

# How do macroinvertebrate communities respond to declining glacial influence in the Southern Alps?

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## ABSTRACT

### How do macroinvertebrate communities respond to declining glacial influence in the Southern Alps?

In the summer of 2018, we compared the macroinvertebrate communities of four streams fed by retreating glaciers and of one pro-glacial pond in the Italian Alps. The general aim was to determine if habitats fed by glaciers with different surface areas host different communities along a longitudinal gradient and to identify which environmental variables can predict these differences. Eight sampling sites were selected with different glacial influences given by the Glacial Index (GI, range 0-1). This index takes into consideration the distance from the snout and glacier surface area. Five were classified as kryal, fed mainly by ice melt and lay within 1 km of the snout; two as glacio-rhithral, with mixed contributions and > 1 km from the snout; and one as a pro-glacial pond, which lays within 50 m from the snout and was fed by ice melt. Chironomids were the most frequent (100 %) and abundant taxa (80 %), followed by EPT (Ephemeroptera, Plecoptera, Trichoptera) which represented 9 % of total abundance. All kryal sites (MA1, CR0, CR1, AG1) were dominated by chironomids (> 98 %), except for C0, which hosts a community resembling that of glacio-rhithral sites (C2, CR2), which are more diversified and have approximately 60 % of their communities composed of chironomids. While C0 is fed mainly by ice-melt, its GI was lower (0.3) than that of the other kryal sites (0.5-1.0) because of the small size of the feeding glacier (0.14 ha). These results emphasise that the glacier surface area affects the benthic fauna and that the GI should be considered when classifying different types of sites. GI was also selected as one of the best predictors of faunal changes by distance-based redundancy analysis (dbRDA), which was performed to investigate the relationships between taxa distribution and environmental conditions, along with the values of maximum water temperature and chlorophyll *a* concentration. Overall, this study provides new insights into how different glacial influences affect the macroinvertebrate communities of freshwater habitats.

**Key words:** Chironomids, Glacial Index, stream type, *bédière*, pro-glacial pond, water temperature, glacier retreating, Trentino Province (Italy)

## RESUMEN

### *Una comparación entre comunidades de macroinvertebrados en ríos de gran altitud: la importancia de la influencia glacial*

*En el verano de 2018 comparamos las comunidades de macroinvertebrados en cuatro ríos alimentados por glaciares en regresión en tres grupos montañosos alpinos. El objetivo principal era determinar si habitats alimentados por glaciares con superficies diferentes albergan comunidades distintas a lo largo de un gradiente longitudinal e identificar qué variantes ambientales pueden predecir estas diferencias. Se seleccionaron ocho sitios de muestreo, con diferentes influencias glaciales que fueron calculados como en Índice Glacial (GI, rango 0-1). Este índice tiene en consideración la distancia entre el frente y la superficie del glaciar. Cinco fueron clasificados como kryal, alimentados mayormente por deshielo y situados a menos de 1 km del frente; dos como glacio-rhithral, con aportación mixta y a más de 1 km del frente; y uno como laguna proglacial, que está situado a menos de 50 metros del frente y alimentada por deshielo. Los Quironómidos han sido el taxón más frecuente (100 %) y abundante (80 %), seguidos por EPT (Ephemeroptera, Plecoptera, Trichoptera), que representaron un 9 % de la abundancia. En todos los sitios kryal (MA1, CR0, CR1, AG1) predominan los quironómidos (> 98 %), con la excepción de C0, que alberga una comunidad parecida a la de los sitios glacio-rhithral (C2, CR2), que son más diversificados y tienen aproximadamente*

el 60 % de sus comunidades compuestas por Quironómidos. Mientras que C0 es alimentado sobre todo por deshielo, su GI fue más bajo (0.3) que el de los otros sitios kryal (0.5-1.0) a causa de las pequeñas dimensiones del glaciar que lo alimenta (0.14 ha). Estos resultados destacan que la superficie del glaciar afecta a la fauna bentónica y que hay que tomar en consideración el GI a la hora de clasificar diferentes tipos de sitios. El GI ha sido también seleccionado como uno de los mejores indicadores de cambios en la fauna a través de Análisis de Redundancia basados en la distancia (dbRDA), que se han realizado para examinar la relación entre la distribución de los taxones y las condiciones ambientales, así como los valores de la temperatura máxima del agua y la concentración de clorofila a. En su conjunto, este estudio ofrece nuevas perspectivas sobre cómo diferentes influencias glaciales afectan a las comunidades de macroinvertebrados en los hábitats de agua dulce.

**Palabras clave:** Quironómidos, Índice Glacial, tipología de los arroyos, bédrière, laguna proglacial, temperatura del agua, retroceso del glaciar, Provincia del Trentino (Italia)

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## INTRODUCTION

Since the end of the LIA – Little Ice Age (circa 1850), glaciers worldwide have been rapidly retreating (Vincent *et al.*, 2005; Zemp *et al.*, 2006). European Alps have lost 54 % of their glacial surface in the last 150 years due to increased temperatures in summer and decreased amounts of snowfall in winter. Furthermore, human activities have already caused a mean global warming of 0.87 °C compared to the pre-industrial period (1850-1900) (IPCC, 2018) and current forecasts suggest that only 4-13 % of the European Alps ice area recorded in 2003 will remain by the end of the 21<sup>st</sup> century (Milner *et al.*, 2017).

High-altitude streams are classified according to the origin of their water as follows: kryal (fed by ice melt), krenal (fed by groundwater), rhithral (fed by groundwater, snow/ice melt, and rainfall) (Füreder, 1999; Brittain & Milner, 2001). Due to differences in their respective environmental conditions, they host different communities of macroinvertebrates. Low richness is expected in the kryal, within the first kilometre downstream of the glacier snout, where extremely low water temperatures (< 4 °C even in summer), high turbidity, low channel stability, and highly variable discharge make these habitats too extreme for life (Milner & Petts, 1994). Only cold stenothermal invertebrates can cope with these habitats that are characterised by high glacial influence. There, Diptera Chironomidae can be the only taxon within the first 500 m from the snout, present with only species of the genus *Diamesa* Meigen (Lods-Crozet *et al.*, 2001; Rossaro *et al.*, 2016).

With increasing distance from the glacier snout and decreasing altitude, the glacier's influence on streams decreases and environmental conditions become less severe. This is especially true downstream of the confluence of non-glacial tributaries (fed by groundwater) in the glacio-rhithral stream (Castella *et al.*, 2001). The glacio-rhithral, typically at a distance > 1 km from the snout, hosts a more diversified invertebrate community that is still dominated or co-dominated by chironomids, but with different genera (mainly Orthocladinae) along with other dipterans (e.g. Limoniidae) and EPT (Ephemeroptera, Plecoptera, Trichoptera). Oligochaeta and Nematoda (mainly parasites of *Diamesa* larvae and pupae in the kryal, but free-living species in the glacio-rhithral) can be found as representatives of non-insect taxa (Gobbi & Lencioni, 2020).

Glacier retreating is leading to a cascade effect on inland ecosystems, from altered hydrological-thermal regimes and bio-geochemical cycles, to biodiversity loss and disruption of trophic chains (Sertić Perić *et al.*, 2015; Milner *et al.*, 2017; Brown *et al.*, 2018). Glacial run-off decreases proportionally to the factors of increasing water temperature and channel stability and decreasing discharge in glacier-fed streams (Robinson *et al.*, 2014). Glacial influence decreases even at relatively short distances from the snout, and environmental conditions become less stressful in the uppermost reach (Lencioni *et al.*, 2021). Meanwhile, eurythermal species move upstream, leading to an increase in local taxonomic richness (Brown & Milner, 2012). However, not all glaciers are retreating at the same rate, even

in the same region, and differences in macroinvertebrate community structure are expected in relation to remnant glacier area (Jacobsen et al., 2012; Sertić Perić et al., 2015).

How do macroinvertebrate communities respond to declining glacial influence in the Southern Alps? To answer this question, we selected, as case study, one pro-glacial pond and four

**Table 1.** Sampling sites and their respective feeding glacier. Glaciological data and distance from the glacier snout from Smiraglia & Diolaiuti (2015). \* see explanation in the text. *Sitios de muestreo y sus respectivos glaciares de alimentación. Datos glaciológicos y distancia desde el frente del glaciar por Smiraglia & Diolaiuti (2015). \* ver explicación en el texto.*

	C0	C2	MA1	AG1	AG_pond	CR0	CR1	CR2	
Glaciers	Mountain Group	Adamello-Presanella		Brenta Dolomites		Ortles-Cevedale			
	Geology	Igneous crystalline rock			Sedimentary calcareous rocks		Metamorphic rocks		
	Name	Conca	Mandrone	Agola			Careser		
	Area (km <sup>2</sup> )	0.14	10.46	0.19			1.66		
	Retreating rate (1987-2015)	-1.1	-0.6	-1.0			-2.3		
	Snout altitude (m a.s.l.)	3031	2584	2597			2938		
	Sites	Latitude (UTM 32)	624408	625164	620717	643496	643419	631528	631608
Longitude (UTM 32)		5106984	5106582	5115876	5112526	5112593	5144516	5144166	5143477
Altitude (m a.s.l.)		2830	2435	2578	2627	2596	2858	2707	2637
Distance from snout (m)		439	1320	50	0	23	747	1163	2287
Habitat type		kryal	glacio-rhithral	kryal	kryal	proglacial pond	kryal	kryal	glacio-rhithral
Sub-basin area (km <sup>2</sup> )		0.08	0.19	13.66	-	0.41	5.22	6.35	8.49
Glacial Index (GI)		0.31	0.14	0.98	1.00	0.95	0.61	0.5	0.34
Pfankuch Index		31 (stable)	30 (stable)	47 (unstable)	39 (stable)		58 (unstable)	51 (unstable)	48 (unstable)
mTmax (°C)		4.1 ± 2.4	11.0 ± 0.5	0.4	0.2 ± 0.1	9.9 ± 6.0	5.5 ± 0.3	7.1 ± 1.2	9.7 ± 0.6
Chl <i>a</i> (µg/cm <sup>2</sup> )		0.21 ± 0.1	0.69 ± 0.3	0.25	0.002 ± 0.0	0.07 ± 0.0	0.01 ± 0.004	0.02 ± 0.0	0.634 ± 0.6

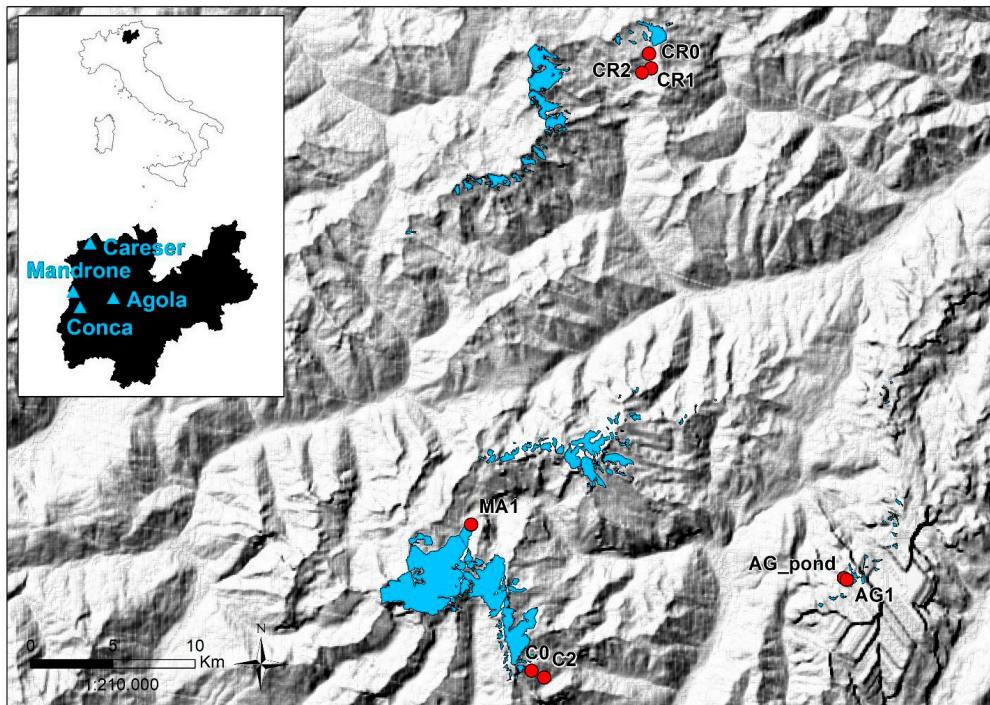
streams fed by retreating glaciers (Conca, Mandrone, Careser, and Agola) in the Italian Alps, with different surface areas (0.14-10.46 km<sup>2</sup>) and different retreating rates (from 0.6 % to 2.3 % of their surface/year in the last three decades; Lencioni *et al.*, 2021). The general aim of this work was to determine if habitats fed by glaciers with different surface areas host different communities along a longitudinal gradient and to identify which environmental variables can predict these differences. Furthermore, physical and chemical variables such as water temperature, channel stability, discharge, and primary production, were included in the analysis, all potentially affecting the benthic fauna (Castella *et al.*, 2001; Kärnä *et al.*, 2019). The classification of stream types, kryal and glacio-rhithral, based on the ice melt contribution and the model of Milner & Petts (1994), were also revised. This conceptual model, that was proposed approximately 30 years ago, suggests distance from the glacier snout as the

best predictor of species richness in glacier-fed streams, with the lowest number of taxa closer to the snout (in the kryal) and the highest number of taxa at a distance from the snout > 1 km (in the glacio-rhithral). Our dataset, including sites close to the snout but with high channel stability due to presence of *roche moutonnées*, or with  $T_{max} > 4$  °C due to reduced surface area of the feeding retreated glacier, suggests that glacial influence (evaluated as Glacial Index, as described in the Material and Methods) instead of distance from the snout plays the major role in predicting the richness trend in glacier-fed streams.

## MATERIAL AND METHODS

### Study Area

The study area is located in northeast Italy (Trentino Province; 46° N, 10° E), and included four glaciers of three mountain groups, namely: the

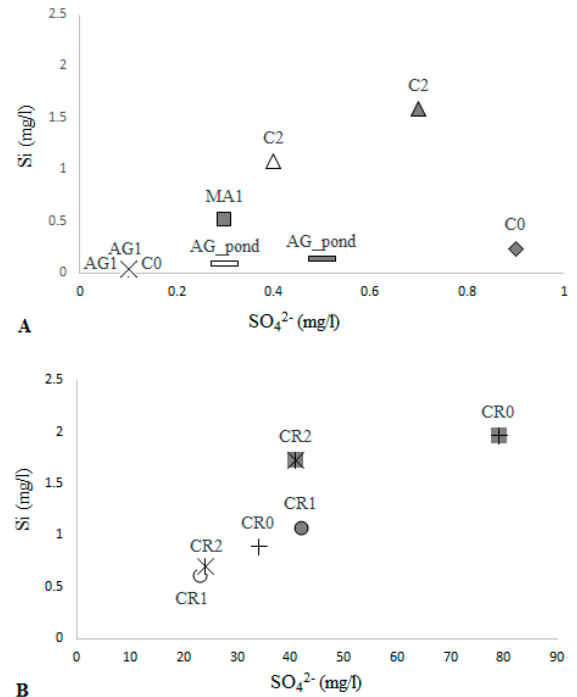


**Figure 1.** Geographical location of study area and detail of sampling sites. Orthophoto from Geo-Service of Trento Province, Italy. Glacier area data source: Smiraglia & Diolaiuti (2015). *Situación geográfica del área de estudio y detalle de los sitios de muestreo. Ortofoto del Geo-Servicio de la provincia de Trento, Italia. Fuente de los datos del área glacial: Smiraglia & Diolaiuti (2015).*



Mandrone and Conca glaciers in the Adamello-Presanella Mts. (siliceous), Careser glacier in the Ortles-Cevedale Mts. (metamorphic), and Agola in the Brenta Dolomites (calcareous) (Fig. 1). These glaciers differ from each other in altitude of the snout, exposure, lithology of the bedrock, and retreating rate (Table 1). Careser lost more than 20 % of its LIA surface area between 2003 and 2015 and has the highest rate of shrinking (Carturan et al., 2013). Agola and Conca lost 64 % and 82 % of their surface areas, respectively, since 1850 and currently have a surface area of < 1 km<sup>2</sup> (Smiraglia & Diolaiuti, 2015). Meanwhile, Mandrone is the largest glacier, with an area of 10 km<sup>2</sup>.

Eight sampling sites were selected with different glacial influences, calculated as Glacial Index (GI expressed in a range from 0–1), based on their distance from the snout and the surface area of the feeding glacier (Table 1). Based on the distance from the snout and the ratio between sulphate (SO<sub>4</sub><sup>2-</sup>) and silicium (Si) concentrations (Fig. 2), we made *a priori* classifications to our study sites. The ratio between Si and SO<sub>4</sub><sup>2-</sup> is generally used to discriminate stream types based on the dominant water contribution (Brown et al., 2003, 2006; Marchina et al., 2020). In streams fed by ice melt (both kryal and glacio-rhithral), the sulphate and silicium concentrations are generally low in early summer ('quick flow' dilutes rapidly routed meltwater *sensu* Brown et al., 2003). In late summer instead, the kryal sites have high sulphate concentrations ('distributed flow' slows routed sub-glacial waters *sensu* Brown et al., 2003) and glacio-rhithral sites have high Si concentrations (groundwater inputs). Based on these results, we attributed the sites C0, CR0, CR1, AG1, and MA1 to the kryal type, while C2 and CR2 were classified as the glacio-rhithral type (Table 1). CR2 was the only site selected downstream of the confluence of a non-glacial tributary. The proglacial pond (AG\_pond) is located at a distance of 23 m from the Agola glacier snout, has an area of 227 m<sup>2</sup> and a depth < 1.5 m. It is fed by ice meltwater (mainly in early summer), groundwater, and rainfall (Marchina et al., 2020). The pond undergoes water level fluctuation (from early to late summer we recorded a decrease of 20 cm in water level) and we can presume that it freezes



**Figure 2.** Si (mg/l) and SO<sub>4</sub><sup>2-</sup> (mg/l) concentrations (white = early summer, grey = late summer) in the sampling sites: A = Conca, Agola, and Mandrone sites; B = Careser sites. *Concentración de Si (mg/l) y SO<sub>4</sub><sup>2-</sup> (mg/l) (blanco = principios de verano, gris = finales de verano) en los sitios de muestreo. A = sitios del Conca, Agola y Mandrone, B = sitio del Careser.*

solid during winter. Among kryal sites, AG1 was selected in a supra-glacial channel (*bédrière*) because, due to the high permeability of the calcareous bedrock, the snow-ice meltwater flows underground downstream of the snout.

All sites were sampled twice during the ablation season in the summer of 2018 (in July and September), except MA1, which was sampled only in September, because the glacier snout could not be accessed safely in July.

### Environmental data

Channel stability was assessed using the Pfankuch Stability Index (Pfankuch, 1975) for running waters, based on the visual evaluation of six substrate variables (rock angularity, bed-surface brightness, particle packing, percentage of stable material, scouring, and aquatic vegetation).

A score was assigned to each variable and the total sum was compared with four stability ranges. High scores represent unstable channels (45-60) and low scores indicate higher stability (15-44). We made an *a priori* assignment of the lowest value of the index to the pond (corresponding to the highest stability being a lentic habitat) to compare this site with the others for this variable.

A current meter (OTT Hydrometrie Z30) was used to determine both the water depth and current velocity. Five measurements were made at each site, which were then averaged to obtain a better estimate.

Discharge was measured at hydrometric stations owned by Meteotrentino (<https://www.meteotrentino.it>) at three sites with the highest flow (CR0, CR2, and MA1), while at the other sites calculations were performed according to the salt dilution method outlined by Hongve (1987).

Turbidity was measured as the amount of suspended solids present and was obtained by filtering a known volume of water (from 250 mL in kryal sites and 500-1000 mL in glacio-rhithral sites) through 0.45 µm membrane MFS filters and drying at 105 °C for 1 hour, according to APAT-IRSA/CNR (2003).

Glacial influence was calculated using the Glacial Index (GI – Jacobsen & Dangles, 2012), which was estimated using the following formula:

$$(1) \quad GI = \sqrt{\text{area}/(\text{dist} + \sqrt{\text{area}})}$$

where ‘area’ is the extension of the glacier surface (km<sup>2</sup>) and ‘dist’ represents the distance of the site from the glacier margin (in km). The maximum glacial influence is represented by GI = 1 (zero distance from the glacier) and it decreases towards zero whilst moving away from the glacier.

Water temperature was measured at 1-hour intervals with digital loggers (Onset Hobo TidbiT®, Escort Junior, TinyTag, and TinyTalk) starting from 1 week before the first sampling date. At three sites (CR0, CR2, and MA1), water temperature was measured at the permanent hydrological stations managed by Meteotrentino (<https://www.meteotrentino.it>). Minimum, maximum, mean, and mean maximum temperatures were calculated over a period corresponding to one week before sampling.

Water samples (1 L) were collected at each site to analyse their chemical composition (pH, conductivity, alkalinity, hardness, nutrients, silica, anions, and cations) according to APAT-IRSA/CNR (2003).

At each site and date, the standing crop of algae was estimated as chlorophyll *a* concentration by scraping an area of 9 cm<sup>2</sup> from three stones selected randomly in the channel, yielding a total of 27 cm<sup>2</sup>. Chlorophyll *a* was extracted with 90 % acetone and the concentration was read at 665 nm and 750 nm, according to Steinman *et al.* (2017). Environmental data are presented in Table 1 and Table S1 (see Supplementary information, available at <http://www.limnetica.net/en/limnetica>).

### Macroinvertebrate sampling and identification

A total of 75 quantitative kick-samples were collected during the summer of 2018 at the eight sampling sites. In the streams, each site was 15 m long. In each of these, benthic macroinvertebrates were collected along the shoreline and on the bottom of the pond by kick-sampling with a 22 × 23 cm (= 0.05 m<sup>2</sup>) pond net (mesh size = 100 µm) in five micro-habitats (for a total of 0.25 m<sup>2</sup>), according to Castella *et al.* (2001). The five micro-habitats were selected as major habitats in proportion to their presence within the 15 m sampling site (Lencioni, 2018). We also considered the occurrence of algal mats or mosses in choosing the micro-habitats that are most representative of the stream site.

The macroinvertebrates were sorted under a stereomicroscope (50 x) and identified to genus (Ephemeroptera and Plecoptera), family (Diptera, Trichoptera, Oligochaeta, Coleoptera), or higher taxonomic levels (Tricladida, Nematoda) according to the national assessment method to assess the ecological status of rivers (D.M. 260/2010, 2011). All samples were preserved in 75 % ethanol and stored in the invertebrate collection (cINV017) of the MUSE - Museo delle Scienze of Trento (Italy).

In the sorting process, benthic particulate organic matter (BPOM, > 1 mm) was separated from each benthic replicate, dried for 1 hour at 60 °C, and ashed at 500 °C in a muffle furnace, according to Galas (1993). The list of taxa identified

is presented in Table S2 (see Supplementary information, available at <http://www.limnetica.net/en/limnetica>).

### Data analysis

Abundance was expressed as individuals per square metre (ind/m<sup>2</sup>) in each sampling unit (= site × date of sampling). A matrix of 33 taxa × 15 sampling units (= 2 sampling units/site for seven sites + 1 sampling unit for MA1) was created.

Alpha diversity (local diversity = number of species found in one site) was calculated as number of individuals, number of taxa, Simpson Index – D, and Shannon-Wiener Index – H. Beta diversity (change in species diversity between sites) was calculated as Whittakers  $\beta_w = \gamma/\bar{\alpha}$ , where  $\gamma$  = gamma diversity (total number of species in the catchment) and  $\bar{\alpha}$  = average number of species at the study sites (Whittaker, 1960).

The Kruskal-Wallis test (multiple comparisons of mean ranks for all groups) and Mann-Whitney non-parametric U test were used to highlight spatial and temporal differences among communities. Values with  $p < 0.05$  were considered significant (Table S3; see Supplementary information, available at <http://www.limnetica.net/en/limnetica>). The environmental variables were tested for a normal distribution using the Shapiro-Wilk test and variables with non-normal distributions were  $\ln(x)$  or  $\ln(x + 1)$  transformed. Furthermore, one-way ANOVA and post-hoc Tukey's HSD (Honestly Significant Difference) tests were performed to evaluate any significant inter-site differences existing among the variables (Table S4; see Supplementary information, available at <http://www.limnetica.net/en/limnetica>). Correlations among environmental variables were estimated using Pearson's correlation coefficient and values with  $p < 0.05$  were considered significant. All analyses were performed using the PAST software (version 3.22) (Hammer et al., 2001) and STATISTICA version 12.0 computer package (Statsoft).

The quantitative faunal data, expressed as total invertebrate density (ind/m<sup>2</sup>) per sample (site × season), were  $[\log(x+1)]$  transformed prior to classification and ordination analyses for normalisation. A cluster analysis (paired group –

UPGMA), based on the Bray-Curtis similarity coefficient, was performed to highlight similarities among sites in relation to the macroinvertebrate community (Sneath & Sokal, 1973; Lencioni et al., 2018). Furthermore, a distance-based redundancy analysis db(RDA) was performed to investigate the relationships between fauna and environmental conditions. Five environmental variables were included in the dbRDA analysis, selected among those that were significantly different among sites (Table S1) and had r-Pearson correlations  $< 0.6$  between each other; these are: Glacial Index, mean maximum temperature, chlorophyll *a* concentration, discharge, and conductivity. Forward selection within the dbRDA was performed to find a set of significant explanatory predictor variables among the environmental factors. The statistical significance ( $p < 0.01$ ) of each environmental variable was assessed independently by means of an unrestricted Monte Carlo permutation test (999 permutations). Notably, for multiple simultaneous testing, the false discovery rate correction (FDR) was considered. The significance of the total model, after diluting the non-significant environmental factors, was also performed using the Monte Carlo permutation test (999 permutations).

The cluster analysis was performed using the PAST software (version 3.22) (Hammer et al., 2001), while the dbRDA was performed with the CANOCO software (version 5.1) (Cajo & Ter Braak, 2013).

## RESULTS

### The environmental conditions

The main environmental data collected (mean ± SD) are shown in Table 1. Thirteen variables were selected as significantly different ( $p < 0.05$ ) among the sampling units (Table S1).

Glacial influence (GI) significantly varied among the sites ( $p < 0.001$ ). The sites with the highest GI were the kryal AG1 (1.00), MA1 (0.98), and AG\_pond (0.95), while those with the lowest were the glacio-rhithral C2 (0.14) and CR2 (0.34), as well as the kryal C0 (0.31).

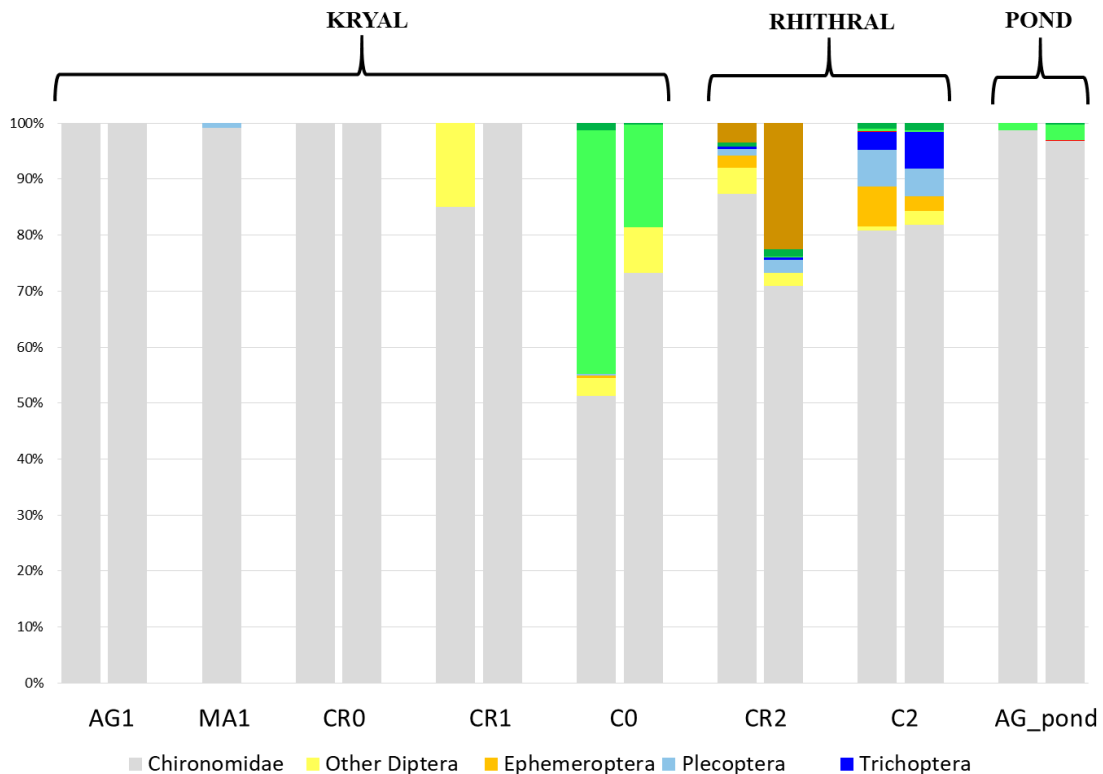
The lowest channel stability (high Pfankuch Index) was calculated for the kryal sites of the

Careser and Mandrone streams (47–58), while the highest stability was found in Conca sites (30–31) (Table 1). The Pfanckuch Index was significantly different among the sites ( $p < 0.001$ ) and positively correlated with turbidity ( $p = 0.03$ ), current velocity ( $p = 0.002$ ), and discharge ( $p = 0.004$ ). Indeed, Careser and Mandrone streams, besides being highly unstable and fed by the largest glaciers, were also the most turbid (maximum value = 166 mg/L in CR1), turbulent (maximum value of current velocity = 0.73 m/s in CR0) and had the highest discharge (maximum value = 3.8 m<sup>3</sup>/s in MA1). A positive correlation was found between discharge and glacier area ( $p < 0.001$ ).

Maximum water temperature, absolute (Tmax,  $p = 0.02$ ) and mean (mTmax,  $p = 0.03$ ), showed significant variation among some sites (Table S1, Table S4). mTmax positively correlated with Tmean ( $p < 0.001$ ), Tmin ( $p < 0.004$ ), and Tmax

( $p < 0.001$ ). Furthermore, the maximum temperature remained  $< 4$  °C in only two sites (AG1 and MA1). In the other kryal sites, Tmax reached up to 8–10 °C, whilst in the glacio-rhithral sites, Tmax was recorded up to 13 °C (Table S1). The mean temperature (Tmean) remained  $< 5$  °C in the other kryal sites (CR0, CR1, and C0); meanwhile, in the glacio-rhithral sites and in the pond, this value was  $< 8$  °C.

Water samples in the streams flowing on tonalite (in the Adamello-Presanella Mts) were the most acidic (pH = 6.2–6.9) and poorest in anions and cations (conductivity  $< 15$  µS/cm). Further, the highest pH (7.4–8.5) was recorded in the glacial pond and in AG1, while the highest conductivity ( $> 70$  µS/cm) was recorded in Agola sites, due to the calcareous nature of the area, and in the Careser stream (86–137 µS/cm) due to the high sulphate concentration. Both parameters were



**Figure 3.** Relative composition of the macroinvertebrate community in the eight study sites obtained on the two sampling dates (ind/m<sup>2</sup>). *Composición relativa de las comunidades de macroinvertebrados en los ocho sitios de estudio obtenida en las dos fechas de muestreo (ind/m<sup>2</sup>).*



consistent in differentiating the sites according to the ANOVA analysis (pH,  $p = 0.005$ ; conductivity,  $p = 0.02$ ).

Sulphate concentration was significantly higher ( $p = 0.03$ ) in all Careser sites ( $27.0 \pm 6.1$  mg/L in early summer and  $54.0 \pm 21.7$  mg/L in late summer) than in the other sites ( $0.23 \pm 0.2$  mg/L in early summer and  $0.5 \pm 0.3$  mg/L in late summer). For this reason, we separately plotted the Careser sites in Fig. 2 to show the ratio between  $\text{SO}_4^{2-}$  and Si that is used to separate the water contributions for each site. Notably, the concentrations of both  $\text{SO}_4^{2-}$  and Si, used as tracers of water origin, were lower in early summer than in late summer (Fig. 2).

Both chlorophyll *a*, expressed as  $\ln\text{Chl } a$  ( $p < 0.001$ ) and BPOM ( $p < 0.001$ ) were significantly different among sites. The chlorophyll *a* concentration was highest in the glacio-rhithral sites ( $0.6\text{--}0.7$   $\mu\text{g}/\text{cm}^2$ ), measured  $< 0.25$   $\mu\text{g}/\text{cm}^2$  in the kryal sites, and was found to equal  $0.07$   $\mu\text{g}/\text{cm}^2$  in the glacial pond. Among the kryal sites, the highest values were recorded in C0 ( $0.21$   $\mu\text{g}/\text{cm}^2$ ) and in MA1 ( $0.25$   $\mu\text{g}/\text{cm}^2$ ) due to the presence of biofilm and mosses, and due to the presence of *Hydrurus foetidus* (Villars), respectively.

Benthic Particulate Organic Matter (BPOM) showed trends similar to chlorophyll *a* concentration (correlated positively but not significantly,  $p = 0.066$ ), being lower in kryal sites and in AG\_pond ( $0.4\text{--}8$   $\text{mg}/\text{m}^2$ ) but higher in glacio-rhithral sites (maximum of  $62$   $\text{mg}/\text{m}^2$  in C2). Among kryal sites, an exception to this trend was seen in C0, where BPOM reached up to  $45 \pm 15$   $\text{mg}/\text{m}^2$ .

### The macroinvertebrate communities

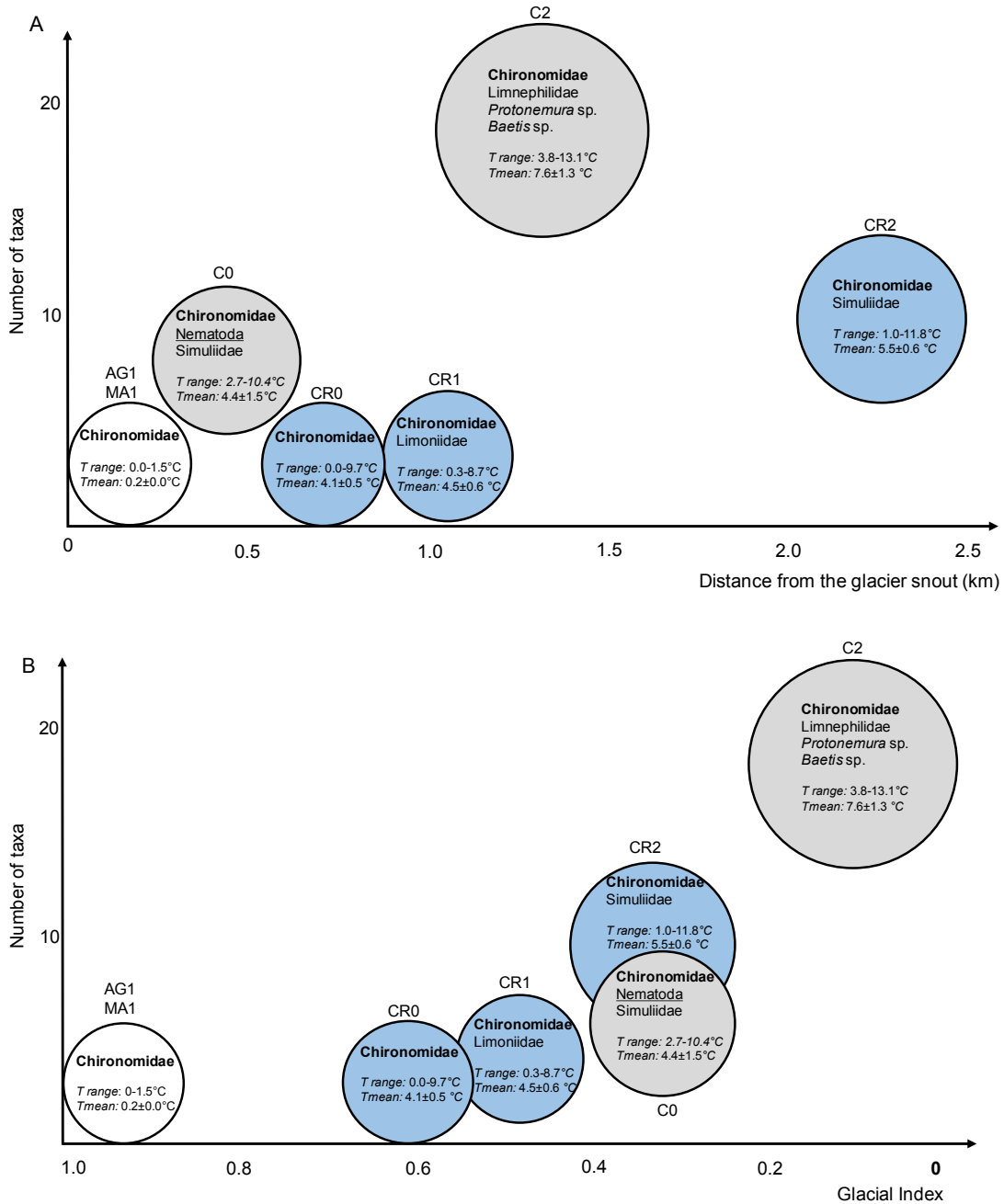
In total, 15 273 specimens were collected, most of which were from the Conca sites (73 %). Thirty-three taxa were identified, among which the Diptera family Chironomidae accounted for 80 % of individuals, followed by EPT (9 %). The remaining 11 % was represented by other Diptera families (Anthomyiidae, Blephariceridae, Ceratopogonidae, Dixidae, Dolichopodidae, Empididae, Limoniidae, Psychodidae, Rhagionidae, Simuliidae, and Tipulidae), as well as Nematoda, Tricladida, Oligochaeta, Elmidae, and Helophori-

dae (Fig. 3, Table S2). The highest values for both taxa richness and Shannon diversity index, as well as the lowest Simpson dominance, were calculated for the glacio-rhithral sites (C2 and CR2) and the kryal site (C0), respectively (Fig. 5, Table S2). The distance from the glacier snout ( $p = 0.020$ ), and the maximum and mean water temperatures ( $p = 0.043$  and  $p = 0.033$ , respectively) were positively correlated with increasing taxa richness.

Site C0 hosted a macroinvertebrate community significantly different from those colonising the other kryal sites, but similar to that found in the glacio-rhithral site, CR2 (Table S3). This is due to the consistent presence of dipterans (Simuliidae, Limoniidae, Tipulidae), EPT, Oligochaeta, and Nematoda. Beta-diversity confirmed these similarities, and the anomaly of site C0, within the kryal sites for the macroinvertebrate community structure (Table S5; see Supplementary information, available at <http://www.limnetica.net/en/limnetica>).

The macroinvertebrate community structure changed with increasing distance from the glacier snout, as shown in Figure 4A: taxa richness increased progressively with increasing distance, with one exception represented by C0, close to the snout but with a richness comparable with that one of CR2. On the other hand, C0 represents no longer an exception if sites are ordered by glacial influence (as GI) instead of distance from the snout (Fig. 4B). It was evident that the taxa richness increased with decreasing GI ( $r = -0.718$ ,  $p = 0.0025$ ), independently of the vicinity of the feeding glacier, with seven taxa at  $\text{GI} = 0.3$  with a  $T_{\text{mean}} = 4.5$   $^{\circ}\text{C}$ , and more than 20 taxa at  $\text{GI} < 0.2$  with a  $T_{\text{mean}} = 8$   $^{\circ}\text{C}$ .

The similarities among sites, based on their macroinvertebrate communities, were highlighted by cluster analysis (Fig. 6). Sampling sites were grouped according to their origin and glacial influence: glacio-rhithral sites (with the lowest GI and the highest biodiversity) were separated from the kryal sites and the glacial pond (with higher GI and lower number of taxa). The only exception was site C0, which is fed by ice melt (Fig. 2A) but has a low GI ( $\text{GI} = 0.31$ , Table 1). This site hosted a community resembling a more glacio-rhithral one (low Chironomidae abundance, presence of other dipterans, and EPT) than a kry-

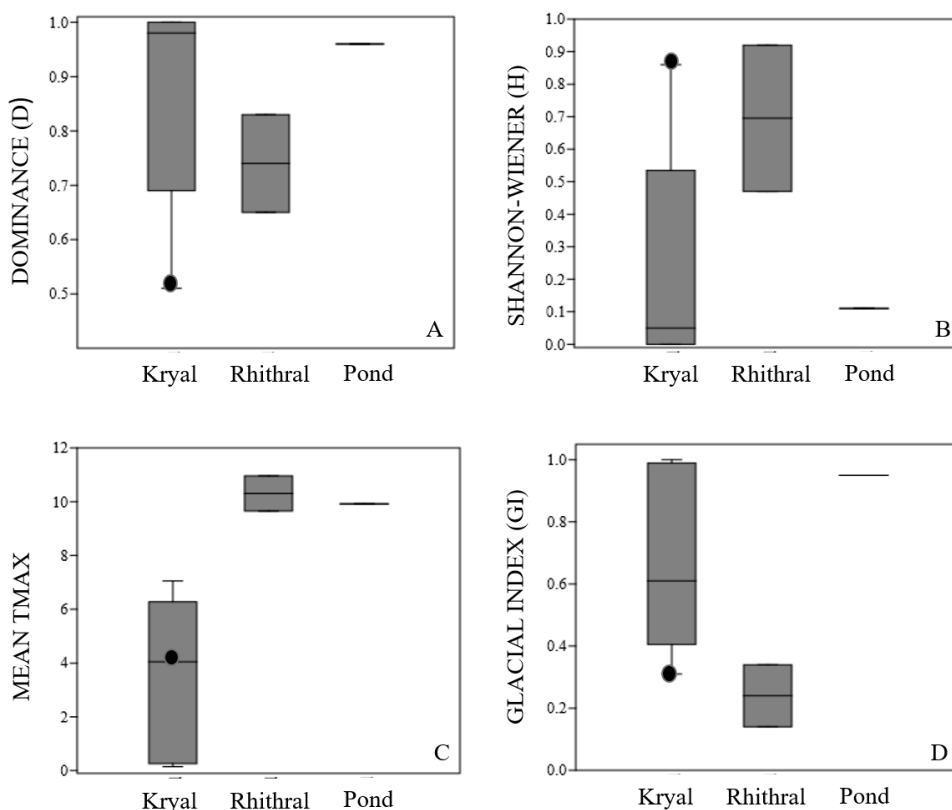


**Figure 4.** The Milner & Petts (1994) model with sites ordered according to distance from the glacier snout (A) and to glacial influence (as Glacial Index) (B). Taxa in bold have a relative abundance > 60 %, taxa underlined are between 20 % and 60 %, taxa not underlined are between 2 % and 20 %; taxa < 2 % were not reported. The ball size is proportional to the number of taxa counted in each sampling site and their colour clusters sites belonging to the same stream: white = Mandrone or Agola; grey = Conca; light blue = Careser. For each site water temperature range and mean water temperature are reported. *Modelo Milner & Petts (1994) con sitios ordenados según la distancia desde el frente glacial (A) y según la influencia glacial (como Índice Glacial) (B). Los taxones en negrita tienen una abundancia relativa > 60 %, los subrayados están entre el 20 % y el 60 %, los no subrayados entre el 2 % y el 20 %, y los < 2 % no han sido registrados. El tamaño de los círculos es proporcional al número de taxones contados en cada sitio de muestreo y sus zonas de color pertenecientes al mismo arroyo: blanco = Mandrone o Agola; gris = Conca; celeste = Careser. Por cada sitio también se muestran rango de temperatura y temperatura media.*

al one (strong dominance of Chironomidae, low biodiversity) (Table S2).

The ordination analysis dbRDA calculated three eigenvalues (dbRDA1 = 0.501, dbRDA2 = 0.021, dbRDA3 = 0.012), explaining 41.6 % of the total faunal variance overall. Three environmental variables were selected by RDA as the best variables to be associated with changes in macroinvertebrate communities in the eight sites. These are: glacial index (GI), mean maximum temperature (mTmax), and chlorophyll *a* concentration (lnChl *a*) (Fig. 7). All kryal sites were grouped in quadrants II and III. The sole exception was the kryal site, C0 that was grouped with glacio-rhithral sites on the right of the diagram due to its relatively high values of water temperature and chlo-

rophyll *a* concentration. dbRDA1 emphasised a glacial influence (increasing from the right to the left of the graph) and a thermal gradient (increasing from the left to the right of the graph) in all sites with GI > 0.5 and mTmax < 8 °C. Exceptions to this arrangement were AG\_pond\_II (grouped on the left, where mTmax reached 14 °C), and C0 (grouped on the right, where Tmax in late summer was 6 °C). This value is similar to that in CR0 and CR1 in early summer. Furthermore, dbRDA2 emphasised a chlorophyll *a* concentration gradient, increasing from the left to the right of the graph. The relatively high values of primary production found in the pro-glacial pond (0.07 µg/cm<sup>2</sup>) and in MA1 (0.25 µg/cm<sup>2</sup>) justifies their separation in quadrant II.



**Figure 5.** Box plots showing the trends of four variables (Simpson Dominance Index, Shannon-Wiener Index, Mean maximum temperature, and Glacial Index) in sampling sites, that were grouped according to the three stream typologies (rhithral = glacio-rhithral; black dots in the kryal column indicate the values of site C0). *Diagramas de caja que muestran la tendencia de: Simpson Dominance Index, Shannon-Wiener Index, la media de las temperaturas máximas e Índice Glacial) en los sitios de muestreo, agrupados en las tres tipologías de ríos (“rhithral” “glacio-rhithral”;* los puntos negros en la columna kryal indican los valores del sitio C0).

## DISCUSSION

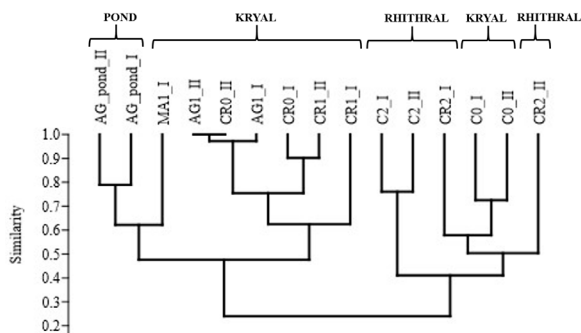
Overall, this study provides new insights on how different glacial influences can serve as proxies for climate change and affect macroinvertebrate communities in high-altitude freshwater habitats.

As documented for other stream ecosystems in the Alps (Lods-Crozet *et al.*, 2001; Lencioni & Gobbi, 2018; Scotti *et al.*, 2019), kryal sites were dominated by chironomids, representing up to 100 % of the macroinvertebrate fauna at two sites (AG1 and CR0). However, the percentage of chironomids decreased with decreasing glacial influence, and with increasing channel stability, water temperature, and food availability (e.g. chlorophyll *a* and benthic particulate organic matter). Where food is more abundant and environmental conditions are less stressful (as in glacio-rhithral sites), chironomids become less abundant because of competition with other insects (mainly EPT) and non-insects (mainly oligochaetes) (Milner *et al.*, 2001). Lencioni *et al.* (2021) highlighted that the decrease in chironomids at these sites

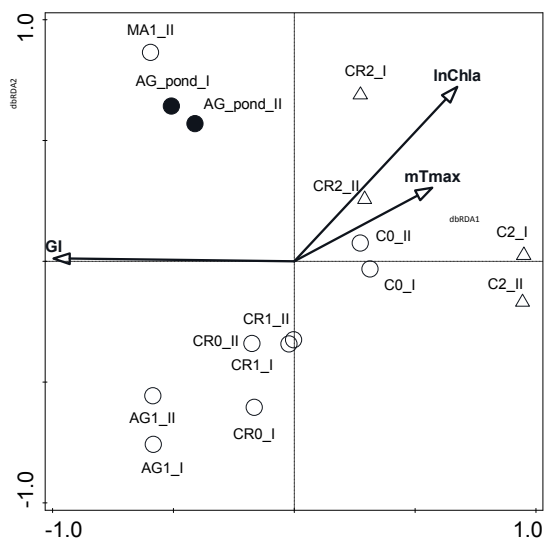
was mainly due to the decrease in the subfamily Diamesinae, prevalent in all typical kryal sites, which are replaced in glacio-rhithral sites by the subfamily Orthocladiinae and other dipterans. In addition, the pro-glacial pond had a high glacial influence (comparable with that of the kryal habitats) and was dominated by chironomids (> 90 %). Nevertheless, within them, Orthocladiinae prevailed as in the glacio-rhithral sites.

The expected temperatures for kryal sites (< 4 °C *sensu* Ward, 1994) were registered at only two sites (AG1 and MA1). In the other kryal sites (CR0, CR1, and C0), the maximum temperature was significantly above the thermic limit of 4 °C, suggesting a change taking place at these sites. However, as stated by Lencioni (2018), in the Southern Alps values of maximum temperature and GI of kryal can be very different from the standard *sensu* Milner & Petts (1994), being comparable in some cases with those typical of glacio-rhithral regions (Tmax = 9 °C, GI = 0.16).

The sites fed during summer, mainly or exclusively by ice melt, also had the highest GI, low water temperatures (mTmax < 6 °C; Tmax < 11 °C), low channel stability, and hosted a community dominated by chironomids. Based on these parameters, these can classify them as typical 'kryal', without solely relying on the basis of the sulphate and silicium ratio. Most of these, fed by the largest glaciers (area > ~2 km<sup>2</sup>), also had a high discharge (1.6-3.8 m<sup>3</sup>/s). Among the sites dominated by ice melt contribution, according to the sulphate and silicium ratio, we found only one site that exhibited exceptions to this trend. That is site C0, on the Conca stream. This result was supported by the site's GI (= 0.31), and high level of food availability (as Chl *a* and BPOM, due to the presence of mosses and biofilm), combined with high channel stability, low discharge (0.04 m<sup>3</sup>/s), and relatively high water temperature (reaching a maximum of 13 °C). In C0, chironomids represented only 60 ± 15 % of the community, which is lower than the one in the other kryal sites and even lower than in glacio-rhithral sites (77 ± 9 %). Overall, these findings suggest that C0 is in a transition from a typical kryal habitat to a glacio-rhithral habitat. We can attribute this transition to the retreating of the Conca glacier. This glacier, with its 0.14 ha, is feeding a small



**Figure 6.** Paired group (UPGMA) clustering based on the Bray-Curtis similarities (ranging from 0 = total dissimilarity, to 1 = complete similarity). Clustering based on species composition (33 taxa). Sampling unit codes: site\_season; I = early summer; II = late summer. Sampling units were grouped based on their *a priori* assigned type (kryal, rhithral = glacio-rhithral, and pond = pro-glacial pond). *Paired Group (UPGMA), agrupación basada en las similitudes de Bray-Curtis (que oscilan desde 0 = disimilitud total, a 1 = completa similitud). Agrupación basada en composición de especie (33 taxones). Código de la unidad de Agrupación: Estación: I = principios de verano; II = finales de verano. Las unidades de muestreo han sido agrupadas basándose en su tipo de asignación a priori (kryal, rhithral = glacio-rhithral, y laguna: laguna proglacial).*



**Figure 7.** Distance-based Redundancy Analysis (dbRDA) biplot (sites  $\times$  environmental variables). I = early summer; II = late summer; white circles = kryal; black circles = proglacial pond; up-triangle = glacio-rhithral. mTmax = mean maximum temperature recorded during the 3 weeks before sampling, InChl *a* = chlorophyll *a* concentration, GI = Glacial Index. *Análisis dbRDA (sitio  $\times$  variables ambientales). I = principios de verano; II = finales de verano; círculos blancos = kryal; círculos negros = laguna proglacial; triángulo = glacio-rhithral. mTmax = media de temperaturas máximas registradas en las tres semanas anteriores al muestreo; InChl *a* = concentración de clorofila *a*; GI = Índice Glacial.*

stream that flows on dark and steep *roche moutonnées*, facing the south, and tends to overheat. Previous investigations reported that at the end of the 1990s, this site had a higher GI ( $\sim 0.5$ ) and hosted a typical kryal community dominated by chironomids, at a mean temperature  $< 4$  °C (Lencioni, 2018).

It is interesting to note that, in contrast with Kärnä et al. (2019), geo-diversity did not support differences observed between communities living in catchments with different lithologies. These authors found that geo-diversity affects diatom and macroinvertebrate richness through changes in stream water chemistry (Chuman et al., 2013), and proposed it as a proxy for predicting stream species richness. In our case, the Careser catchment significantly differed from the Adamello-Brenta Mts. in lithology and, consequently, water chemistry (i.e. higher conductivity and sul-

phate concentration) but this did not influence the grouping of kryal sites.

The Mandrone site (MA1) emphasised another interesting aspect. Although its concentration of chlorophyll *a* is high ( $0.25 \mu\text{g}/\text{cm}^2$ ) due to the presence of the crysophita *H. foetidus*, its high GI (0.98) and low temperature ( $< 2$  °C) results in a macroinvertebrate community dominated by chironomids (99 %) (all belonging to the genus *Dia-mesa*, Lencioni et al., 2021). This highlighted the fact that the community structure and diversity were controlled, in a determinist way (Milner et al., 2017), by glacial influence, notwithstanding the high abundance of food resources (in accordance to Niedrist & Füreder, 2017).

According to the model by Milner & Petts (1994) the community shifts were mainly linked to water temperature and the stability of the stream bed, with increasing biodiversity downstream from the glacier snout. But with one exception, represented by one site (C0) fed by a vanishing glacier and dominated by *roche moutonnées*, making the substrate highly stable. Due to these features, C0 was warmer than expected based on its distance from the snout and had a taxa richness comparable with that one of sites at a distance  $> 1$  km from the snout. This exception may be solved if sites are ordered according to their glacial influence instead of their distance from the snout. Specifically, excluding Conca sites, within about 1 km downstream of the snout, there is high glacial influence ( $\text{GI} \geq 0.5$ ), Tmean remains below  $4.5$  °C, as expected for the kryal region, and the community is represented by chironomids as the sole or dominant ( $> 90$  %) taxon. Only after 2 km from the snout, and downstream of the confluence of non-glacial tributaries, did the glacial influence decrease ( $\sim 0.3$ ) and the Tmean was  $> 5$  °C, resulting in a richer community ( $> 10$  taxa). Including Conca sites, the model is not validated. This means that, we have an unexpected high richness close to the glacier snout (within 500 m) and a comparable richness ( $> 20$  taxa) with that of non-glacial streams (Lencioni, 2018) at only about 1 km downstream from the snout. The richness trend responds better to the glacial influence, that is based not only on the distance from the glacier snout but also on the glacier surface area. These data suggest that GI might be used instead



of distance from the glacier snout on the x-axis to redraw the model of Milner & Petts (1984), to also include “atypical” kryal sites in transition to the glacio-rhithral type, due to climate change.

Contrary to what was expected, channel stability did not decrease in all glacial systems with increasing distance from the snout. This might also be due to natural causes, as in the Conca system, where channel stability is as high as it was 20 years ago (Maiolini & Lencioni, 2001) because of the geomorphology of the site. This occurrence is independent from the activity of the on-going retreating glacier. The flow of the kryal reaches on *roche moutonnées* is highly stable and south-exposed. This exposure also justifies the high maximum temperature recorded within the first kilometre of the glacier-fed stream, which is already higher than what was expected 20 years ago for this same reason (Maiolini & Lencioni, 2001).

In conclusion, we showed that streams fed by glaciers with different surface areas host different benthic communities along a longitudinal gradient. We also noted that compared to distance from the snout, as originally proposed by the Milner & Petts model, the Glacial Index (GI) is a better predictor of changes in benthic communities. Therefore, the GI should be considered in assigning a type category to stream reaches when ice melt is one of the water contributors.

The decrease in the percentage of chironomids is the most evident change that occurs in the community, as expected (Burgherr & Ward, 2001), and we found typical kryal communities (i.e. with percentage of chironomids > 80 %) only at a GI > 0.5.

We also confirmed that the identification of chironomids to the species level, or at least the genus level is necessary to distinguish the influence of glaciers in the macroinvertebrate communities, as observed by other authors (Hamerlík & Jacobsen, 2012; Rossaro *et al.*, 2016). In fact, dbRDA analysis plotted the MA1 and AG\_pond together, but if we had considered a lower level of identification for chironomids, they would have been separated. This is most likely because MA1 fauna are represented only by the genus *Diamesa* (Diamesinae), while the pond was dominated by the genus *Metriocnemus* (Orthocladiinae) (Lencioni *et al.*, 2021).

This survey suggests that small vanishing

glaciers (with a surface < 1 km<sup>2</sup>) are particularly suitable to monitor the effects of climate change being expected to disappear in a few years. Studying them allows us to predict the future of aquatic glacial biodiversity. Within this context, ecological studies are claimed not only on macroinvertebrates, but also on other communities, such as bacteria and algae and their trophic relationships to have a holistic view of the effects of glacier retreating on alpine ecosystems (Battin *et al.*, 2016; Fell *et al.*, 2017; Sertić Perić *et al.*, 2021).

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## Author contributions

**Daniele Debiasi:** participated in field activity, sorted biological samples, identified macroinvertebrates, edited part of data, performed data analysis, and wrote the first draft of the manuscript; **Alessandra Franceschini:** participated in field activity and analysed water samples; **Francesca Paoli:** participated in field activity, edited part of data, and made Fig. 1; **Valeria Lencioni:** conceived and supervised the research, designed protocols, participated in field activity, performed data analysis, wrote and revised the manuscript. All co-authors approved the final version of the manuscript.

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