ORIGINAL ARTICLE

Oligochaete distribution in alpine freshwaters: not a mere question of altitude

Elzbieta Dumnicka,[1] Kamil Najberek,[1] Valeria Lencioni[2*]

[1]Institute of Nature Conservation, Polish Academy of Sciences, al. Adama Mickiewicza 33, 31-120 Krakow, Poland; [2]Climate and Ecology Unit, Research and Museum Collection Office, MUSE-Science Museum of Trento, Corso del Lavoro e della Scienza 3, 38122 Trento, Italy

ABSTRACT

Alpine freshwater biodiversity is at risk of extinction due to climate change and some species could remain undescribed before they go extinct. These species are not yet included in red lists of protection by law, such as annelids in alpine habitats. Within this context, we studied the annelid fauna in 20 sites located between 1223 and 2703 m asl, belonging to different habitat types (kryal, glacio-rhithral, non-glacial streams, springs, littoral lake zone), in three glaciated catchments of the Italian Alps (Noce Bianco, Careser and Conca-Carè Alto; Trentino Province). The aim of this study was to describe the annelid communities of the different habitat types collected with different sampling methods (kick sampling and drift) and investigate the relationships between species richness and abundance with altitude. Between the years 2000 and 2005, we collected 4,765 individuals in 418 samples. One genus of Polychaeta (*Aeolosoma*) and 36 species of Oligochaeta were identified, distributed in four families: Enchytraeidae (28), Lumbriculidae (4), Naididae (3 and Tubificinae juveniles) and Haplotaxidae (1). Five species were new to Italy: *Cernosvitoviella carpatica*, *Cernosvitoviella* cf. *crassoductus*, *Henlea brucei*, *Henlea glandulifera* and *Mesenchytraeus sanguineus*. As expected, Enchytraeidae prevailed in all sites with the genera *Cernosvitoviella* and *Cognettia* the most abundant and frequent. The regression and cluster analyses and the generalized linear mixed models we performed, highlighted that the differences in species richness and abundance among sites are explainable partly by altitude but mainly by habitat type. A clear longitudinal species turnover was evident only in the Noce Bianco and Careser glacier-fed streams, where taxonomic diversity (tested by Seriation analysis) and abundance increased with increasing distance from the glacier front and decreasing altitude. Also expected, the harsh kryal habitat was the poorest in species and individuals, with a dominance of semi-aquatic and terrestrial enchytraeid taxa. Drift was found to be more effective than kick sampling in collecting oligochaetes, especially in glacier-fed streams where the use of the pond net is generally hindered by their high and highly variable discharge. In conclusion, this study provides new data on oligochaete fauna in alpine freshwaters, useful to implement the prevision models for Alpine biodiversity up to date not including annelid fauna.

Corresponding author: valeria.lencioni@muse.it

Key words: Enchytraeidae; glacier-fed streams; springs; new records; taxonomic diversity; Italian Alps.

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INTRODUCTION

Oligochaetes are the most diverse and widely distributed group of annelids in all kinds of terrestrial and aquatic habitats. About 5000 species of oligochaetes have been described so far, of which about 1100 colonize freshwaters (Martin *et al*., 2007). Aquatic oligochaetes are used as biological assessment indicators as well as in the biological management of organic-enriched systems. However, most studies have been carried out on their taxonomy, while knowledge on their ecology is still fragmentary, especially for those living in alpine freshwater which few works have been entirely devoted (Malard *et al*., 2001; Lencioni *et al*., 2004; Sambugar *et al*., 2005; Dumnicka and Boggero, 2007; Stoch *et al*., 2011; Dumnicka *et al*., 2015).

Oligochaetes colonize all mountain freshwater habitats, even if their abundance is usually lower than 10% of the whole benthic fauna above 2000 m asl (Lods-Crozet *et al*., 2001; Maiolini *et al*., 2006). They are more common in non-glacial habitats such as springs and springbrooks. Here mosses and periphyton generally thrive in still and transparent waters, and oligochaetes find amounts of organic debris in the depositional areas (Klein and Tockner, 2000; Becquet *et al*., 2022). With a lower diversity they have invaded also glacial habitats, in the benthic and hyporheic zone (Malard *et al*., 2001; Lods-Crozet *et al*., 2001; Lencioni and Spitale, 2015). Here, especially in the uppermost sectors of glacier-fed streams (=kryal), high turbidity, discharge and channel instability hinder algal growth and debris formation and deposition, making the habitat unsuitable for oligochaetes and many other invertebrates, apart from chironomids (*Diamesa* species) (Milner *et al*., 2001; Maiolini and Lencioni, 2001). Today there is a major concern on glacial biodiversity, including cold-adapted species not yet known to science that may become extinct before they are described due to climate change (Gobbi and Lencioni, 2020). Among endangered species, there are also oligochaetes that only recently have been discovered and that seem to be restricted to Alpine headwaters, such as *Cernosvitoviella tridentina* Dumnicka, *Cernosvitoviella longiducta* Dumnicka and *Cognettia valeriae* Dumnicka (Dumnicka, 2004, 2010).

Within this scenario, there are taxa not included in red lists of protection or from habitats still little investigated. Due to the still fragmentary information on alpine oligochaetes, they have not been included in provisional models performed to predict "loser" and "winner" taxa in a changing landscape, where glaciers will disappear (Wilkes *et al*., 2023). The aim of this work is to implement this knowledge gap, studying the oligochaete diversity (as species richness and phylogenetic diversity) in the different habitat types, located along an altitudinal gradient, from 1223 and 2703 m asl. Phylogenetic diversity, evaluated as taxonomic diversity index, is a measure of biodiversity which incorporates phylogenetic difference between species, and it is closely related to ecological diversity and functionality. It is evident that evaluating this parameter may be crucial to evaluating the ecological complexity and degree of disturbance of a certain ecosystem (Heino, 2008; Paschetta *et al*., 2013; Lencioni and Gobbi, 2018). Data refer to samples collected over six years with two sampling techniques (kick and drift) and lend themselves well to testing the time and technique effects on species occurrence per habitat type. In this way, we give some suggestions on how to set up oligochaete monitoring in high altitude freshwater habitats.

METHODS

Study area and sampling fauna

Twenty sites were selected in three stream catchments: Conca-Bedù in the Adamello Mt. Group (Adamello-Brenta Natural Park): Noce Bianco and Careser in the Ortles-Cevedale Mt. Group (Stelvio National Park) (Trentino, Italian Alps, 46°N, 10°E) (Fig. 1).

Conca-Bedù, Noce Bianco and Careser are three glacier-fed streams, fed, respectively, by the glaciers Conca, Vedretta de la Mare and Careser. We selected 12 sites in these streams (C3, C4, C8 in Conca-Bedù; NB1, NB2, NB3, NB4, NB5, NB7 in Noce Bianco; CR1, CR2, CR3 in Careser) and 5 in their non-glacial tributaries (Conca tributary: C7; Noce Bianco tributary: NB1bis, NB2bis, NB3bis; Careser tributary: CR1bis). The other sites were represented by one lake inlet (InL), the littoral zone of a lake (LittL) and a system of springs. The springs (called altogether NBspr) were all rheo-limnocrene, interconnected, located in the Noce Bianco floodplain at 2270 m asl in a restricted area (about 10 m^2).

On the basis of the distance from the glacier snout and general composition of the macroinvertebrate community described in Lencioni *et al*. (2004) and in Lencioni (2018), three main sectors were identified in glacier-fed streams: metakryal, hypokryal and glacio-rhithral. Metakryal is located within 300-500 m downstream from the glacier snout with maximum temperature <4°C (CR1, NB1, NB2). Hypokryal is located further downstream but within 1 km from the glacier snout and upstream of the confluence with a non-glacial stream with maximum temperature <6°C (NB3, C3). Glacio-rhithral is the stream sector at a distance >1 km from the glacier front, typically downstream of the confluence with a springbrook where maximum temperature was >6°C or downstream of the confluence with a non-glacial stream (C4, C8, CR2, NB4, NB5, NB7).

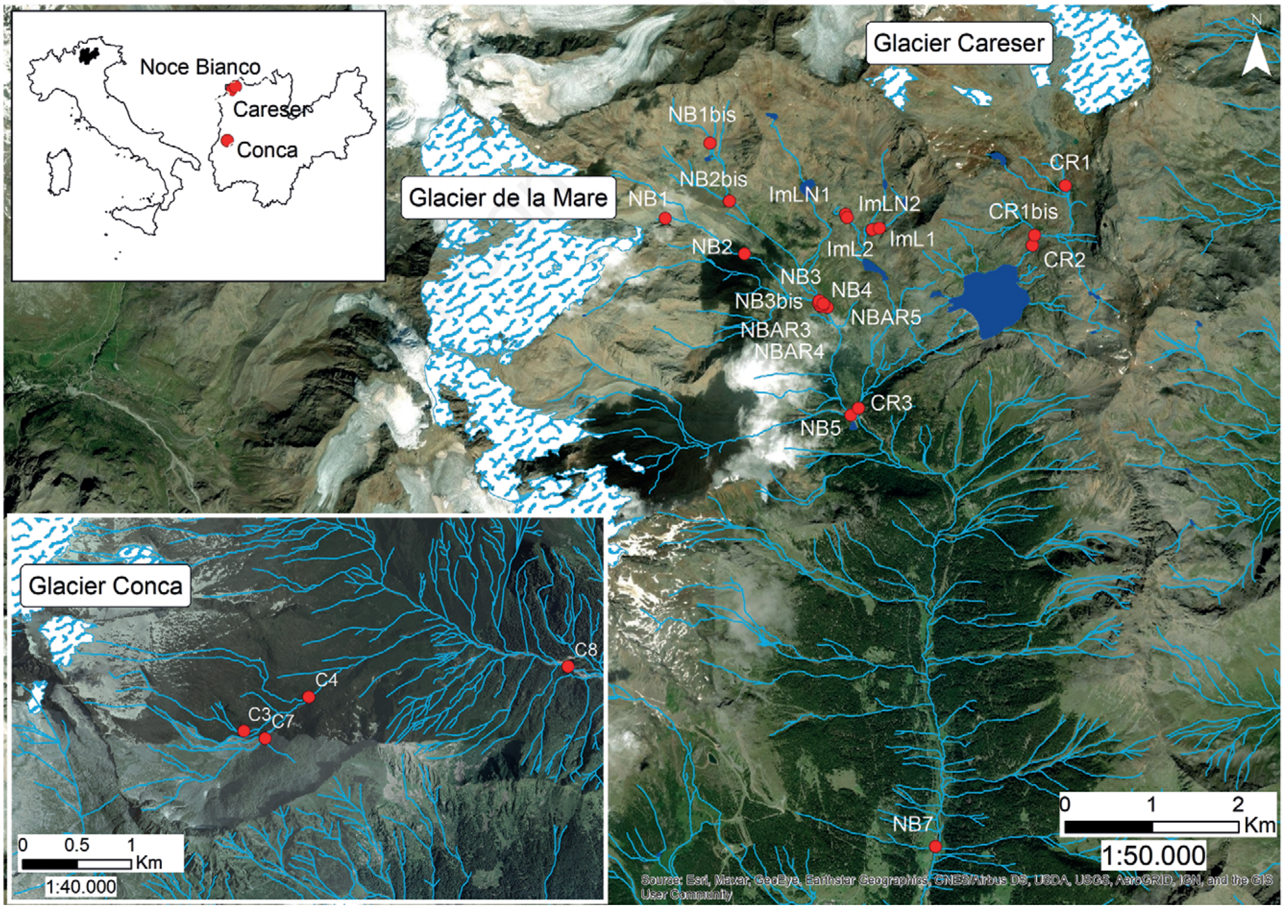
Macroinvertebrates were sampled between the years 2000 and 2005, with a major sampling effort in 2001-2002 in the Stelvio National Park and in 2005 in the Adamello-Brenta Natural Park (*Tab. S1*). Samples were collected on two to five occasions from May to October by kick and drift sampling (*Tab. S1*). In each stream 15 m-long site, five quantitative kick samples were taken in five different microhabitats using a pond net (30x30 cm, 100 µm mesh size). The same net was used to collect macroinvertebrates form the littoral zone of the Lungo Lake, investigating an overall area of 0.5 m^2. In the spring area, in all five quantitative integrate samples were taken with a smaller pond net (10x10 cm, 100 µm mesh size), investigating an overall area of 0.5 m^2. Drift samples were collected in all streams and rheocrene springs using from one to three nets (diameter 10 cm, length 1 m, mesh size 100 µm) exposed for 30 min on the surface (Lencioni *et al*., 2002). All samples were preserved in 75% ethanol.

During sorting under the stereomicroscope (50x), oligochaetes were isolated, mounted permanently in Canada balsam on microscope slides and identified by the first author according to Timm (2009), Schmelz and Collado (2010) and original taxonomic papers. Part of the collection is deposited at the MUSE-Science Museum of Trento (cINV0017) and part at the Institute of Nature Conservation, Polish Academy of Sciences in Krakow.

Statistical analyses

Species richness was calculated as the sum of the number of species (count data) identified in each site, and as a sum of individuals found in all samples collected in each site and date. Taxonomic diversity index, which indicates the average taxonomic distance (length of the path connecting two organisms along the branches of a phylogenetic tree) between any two organisms chosen at random from a sample (Clarke and Warwick, 1998), was calculated using the equation reported in Hammer *et al.* (2001) on the presence-absence matrix and including taxonomic information also above the species level (genus and family), entered for each species. Taxonomic diversity index is able to incorporate, for each species assemblage, other taxonomic information like the number of different genera or families. A higher taxonomic index value identifies a species assemblage comprising species "taxonomically distantly related" thus belonging to different genera or families, whereas a lower index value identifies a species assemblage composed of species taxonomically closely related, belonging to the same genus. Therefore, with respect to species richness, taxonomic diversity emphasizes the taxonomic relatedness between species in a community (Clarke and Warwick, 1998). A major benefit of this index over species richness is its virtual independence of sampling effort (Heino, 2008).

The effect of the altitude (independent variable) on oligochaete species richness and taxonomic diversity (dependent variables) was tested by Linear Regression Analysis (Gotelli and Ellison, 2004; Hammer *et al.*, 2001). The presence of species turnover with decreasing altitude was tested by Seriation analysis, which is a reciprocal averaging analytic technique aimed at arranging comparable units (species and sites) in a single dimension (that is, along a line) such that the position of each unit reflects its similarity to other units (Liiv, 2010). An absence-presence (0/1) matrix including rare species (= occurring in only one site) was used and sites were ordered along an altitudinal gradient to apply the constrained algorithm (Brower and Kile, 1988). The seriation routines attempt to reorganize the data matrix such that the presences are concentrated along the diagonal. In constrained optimization, only the rows (taxa) are free to move. Given



Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Fig. 1. Study area. Twenty sampling sites in the Conca, Careser and Noce Bianco stream catchments (Trentino, Italian Alps).

the ordering of the columns (= sites), this procedure finds the 'optimal' ordering of rows, that is, the ordering of taxa which gives the prettiest range plot. The probability of obtaining the observed spatial turnover between species was calculated using a Monte Carlo simulation generating and seriating 30 random matrices with the same number of occurrences within each taxon, and compares these to the original matrix to see if it is more informative than a random one (Hammer *et al*., 2001). The Linear Regression analyses and Seriation analysis were performed using PAST 4.11 (Hammer *et al*., 2001).

A cluster analysis (paired group – UPGMA), based on the Bray-Curtis similarity coefficient, was performed to highlight similarities among sites in relation to the oligochaete assemblages (Sneath and Sokal, 1973) on the matrix of log (x+1) abundance was prepared considering the total abundance of individuals collected in each site, as the sum of replicates and drift.

Additionally, we used two generalized linear mixed models (GLMM) with negative binomial distribution to compare richness and abundance of annelids: in different habitat types (kryal, glacio-rhithral, springbrook, spring; littoral lake was excluded from the analysis because it was represented by only one site); in sites at different altitude; in samples collected using different sampling techniques (drift, kick). Case studies referred to the Conca catchment, sampled only in 2005, were deleted from the matrix because contributing too little data compared with the other catchments. The dependent variables were the number of species in the first model and the number of individuals in the second one. In both models the year variable was used as a random factor. In each model the sample size was 345. Pairwise contrasts were applied for between-group comparisons with adjustment for multiple tests using least significant difference (significance level of p=0.05). GLMM analysis was performed with SPSS ver. 26.0 (IBM Corp., 2016).

RESULTS

Assemblages composition

In all, 4765 individuals were counted in 418 samples, belonging to one genus of Annelida incertae sedis or Polychaeta (*Aeolosoma*) and to 36 species of Oligochaeta distributed in four families: Enchytraeidae (28), Lumbriculidae (4), Naididae (3 and juveniles of the subfamily Tubificinae) and Haplotaxidae (1) (Tab. 1). Eighteen species occurred in only one site. The most frequent species was *Cognettia sphagnetorum* Vejdovský, found in 17 sites.

Enchytraeidae was the most abundant and frequent family. Within it, the genera *Cognettia* and *Cernosvitoviella* occurred in all sampling sites and represented 32.3% and 29.4% of the whole oligochaete fauna respectively (Tab. 1). Fifteen enchytraeid species were exclusively from one site and they were represented by one or a few individuals. Five enchytraeid species were found as new for Italy (Tab. 1): *Cernosvitoviella carpatica* Niel. and Christ., *Cernosvitoviella* cf. *crassoductus* Dózsa-Farkas, *Henlea brucei* Stephenson, *Henlea glandulifera* Nurminen, *Mesenchytraeus sanguineus* Niel. and Christ. Three mature, partly worn individuals were identified as *Marionina argentea* complex, probably belonging to *M. argentea* s.str. or *M. deminuta* according to Rota (2013). In the kryal zone (sites NB1, NB2, NB3, CR1, C3) almost exclusively enchytraeids were present, whereas in the glacio-rhithral habitat and in springbrooks their percentage varied from 46.1% to 96.0% and 56.4% to 99.6 % respectively. In the springs, enchytraeids represented only 27.5% of the annelid assemblage. Lumbriculidae were present in 12 sites and they represented the highest percentage of oligochaetes (72.0%) in the littoral zone of Lungo Lake with the sole species *Lumbriculus variegatus* (O.F. Müller). The abundance of lumbriculids varied greatly in the stream types: they accounted from 0.2% to 36.6% of the oligochaete community in springbrooks; in glacio-rhithral sites they were less numerous (2.6% – 18.1%), whereas in springs they reached 19.4% of the annelid fauna. Naididae were present in 10 sites, and the subfamily Tubificinae in three stations only. Three species of the *Nais* genus were identified, with many damaged individuals (*Nais* spp.), unidentifiable to species. The two most abundant and frequent morphospecies were *Nais communis* Piguet and *N. variabilis* Piguet. Representatives of family Naididae dominated in the springs (48%) and in some glacio-rhithral sites. They were less numerous in springbrooks (up to 17.6%) and absent in the kryal zone. *Haplotaxis gordioides* (Hartmann) (family Haplotaxidae) was found in seven sites belonging to all stream types.

The similarities among sites, based on their oligochaete assemblages, were highlighted by cluster analysis (Fig. 2). Sampling sites were grouped, at least partly, according to their origin with metakryal sites (those closer to the glacier snout, poorest in fauna: CR1, NB1 and NB2) on the left and the unique lentic habitat, the littoral zone of the Lake Lungo, on the right of the diagram. NB5 and CR2 were the two glacio-rhithral sites more similar to the metakryal ones. The other glacio-rhithral sites were grouped along with non-glacial sites. No separation among catchments was emphasised.

Species richness, taxonomic diversity and abundance

The GLMM model highlighted a significant positive relationship between number of individuals and altitude (F=9.56, df= 339, p=0.002; Fig. 3; *Tab. S3*) but not with species richness (F=0.16, df=339, p=0.68; *Tab. S3*). The same was confirmed by linear regression analysis for taxonomic diversity and species richness (linear regression: $R^2=0.001$; t= -0.15; p=0.88; *Figs. S1A* and *S1B*; *Tab. S2*).

The spatial turnover in species distribution in sites was significantly higher than expected by random chance (p< 0.001), with a nested pattern of the analysed meta-community. There are species whose presence are mutually exclusive (Fig. 4): it is evident that some are exclusive of lower or higher altitude, but most species occur at all

Tab. 1. List of species and relative abundance. In the third column, the code of the species included in the Seriation analysis (Fig. 4).

	Occurrence	Abundance	Species code
Family Lumbriculidae			
Stylodrilus sp. juv. Claparède, 1862	9	551	
Stylodrilus heringianus Claparède, 1862	7	112	St.herin
Stylodrilus cf. *parvus* (Hrabě and Čern., 1927)[§]	1	1	St.parvu
Stylodrilus lemani (Grube, 1879)	1	12	St.leman
Lumbriculus variegatus (O.F. Müller, 1774)	1	9	Lu.varie
Lumbriculidae gen. spp. juv.	4	12	
Family Enchytraeidae			
Cernosvitoviella spp. juv. Nielsen & Christensen, 1959	20	1012	
Cernosvitoviella tridentina Dumnicka, 2004[*]	11	232	Ce.tride
Cernosvitoviella atrata (Bretscher, 1903)	12	75	Ce.atrat
Cernosvitoviella longiducta Dumnicka, 2010[**]	9	32	Ce.longi
Cernosvitoviella ampullax Klung. and Abrah., 1981[§]	4	27	Ce.ampul
Cernosvitoviella omodeoi Rota, 1995	1	1	Ce.omode
Cernosvitoviella carpatica Niel. and Christ., 1959[#]	1	1	Ce.carpa
Cernosvitoviella cf. *palustris* Healy, 1979[+]	1	13	Ce.palus
Cernosvitoviella cf. *crassoductus* Dózsa-Farkas, 1990[#]	1	7	Ce.crass
Cognettia spp. Niel. and Christ., 1959	19	847	
Cognettia sphagnetorum Vejdovský, 1878	17	338	Co.sphag
Cognettia glandulosa (Michaelsen, 1888)	12	167	Co.gland
Cognettia cognetti (Issel, 1905)	12	180	Co.cogne
Cognettia valeriae Dumnicka, 2010[**]	4	4	Co.valer
Cognettia cf. *paxi* (Moszyński, 1938)[§]	1	1	Co.paxi
Enchytraeus buchholzi complex Vejdovský, 1879	4	4	En.buchh
Fridericia spp. juv. Michaelsen, 1889	5	9	Frideric
Fridericia bulbosa (Rosa, 1887)	4	12	Fr.bulbo
Fridericia perrieri (Vejdovský, 1878)	1	1	Fr.perri
Fridericia leydigi (Vejdovský, 1877)	1	1	Fr.leydi
Fridericia bulboides Niel. and Christ., 1959	1	1	Fr.bulbo
Fridericia maculata Issel, 1905	1	2	Fr.macul
Henlea spp. juv. Michaelsen, 1889	6	8	Henlea_j
Henlea perpusilla Friend, 1911	3	6	He.perpu
Henlea nasuta (Eisen, 1878)	1	1	He.nasut
Henlea brucei Stephenson, 1922[#]	1	1	He.bruce
Henlea ventriculosa (d'Udekem, 1854)	1	1	He.ventr
Henlea similis Niel. and Christ., 1959	1	1	He.simil
Henlea glandulifera Nurminen, 1970[#]	1	1	He.gland
Marionina argentea complex (Michaelsen, 1889)	3	3	Ma.argen
Mesenchytraeus spp. juv. Eisen, 1878	6	13	Mesenchy
Mesenchytraeus armatus (Levinsen, 1884)	12	77	Me.armat
Mesenchytraeus sanguineus Niel. and Christ., 1959[#]	1	1	Me.sangu
Enchytraeidae gen. spp. juv.	19	151	
Family Naididae			
Nais spp. O.F. Müller, 1774	9	114	Nais_
Nais communis Piguet, 1906	7	506	Na.commu
Nais variabilis Piguet, 1906	6	132	Na.varia
Nais pseudobtusa Piguet, 1906	3	6	Na.pseud
Tubificinae gen. spp. juv.	3	37	
Family Haplotaxidae			
Haplotaxis gordioides (Hartmann, 1821)	7	35	Ha.gordi
Polychaeta, Family Aeolosomatidae			
Aeolosoma spp. Ehrenberg, 1828	2	7	Aeolosom

Occurrence, number of sites in which each taxon was recorded; abundance, number of counted individuals; [§]reported by Lencioni et al. *(2004) in headwaters of the same catchment; *described by Dumnicka (2004) and **described by Dumnicka (2010) from the same catchment; [+]reported by Stoch* et al. *(2011) in the Italian Alps; [#]new for Italy.*

altitudes. The analysis was repeated separating glacial from non-glacial sites, with the aim to investigate if there is a longitudinal gradient in each stream type downstream of the source/snout *(Figs. S2A* and *S2B*). The turnover was emphasized for both, but significant only for glacial sites (p=0.02).

GLMMs models stressed the effect of habitat type on species richness and also on individual abundance, emphasizing kryal sites as significantly less rich in species (5-14) and in number of individuals (9-179) with respect to the other habitat types (contrasts at p< 0.031; *Tab. S4*) (Fig. 5 A,B). The number of species found in springsbrooks (11-26) was significantly higher than in glacio-rhithral sites (7-24) (contrast: estimate=0.79, SE=0.29, t=2.69, df=338, p=0.008; Fig. 5B), whereas the differences in individual abundance among glacio-rhithral, springbrooks and springs were not statistically significant (Fig. 5A).

GLMMs models for species richness and abundance revealed significant differences (p<0.001) also between the two methods of sampling (Fig. 5 C,D; *Tab. S3*). Higher numbers of species and individuals were collected with drift than pond net in all cases in which both methods were used (Fig. 5 C,D; *Tab. S3*).

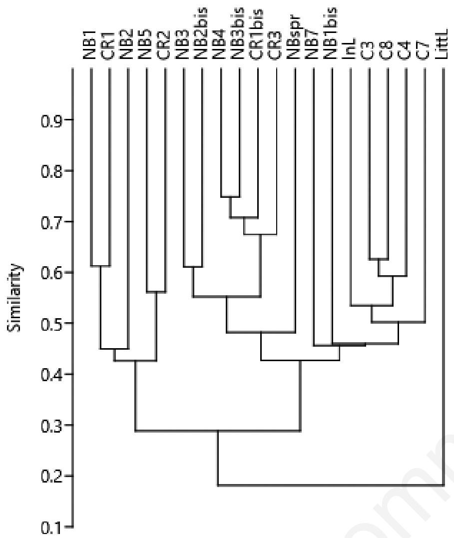


Fig. 2. Paired group (UPGMA) clustering based on the Bray-Curtis similarities (ranging from 0=total dissimilarity, to 1=complete similarity).

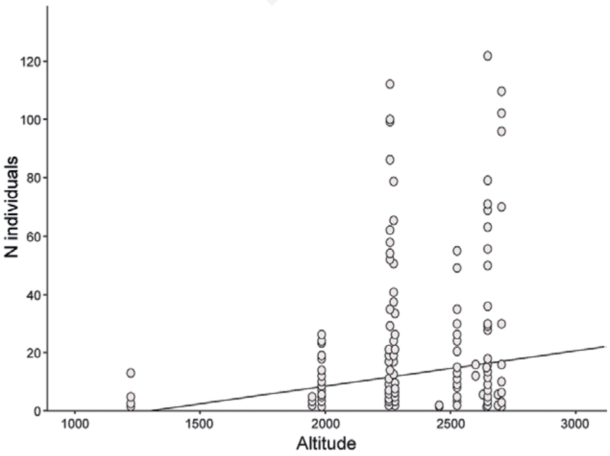


Fig. 3. Simple scatter with fit line for the number of individuals by altitude. Coefficient of determination R^2 Linear: 0.046.

	1223_gr	1300_gr	1950_gr	1985_sb	2115_gr	2170_sb	2252_hy	2257_gr	2270_hy	2270_sp	2270_sb	2455_me	2526_sb	2550_lak	2642_gr	2647_sb	2650_me	2665_inl	2694_me	2703_sb
Fr.leydi			■																	
He.nasut				■																
Me.sangu				■																
St.parvu				■																
Co.paxi				■																
En.buchh	■			■		■			■											
St.leman					■															
Ce.crass						■														
Ce.palus							■													
Fr.bulbo							■													
He.perpu		■						■			■									
He.ventr							■													
Ma.argen				■				■			■									
He.balca								■												
Nais_	■	■	■	■				■		■	■					■		■		
Na.varia	■			■				■		■	■							■		
Co.cogne		■	■	■	■		■	■	■	■	■		■			■		■		
Ce.atrat		■	■	■		■	■	■	■	■			■	■	■	■				
Ce.ampul	■							■			■					■				
Me.armat	■	■		■	■		■	■	■	■	■					■		■	■	
Ha.gordi		■				■		■	■		■		■			■				
Aeolosom								■			■									
Ce.longi	■				■	■	■			■	■		■					■		■
Co.gland		■		■		■	■	■	■	■	■		■			■		■		■
Fr.bulbo								■	■			■	■							
Co.sphag		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■		■
St.herin				■	■			■			■			■		■		■		
He.simil											■									
He.gland											■									
Na.commu	■							■		■	■				■	■		■		
Fr.perri												■								
Co.valer											■		■							
Na.pseud										■	■				■					
Mesenchy						■		■			■					■	■	■		
Ce.tride				■	■			■		■	■		■			■	■	■	■	■
Ce.carpa													■							
Fr.macul													■							
Lu.varie														■						
Ce.omode																				■

Fig. 4. Species distribution in relation to altitude (m asl). Species are ordered according to the first axis obtained from the Seriation analysis. Species codes are in Tab. 1. Site code: altitude, (m asl), habitat type; me, metakryal; hy, hypokryal; gr, glacio-rhithral; sb, springbrook; sp, spring; lak, lake littoral; inl, lake inlet.

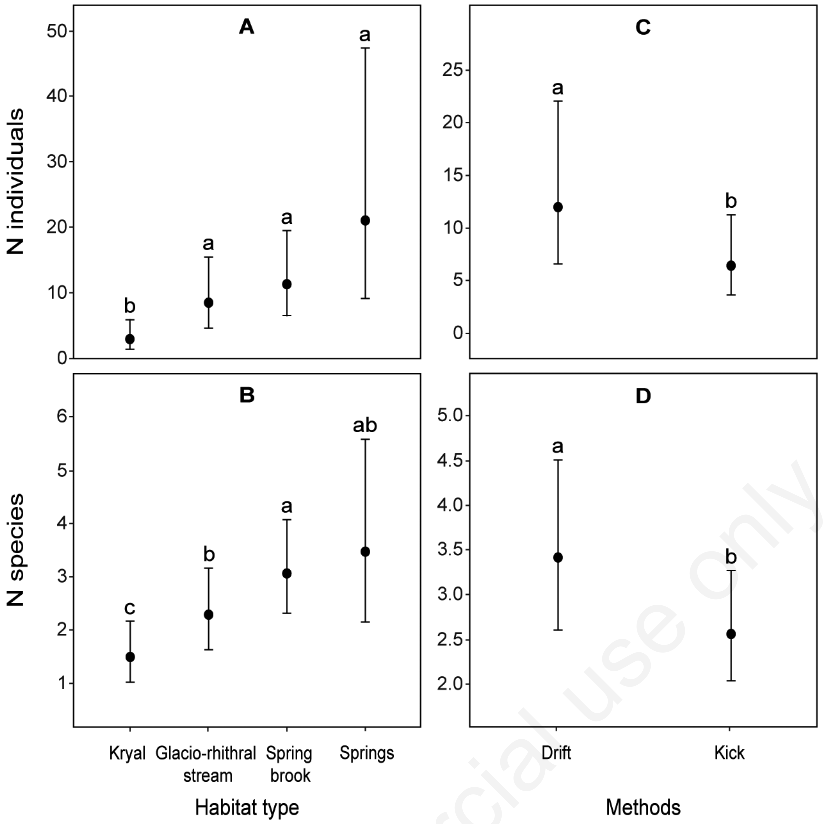


Fig. 5. Estimated mean values (±confidence intervals) of the stream type (A,B) and sampling methods (C,D). Two dependent variables were considered: the number of species and the number individuals. Groups with the same letter above the T-bars are not significantly different at $p<0.05$.

DISCUSSION

Faunistic records and remarks

As observed for other macroinvertebrates in Alpine headwaters (e.g., chironomids; Lencioni, 2018), a high percentage (50%) of rare taxa (i.e., recorded in only one site) was found, with a few species accounting for most individuals, suggesting a nested distribution. As expected (Dumnicka and Boggero, 2007), the most common and abundant family was Enchytraeidae and within it the genera *Cernosvitoviella* and *Cognettia*. Their representatives are frequently abundant and diverse in Alpine streams and lakes (Dumnicka and Boggero, 2007; Dumnicka *et al*., 2015; Oertli *et al*., 2008, Thaler *et al*., 2015), and in cold springs (Stoch *e. al*., 2011). This family accounts also for semi-aquatic or terrestrial species living or moving to the riparian zone of running (Erséus *et al*., 2005) or stagnant waters (Timm, 1996, 2009) and into the soil (Nurminen, 1977; Rota, 1995).

It is interesting to remark that five species, all enchytraeids, were found as new for Italy: *C. carpatica*, *C.* cf. *crassoductus*, *H. brucei*, *H. glandulifera* and *M. sanguineus*. *C. carpatica* lives mainly in mountain rivers, bogs and damp soil (Dumnicka and Boggero, 2017). In the Carpathian Mts. it was recorded in a few localities; specifically, in the Alps, the first record was in the hyporheic zone of the Roseg glacial stream in Switzerland (Lafont and Malard, 2001). We collected it with only one specimen in one non-glacial site (NB2bis). *C.* cf. *crassoductus* is known from stagnant waters of northeastern Hungary (Dózsa-Farkas *et al*., 2017) and from *Sphagnum* mat on a lake shore in Estonia (Timm, 2007). Our specimens, seven in all, were collected in a non-glacial site (C7), with low current velocity and presence of mosses (Lencioni, 2018), confirming its known autecology. The specimens we collected share the most characteristic features with the holotype (for example vas deferens has a stout proximal dilatation) with some differences. For example, the spermathecal duct is 3 times longer than the almost spherical ampulla instead of 2-2.5 times longer than the oval ampulla (Dózsa-Farkas, 1990). Further analyses are necessary to clarify the relationships between the holotype and specimens living in this Alpine region. *H. brucei*, one specimen only being found in one glacio-rhithral site (NB4), is typical for soil rich in organic matter (detritus) and was known before from Spitsbergen and Bosnia (Dózsa-Farkas,1999). This species is treated as a junior synonym of *H. perpusilla* (Schmelz and Collado, 2010), according to Dózsa-Farkas' (1999) suggestion. However, our specimen of *H. brucei* differs from the typical *H. perpusilla*by possessing two well developed intestinal diverticula in segment VIII and an abrupt dilatation of the gut in the next segment, while *H. perpusilla* does not have intestinal diverticula. The "true" *H. perpusilla* was collected in the same site (NB4) and in another two sites, one non-glacial (NB3bis) and one glacial (C8). These findings and the evidence of occurrence of different cytotypes in *H. perpusilla* (Schmelz and Collado, 2010), suggest the existence of different hidden species and the need of a revision of this species complex. *H. glandulifera*, one specimen collected in one non-glacial site (NB3bis), is a species typical of cold habitats, previously found in the soil of the Arctic (Iceland, Greenland) and in the Austrian Alps (Nurminen, 1977). *M. sanguineus*, one specimen collected in one non-glacial site (CR3), is a semi-aquatic species known from many European countries, but it has not been previously recorded in Italy. Overall, these findings update the Italian enchytraeid list by Rota *et al*. (2014) who intensively studied this family (mainly terrestrial species) over the last 30 years (Rota, 1994, 1995, 2013, 2015).

Generally, the prevalence of Enchytraeidae was expected in all sites, considering that for many species of this family freezing tolerance and dehydration have been demonstrated (Dózsa-Farkas, 1973; Sømme and Birkemoe, 1997; Glasby *et al*., 2021). Specifically, our findings confirm the association of some species with cold habitats. For example, *Fridericia bulboides* Niel. and Christ., *Henlea ventriculosa* (d'Udekem) and *Cernosvitoviella* cf. *palustris* Healy were collected only in hypokryal sites. *F. bulboides* and *H. ventriculosa* were previously found in cold waters in Iceland (Nurminen, 1973) while *C. palustris* in two cold Alpine springs (Stoch *et al*., 2011). *C. tridentina,* C *longiducta* and *C. valeriae*, collected in both glacial and non-glacial habitats but preferentially above 2000 m asl as expected, having been found to date only in headwaters of the Alps. These three species were described by the first author as new for science in two previous works (Dumnicka, 2004, 2010), based on specimens collected in some sites in the Noce Bianco and Careser catchments: *C. tridentina* in CR1bis, CR3, NB2bis, NB3bis, and NB4; *C. longiducta* in NB1bis, NB2bis and in the Lungo Lake littoral zone; *C. valeriae* in NB2bis and NB3bis (Dumnicka, 2004, 2010). Among enchytraeids occurring in the kryal, to be mentioned is *Fridericia perrieri* (Vejdovský), a semi-aquatic species widespread and common in wet habitats (Schmelz and Collado, 2010) including Alpine meadows at altitude 2500 m asl (Nurminen, 1977). Our findings suggest that this species complex might be considered ubiquist, and its occurrence was occasional in kryal habitats.

As regards the other families, Naididae were little represented in running waters compared to the springs, as expected. In fact, in Alpine running waters, naidids are generally scarce or even absent (Lencioni *et al*., 2004; Malard *et al*., 2001; Oertli *et al*., 2008) but dominant in springs (Sambugar *et al*., 2006; Stoch *et al*., 2011). Their known sensitivity to freezing might explain their habitat selection, the risk of freezing and desiccation being higher in streams than in springs during winter (Lencioni, 2004). Contrary to literature (Dumnicka and Boggero, 2007; Dumnicka *et al*., 2015), we did not find them in the lake littoral but only in the lake inlet. However, this might be due also to the few examples of this habitat type in our sites. The family Naididae was represented by the genus *Nais,* with the *Nais communis/variabilis* complex including two morphotypes (Envall *et al*., 2012) considered separately in the literature and species checklists (Timm and Giani, 2013; WoRMS, 2023). Both morphotypes were common in streams whereas, in the lake and in the springs, the *N. communis* morphotype prevailed, as expected (Dumnicka and Boggero, 2007; Dumnicka *et al*., 2015; Stoch *et al*., 2011). Within the Naididae we recorded also some juvenile individuals of the subfamily Tubificinae, with morphological features typical of *Tubifex tubifex*, a species found in stagnant Alpine waters earlier (Oertli *et al*., 2008, Dumnicka *et al*., 2015).

The family Haplotaxidae was represented only by *Haplotaxis gordioides* (Hartmann), typical of cold waters (Timm, 2009) while the thermophilic family Lumbriculidae was represented only by the genus *Stylodrilus* and the species *Lumbriculus variegatus* (Müller). The latter is ubiquitous and eurythermal, adapted to live in different environmental conditions (Timm, 1980). We found it abundant only in the littoral zone of the Lungo Lake. Among the *Stylodrilus* species, *Stylodrilus heringianus* Claparède was collected in the lake and in several non-glacial sites. It is most often found in cold, oligotrophic profundal habitats, mainly in lakes (Dumnicka, 1994). Polychaeta were represented by a few individuals of the genus *Aeolosoma*, that contains eurythermal species colonizing freshwaters (Timm, 2009).

A major missing family is the Propappidae: *Propappus volki* (Michaelsen) is a species known from various epigean and subterranean waters (Timm, 2009) distributed in all European countries, even in Italy. It was found also in glacio-rhithral habitats in Val Roseg (Swiss Alps) (Malard *et al*., 2001). Our not finding it in Trentino headwaters might indicate the absence of this species in this Alpine region. Further sampling campaigns are necessary to confirm this statement.

Altitude, habitat type and sampling method effects on diversity and abundance

There is evidence that the macroinvertebrate community is simpler at high than at low altitude, i.e., fewer taxa are present, generally with low abundance, especially for non-insect taxa (Lencioni *et al*., 2007). Our study highlighted that only if we consider the habitat type "glacier-fed stream", including metakryal, hypokryal and glacio-rhithral sectors, a clear turnover and change was evident, with taxonomic diversity and species richness increasing with increasing distance from the glacier front and decreasing altitude. Considering all sites together, only the GLMM model emphasized a positive inverse relationship between abundance and altitude, with maximum abundance at medium-high altitude. Other factors, i.e., habitat type and sampling method, explained the assemblages we observed better than altitude.

Regarding the habitat type, as expected (Malard *et al*., 2001; Lods-Crozet *et al*., 2001), the habitat type poorest in species and abundance of oligochaete fauna was the kryal. Here semi-aquatic and terrestrial enchytraeid taxa prevailed, while true aquatic oligochaetes were absent, as typical of glacier-fed streams. This is due to the harsh hydrological conditions hindering colonization to taxa without specific adaptation to current velocity such as is the case in annelids (Lencioni, 2018). The highest diversity and abundance were observed in springbrooks and springs, where low and less variable discharge and current velocity and the presence of depositional areas create more suitable venues for oligochaetes (Stoch *et al*., 2011). The glacio-rhithral habitat was just in between, showing intermediate values between kryal and non-glacial habitats. Regarding sampling method, drift netting was more effective than kick sampling in collecting oligochaetes in running waters where both methods were used. This was true especially in glacier-fed streams where kick sampling is generally hindered by their stressful hydrological conditions (Robinson *et al*., 2002).

CONCLUSIONS

This study gives new insights on oligochaete fauna in alpine freshwaters, with new interesting records for Italy that supplement the Italian Fauna Checklist (https://www.faunaitalia.it/checklist/introduction.html) and new ecological notes on alpine species. Some species seem to be restricted to the uppermost sectors of glacier-fed streams, others in springbrooks, and they could be differently threatened with extinction due to global warming. Thus, these data might be useful to implement prevision models for Alpine biodiversity up to date not including annelid fauna (Wilkes *et al*., 2023).

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Online supplementary material:

Fig. S1. Species richness and taxonomic diversity in relation to altitude.

Fig. S2. Species distribution in relation to altitude in non-glacial and glacial sites.

Tab. S1. Macroinvertebrates sampling.

Tab. S2. Model coefficients of linear regression analysis.

Tab. S3. Model coefficients of the two generalized linear mixed models (GLMMs).

Tab. S4. Pairwise contrasts for stream type variables from the two GLMM models.