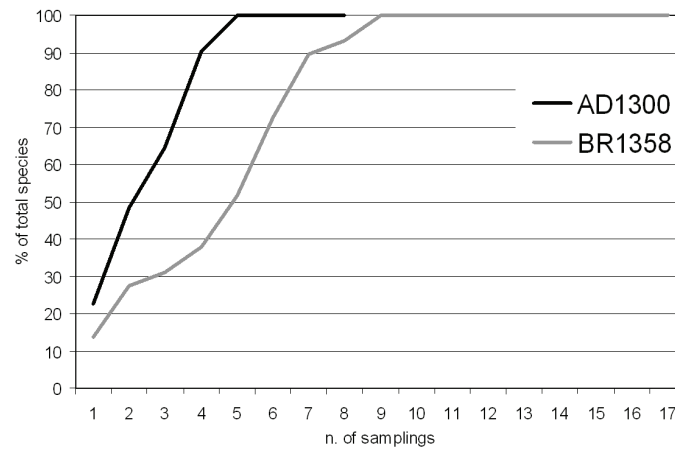
The two long-term monitoring springs (AD1300 and BR1358 of the RhHA type) contributed 13 taxa, that were not found in other springs, to the CRENOTAT EPT species list. These two springs are situated on the opposite sides of the Rendena Valley, at similar elevation (1330 and 1358 m a.s.l. respectively) and differ

mainly in their geological setting: siliceous rocks for AD1300 (Adamello-Presanella Mountain Group), and carbonate strata for BR1358 (Brenta Mountain Group). AD1300 and BR1358 hosted a total of 31 and 29 EPT species, respectively. The maximum taxonomic richness was attained in AD1300 after five sampling occasions, and in BR1358 after nine (Fig. 2).

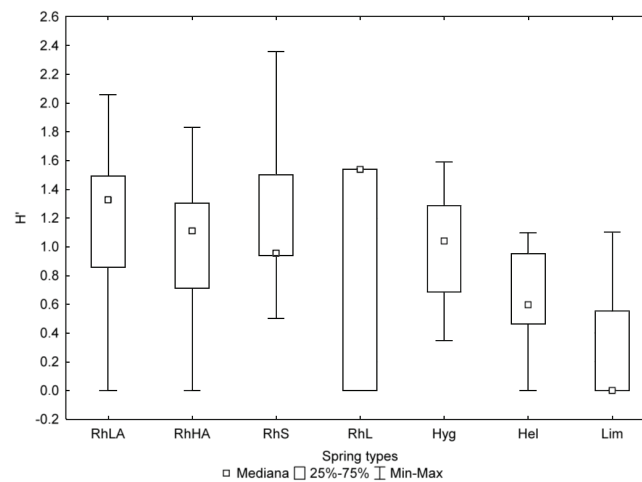
A total of 47 EPT species was recorded in the two long-term springs, but only 13 species were common; 18 were found only at AD1300 and 16 only at BR1358. None of the species found only in the carbonate spring (BR1358) were prevalent in the other short term carbonate springs.

In the same way, the species found in AD 1300 but not in BR1358 were not more frequent in other siliceous springs than in the other carbonate ones.

Some preferences, however, appeared for few species in the dataset. For example, some taxa were more abundant in carbonate springs, such as, *Leuctra braueri*, recorded in 21 springs 71% of which were carbonate, *Ecdyonurus* gr. *helveticus* (13 springs, 77% carbonate), *Sericostoma personatum* (11 springs, 73% carbonate), *Nemoura flexuosa* (13 springs, 77 carbonate) and *Synagapetus dubitans* (8 springs, 75% carbonate). On the other hand, some taxa favoured springs on siliceous substrata: *Lithax niger* (present in 17 springs, 67% siliceous) and *Leuctra major* (11 springs, 64% siliceous).



**Fig. 2.** EPT Species accumulation curves (% of total species) relative to the two long-term monitored springs AD1300 (7 samplings) and BR1358 (17 samplings).



**Fig. 3.** Box plot of the Shannon-Wiener diversity index among spring types.

The springs were quite evenly distributed along the altitudinal range, with the median value around 1300 m a.s.l. (Fig. 1). We used this arbitrary threshold to separate species according to their altitudinal preferences. Of the 90 springs considered, 48 (55%) were below and 40 (45%) above this threshold. Of the 88 taxa, only 16 (18%) were restricted to lower elevation springs, five (17%) Plecoptera and 11 (21%) Trichoptera, while 29 (33%) were found exclusively in higher ones, 10 (33%) Plecoptera and 19 (37%) Trichoptera. Among these, *Halesus rubricollis*, *Drusus discolor* and *D. destitutus* were relatively common in four, five and six high elevation springs, respectively. None of the six Ephemeroptera taxa were restricted to above or below the threshold, although *Rhitrogena* gr. *loyolaea*, *R.* gr. *hybrida* and *Serratella ignita* were more widespread at higher elevations and *Ecdyonurus* gr. *helveticus* and *Baetis alpinus* at lower elevations. The highest spring (2792 m a.s.l.) hosted only four species of Trichoptera: *Acrophylax zerberus*, *Halesus rubricollis*, *Drusus destitutus* and *Pseudopsilopteryx zimmeri*.

### 3.2. Statistical analysis

Using Anova, the Shannon-Wiener diversity index differed significantly among spring types ( $F = 2.3991$ ,  $p = 0.036$ ; Fig. 3), with the RhL group having the highest median value. However, a significant difference was only recorded between RhLA and Lim types ( $p = 0.049$ ). Taxonomic richness also differed significantly between groups ( $p = 0.026$ ), most significantly between RhLA and Lim ( $p = 0.031$ ), but also significantly between RhS and Lim ( $p = 0.050$ ) (Fig. 4). The difference in EPT abundance was highly significant ( $F = 3.1374$ ,  $p < 0.001$ ). In particular, RhLA, RhHA, RhS, and Hyg differed significantly or very significantly from Lim springs [ $p = 0.005$ ;  $p = 0.010$ ;  $p = 0.040$ ;  $p = 0.010$ , (Fig. 5) respectively].

The CANCOR analysis results were non-significant ( $R = 0.36$ ;  $p = 0.64$ ). Abundance and species richness were weakly correlated with the environmental variables, i.e. altitude, pH, shading, current velocity, dis-

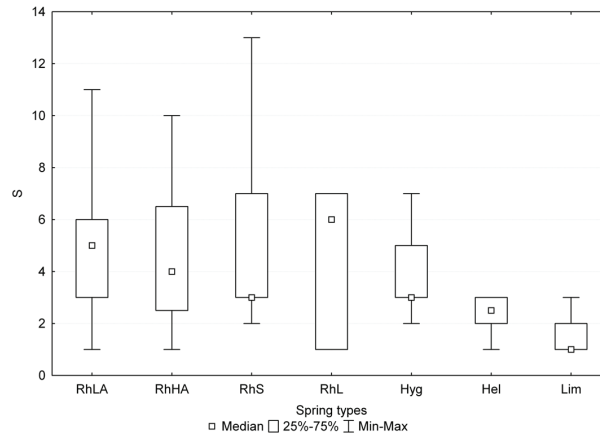


Fig. 4. Box plot of the taxonomic richness among spring types.

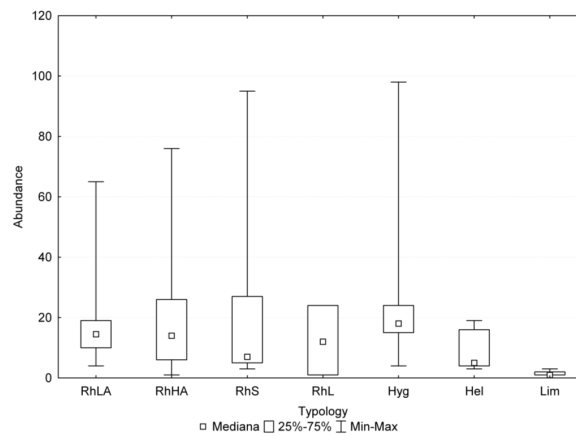


Fig. 5. Box plot of the abundances among spring types.

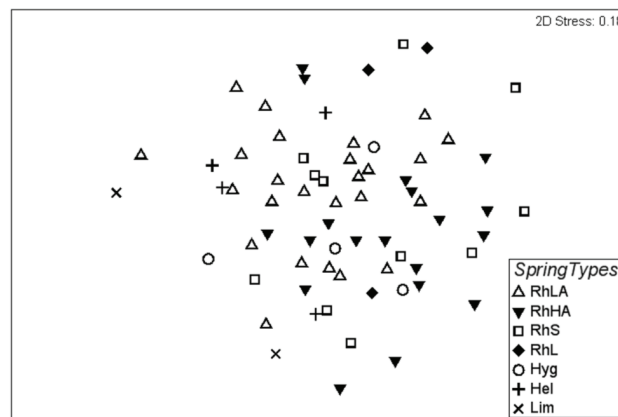


Fig. 6. NMDS (non-metric multidimensional scaling) analyses of EPT taxa assemblages according to the spring typologies.

charge, and substratum composition (Maximum correlation coefficient  $R = 0.16$ ).

The MDS ordination of the total invertebrate assemblage did not show a clear spatial trend in community composition (Fig. 6). However, some differences could be highlighted. The two Lim spring types (the other two were excluded as outliers) are separated from the other

groups, in particular from rheocrenal groups. The RhS group lies mostly inside the RhHA group, which is quite clearly separated from RhLA. Hel is enclosed in RhLA, while Hyg and RhL do not seem to form clear patterns.

The one-way ANOSIM (Tab. 3) showed a significant difference in species assemblages among spring typologies ( $R = 0.136$ ;  $p < 0.001$ ); the pairs of springs



which had high  $R$  values and differed significantly ( $p < 0.05$ ) were: Lim vs RhS, Lim vs RhLA, Lim vs RhHA, RhS vs RhLA, RhHA vs Hel and RhLA vs RhHA.

**Tab. 3.** ANOSIM pairwise comparison between the groups. The bold values have significant  $R$  and significant differences ( $p < 0.05$ ). ANOSIM analysis is slightly significant for  $R$  value ( $R = 0.136$ ,  $p < 0.001$ ).

Pairwise comparison between groups	$R$	$p$
<i>RhHA, Lim</i>	<b>0.327</b>	<b>0.003</b>
<i>RhHA, Hel</i>	<b>0.177</b>	<b>0.03</b>
<i>RhHA, RhS</i>	-0.028	0.675
<i>RhHA, RhLA</i>	<b>0.061</b>	<b>0.043</b>
<i>RhHA, RhL</i>	-0.048	0.618
<i>RhHA, Hyg</i>	0.019	0.411
<i>Lim, Hel</i>	0.004	0.648
<i>Lim, RhS</i>	<b>0.222</b>	<b>0.036</b>
<i>Lim, RhLA</i>	<b>0.444</b>	<b>0.001</b>
<i>Lim, RhL</i>	0.111	0.429
<i>Lim, Hyg</i>	-0.075	0.762
<i>Hel, RhS</i>	0.096	0.14
<i>Hel, RhLA</i>	0.159	0.074
<i>Hel, RhL</i>	0.056	0.345
<i>Hel, Hyg</i>	0.009	0.396
<i>RhS, RhLA</i>	<b>0.202</b>	<b>0.003</b>
<i>RhS, RhL</i>	-0.113	0.821
<i>RhS, Hyg</i>	0.083	0.207
<i>RhLA, RhL</i>	0.202	0.08
<i>RhLA, Hyg</i>	0.175	0.068
<i>RhL, Hyg</i>	0.015	0.5

The similarities within groups were very low (SIMPER analysis, table 4). The lowest dissimilarity was recorded for RhLA and RhS (Bray-Curtis dissimilarity: 91.58 and 94.15 respectively), followed by RhHA, RhL and Hyg springs (Bray-Curtis dissimilarity: 95.25, 97.22, 97.55 respectively). Finally, Hel showed the highest dissimilarity (97.64). Dissimilarity between spring types was mostly explained by species with low abundance.

**Tab. 4.** Results of SIMPER analysis; factor: spring typology. RhLA: rheocrene springs at low altitude; RhHA: rheocrene springs at high altitude; RhS: rheocrene springs on siliceous substratum; RhL: large rheocrene springs; Hyg: hygropetric springs; Hel: helocrene springs; Lim: limnocrene springs. *Leubra* = *Leuctra brueri*, *Rhypro* = *Rhyacophila* (cf.) *producta*, *Litnig* = *Lithax niger*, *Pronim* = *Protonemura nimborum*, *Nemcin* = *Nemoura cinerea*.

Group	Similarity %	Main Species	Contribution %
<i>RhLA</i>	9.30	<i>Leubra</i>	21.12
<i>RhHA</i>	4.75	<i>Rhypro</i>	19.10
<i>RhS</i>	5.85	<i>Litnig</i>	14.66
<i>RhL</i>	2.78	<i>Pronim</i>	100.00
<i>Hyg</i>	2.40	<i>Nemcin</i>	57.35
<i>Hel</i>	2.37	<i>Leubra</i>	70.37
<i>Lim</i>	-	-	-

## 4. DISCUSSION

### 4.1. Species assemblages

The springs were classified into seven groups based on the environmental variables (Spitale *et al.* 2011, submitted). However, the pattern of EPT species richness did not match this classification: only the limnocrene springs differed significantly from the RhLA springs. These two groups differed mainly in substratum composition (Spitale *et al.* 2011, submitted), which is known to be a main driver for macroinvertebrate assemblages (Ilmonen & Paasivirta 2005; von Fumetti *et al.* 2006). As described by von Fumetti *et al.* (2006), substratum composition is paramount in determining species assemblages patterns, with discharge and water velocity acting as substratum selectors. These authors also suggest that leaf litter is one of the most important substratum components for macroinvertebrate assemblage in springs. As also observed by Ilmonen & Paasivirta (2005), who associated limnocrene springs with low diversity, springs of this type (except for one case) supported very low EPT diversity, and the data were not suitable for statistical analysis.

The differences in abundance between some of the spring groups were significant. The higher abundance of EPT taxa in rheocrene (with the exception of RhL) and hygropetric, compared with limnocrene springs highlights the importance of discharge for the crenic fauna. These two groups differed mainly in altitude, but also in discharge, which was much higher in Lim. Altitude is generally considered to be an important factor for macroinvertebrate taxonomic richness, but not for taxon abundances (Barquín & Death 2006). On the other hand, discharge and habitat stability (which can also be dependent on discharge), are considered as predictors of abundance and richness (Hoffsten & Malmqvist 2000; Ilmonen & Paasivirta 2005; Barquín & Death 2006; von Fumetti *et al.* 2006). In this study, the CANCOR analyses did not identify any good predictors for EPT species richness and abundance. In fact, the correlation between these two variables and the considered environmental drivers was very low and not significant, suggesting that no clear gradient was present.

The Hyg group differed significantly in abundance from the Lim group. The two spring groups differed mainly in water velocity, higher in hygropetric springs, and discharge, higher in limnocrene springs. Limnocrene springs had very low species richness, as also reported by other authors (e.g., Ilmonen & Paasivirta 2005).

The MDS ordination did not identify a very clear pattern in EPT spring assemblages, although some separations were highlighted. The ANOSIM analysis gave a significant result, but the  $R$  value was low, indicating that EPT species assemblages often overlapped (Clarke & Gorley 2006). The limnocrene springs were significantly different from most rheocrene springs. Also, as above, high discharge values in limnocrene springs

seemed to act as substratum sorting and species assemblages drivers (von Fumetti *et al.* 2006). The two spring types characterized by higher discharge (Lim and RhL) were not significantly different. RhS differed significantly from RhLA, in altitude, and also pH and substratum (siliceous vs carbonate). However, pH and substratum also differed between RhS and RhHA, although they were not significantly different in their species assemblages. Consequently, altitude seemed to be the main variable separating these two groups. This agrees with Zöllhöfer (1999) and Barquín & Death (2009), who considered altitude a predictor of species richness and species composition.

Hel springs were significantly different from RhHA groups. In this case, the environmental variables which differed the most were substratum composition (mainly clay and sand in Hel, stones and rocks in RhHA), although substratum composition of Hel also differs from that of other rheocrene groups. However, these two groups were different also in the organic substratum, representing the two extreme values for the dataset (highest in Hel, lowest in RhHA); differences in organic matter can lead to significantly different habitat preferences for different species (Scarsbrook *et al.* 2007).

With the SIMPER analysis, we did not detect any consistent EPT species assembly pattern related to the spring typology, as shown by the low similarity recorded within each group of species. Some taxa contributed to the similarity within the springs of each group (because they were most abundant in the springs of each group) with the exception of Lim springs, for which no indicative taxon was identified. All the taxa are known as crenophiles (Bauernfeind *et al.* 2002; Graf *et al.* 2002a; Graf *et al.* 2002b), but not as spring-specialists (crenobiontic). No species was exclusive to one spring group.

A random pattern in EPT spring species assemblages has been reported elsewhere (Erman & Erman 1990; Myers & Resh 2002). Abiotic variables have not been considered good drivers of creno-coenoses (Staudacher & Füreder 2007); each spring has its own history and its own abiotic characteristics which select unique community patterns (Myers & Resh 2002).

#### 4.2. Faunistic considerations

In the Italian Alps, the diversity and assemblages of EPT taxa of springs is only known from few areas (Cantonati *et al.* 2006; Sambugar *et al.* 2006; Dumnicka *et al.* 2007).

Diptera, Trichoptera and Plecoptera are generally dominant in Alpine springs, while Ephemeroptera are less abundant than Plecoptera and Trichoptera (Cantonati *et al.* 2006; Gerecke & Franz 2006). Ephemeroptera are rarely found in springs, with *Baetis rhodani* or *B. alpinus* relatively abundant where periphyton is well developed (Thorup & Lindegaard 1977; Ilmonen *et al.* 2009). This was confirmed in our research, although the

Heptageniidae were abundant in several springs, particularly at high elevations. Plecoptera, especially herbivorous species, are usually recorded in crenal habitats (Ilmonen & Paasivirta 2005). The most abundant and frequent species that we recorded belonged to the detritivore genera, *Nemoura*, *Protonemura* and *Leuctra*, but five predatory species were also quite frequent and abundant. Based on existing literature (e.g., Erman & Erman 1990; 1995; Glazier 1991) our results confirm that the Trichoptera are the most species-rich EPT order in springs, although they were generally less abundant than the Plecoptera in this study.

Some species (for example the Ephemeroptera *Serratella ignita* and *Baetis rhodani*, the Plecoptera, *Protonemura intricata*, *P. auberti* and the Trichoptera, *Rhyacophila* (cf.) *producta*, *Ecclisopteryx* (cf.) *asterix*, *Drusus destitutus* and *D.* (cf.) *felix* were recorded in springs above their known altitudinal range (Stoch 2000–2005), suggesting the role of Alpine springs as *refugia*. In fact, due to their stable ecological features and their interface with freshwater and groundwater systems, they can support a variety of relatively rare and unusual fauna (Glazier 1991; Di Sabatino *et al.* 2003), providing refuge from disturbance events or extreme seasonal conditions. Furthermore, they may act as a source of downstream colonizers and a permeable ecotone for the interaction of phreatic and surface communities (Stanford & Ward 1983).

The two springs with long term data highlight the fact that repeated sampling campaigns are necessary to approach a complete species list. Thus, it is likely that results based on a single sampling occasion could strongly underestimate species assemblages and the possible differences/similarities among springs. Furthermore, seasonal differences (Tab. 1) may also have influenced species assemblages.

Temperature is recognised as a major driver for species distribution and as highly variable in Alpine streams (Brown & Hannah 2008). Only spot temperature readings were available for all springs, and species assemblages could not therefore be analysed in relation to parameters such as annual or seasonal degree days. This could explain the presence of species above or below their known altitudinal limits. It would be expected that low elevation crenal headwaters will have cooler summer water temperatures than surrounding streams, and that higher elevation ones will have milder temperatures with respect to glacial-snowmelt or rain-fed streams in the same areas.

## 5. CONCLUSIONS

The analysis of EPT taxa from 90 Alpine springs confirms that EPT taxa have a rather patchy distribution in Alpine springs. Though altitude appeared to be a main factor determining species distribution, significant physicochemical drivers for EPT species assemblages were not identified. Our results indicate the important

role of springs for regional biodiversity, as fifteen Trichoptera and three Plecoptera species were new for the Trentino, and several taxa were recorded above or below their previously known altitudinal range. The importance of long term monitoring to evaluate species assemblages of Alpine springs is emphasized.

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