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Spatial and temporal community differences in Lazaun alpine streams

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SUMMARY

1. INTRODUCTION

1.1 Glaciers

A glacier is a plurimetric permanent mass of natural ice, derivative to the metamorphism of snow, which is object of gravitational deformations (Casarotto, 2010).
For a glacier to be formed are needed a snow intake and cool summer temperatures, to avoid the complete melting of the winter snow. At our latitudes it takes around 3-5 years for the snow to become ice. That is due to snow compaction and freeze-thaw cycles during spring. In places such as Antarctica it takes almost a century for ice to form, as the temperatures are low and more stable, causing scarce freeze and thaw cycles.
Gravity not only helps to form ice from snow compaction but is also responsible for the downward moving of the glacier. Moving to valley the glacier transfers the ice accumulated on top, in the accumulation basin, to underlying zones, the ablation zone, where the ice melts and gives life to glacial streams (Casarotto, 2010). The accumulation basin is the zone where snowy precipitations endure also in summer months and so snow accumulates. In contrast the ablation basin is the zone where more ice melts than the one that it is formed. The line that divides these two areas is called the equilibrium line, where the annual balance of the glacier is none (Benn, Lehmkul, 2000). The annual balance for a glacier can be positive, where the accumulation basin expands, or negative, where the accumulation basin retreats, and the ablation zone expands. If the ablation zone expands too much, with time, the glacier can become extinct.
The factors that influence the growth and the retreat of a glacier are winter snows and summer temperatures. Winter snows give the glacier the ‘food’ with which it can grow, form more ice, and potentially expand itself. The summer temperatures affect the rate and the degree of melting. A positive balance suggests that the glacier is healthy, moving and growing (Casarotto, 2010).
Another aspect to take in consideration is the mass balance of a glacier, which is the difference between the intakes, winter snows, and the losses, summer melting. The intakes are calculated by measuring the depth and the density of the snow, converting the data in millimeters equivalent of water. Measurements are made approximately during May/June. The losses are also calculated in mm eq. of water, with poles planted on the glacier at the end of the summer of the year before. The melting of the next summer makes the poles emerge, and each meter corresponds to a loss of 910 mm of equivalent water (Casarotto, 2010).
A peculiar type of glacier is the rock glacier, which is a debris landform created by the past or current creep of permafrost, detectable in the landscape by a front, lateral margins and optionally ridge-and-furrow surface topography (RGIK, 2022). They can be distinguished in intact or relict rock glaciers. An active intact glacier is capable of motion, an inactive has lost his motion, but still

maintains internal ice. A relict RG (Rock Glacier) has lost its ice thousands of years ago hence they are no longer in motion (RGIK, 2022).

Globally the glacier situation is dramatic. The Fifth Assessment Report (AR5) of the United Nations Intergovernmental Panel on Climate Change (IPCC) reported a decrease in snow cover over most of the Northern Hemisphere, decreases in the extent of permafrost, increases in its average temperature, and glacier mass loss in most parts of the world. Recent studies confirmed with high confidence that snow cover extent continues to decrease across the northern hemisphere in all months of the year (IPCC, 2022).

The glaciers with the most negative mass balances are in the European Alps and at low latitudes in the South American Andes (Milner et al., 2017).

Recent previsions point out that possibly just 4-13% of the 2003 European Alps ice area will remain by 2100. This projection is considering previous trends, as the atmospheric warming of the last 30 years combined to a decrease in snowfalls led to 54% loss of ice area since 1850 (Milner et al., 2017).

In Italy from the second half of 19^{th} century, we can observe a 40% decrease in the number of glaciers (Casarotto, 2010).

Glaciers are fundamental as they represent one of the biggest sources of freshwater, which can be used for various aims, as agricultural, civil, or industrial purposes. They can also be used for hydroelectric energy production. In second place they serve also as an attraction for tourists, alpinists, summer skiing, which for mountain regions can be a large source of income (Casarotto, 2010).

The potential changes generated by the melting of glaciers is immense, both for downstream ecosystems and for society. The downstream impacts will range from the glacier to rivers and to the ocean, affecting various ecosystems levels ranging from biogeochemical shifts and sediment fluxes to loss of biodiversity and changes in the food web dynamics (Milner et al., 2017).

Furthermore, the importance of glaciers has been recognized at a legislative level as they have been included in Rete Natura 2000 network with the code 8340 as they are a highly fragile habitat that cannot be replaced and could likely go extinct in the next decades (Gobbi et al., 2021)

1.2 Alpine streams

Headwater streams who are situated above the tree line and under the permanent snowline are called alpine streams and are subject to a different classification (Brown et al., 2003). The tree line is defined as, in undisturbed areas, the 10C isotherm for mean daily temperatures in the warmest month, which divides the subalpine/subartic woodland and the alpine/tundra (Remmert, 1980).

Above the treeline, the alpine environment is harsh and is characterized by bare rock surfaces, snow beds, pigmy shrubs, skeletal soils (Brittain et al., 2000).

These alpine river streams are fed by glacial icemelt, snowmelt, and groundwater (Brown et al., 2003). Even if streams originated from these systems share common characteristics, as steep gradients, high flow velocities, and high dissolved oxygen concentrations, each source has characteristic physical and chemical features and discharge regime (Ward, 1994) (Brown et al., 2003).

Their water source and temperature define their classification, although in some studies are included also habitat conditions, as turbidity and flow regimes as discriminants (Ward, 1994) (Brown et al., 2003). Three general stream biotypes have been distinguished using those parameters: kryal, rhithral, and krenal ward 1994 (Brittain et al., 2000) (Brown et al., 2003). In addition to these categories, Brighenti et al. (2019) suggests including alpine permafrost as a water source. Permafrost waters exhibit typically clear waters, low and stable temperatures (0.5-1.5°C), high conductivity due the concentrations of ions and cations as Ca^{2+} and SO_4^{2-} (Brighenti et al., 2019) and higher α diversity in the microbiota (Fegel et al., 2016). So, in this work we will also address to active and relict rock glacier water streams.

Kryal streams are glacier-melt-dominated, occur at high altitude near to the glacier terminus and have high daily flow fluctuations during summer and the coldest water temperatures (Ward, 1994) (Brown et al., 2003). Those are usually divided into metakryal and hypokryal, based on different faunal compositions; respectively dominated by *Diamesa* sp. and Diamesinae/Orthocladiinae (Debiasi et al., 2022). Krenal streams originate from groundwater and possess constant water temperature. Rhithral streams are characterized by conditions intermediate between kryal and krenal as they are seasonal snowmelt-fed streams (Brown et al., 2003).

Another approach in classifying alpine streams was presented by Brown et al. 2003, in which they suggested that for further studies, instead of assigning categories based upon their principal water source and temperature regime, it should be determined the actual relative contribution of different water sources. Nine categories were proposed, and the principal ones are kryal, nival and krenal. The other categories are intermediate with the three major ones. This classification points out the differences in water chemistry between different water sources, which give the streams different properties. In this paper we will use the classification proposed by Ward in 1994.

Regional climate influences the dynamics of alpine streams by adding or removing mass and energy from the glacier and snow surface, determining variations in accumulation and ablation rates of ice and snow, therefore changing the meltwater inputs to glacial streams throughout the year. Accordingly seasonal changes in streams occur (Brittain et al., 2000). The seasons' influence is most evident during late summer, where the glacier runoffs and ice melting reach their peak, making creeks display their unique characteristic as high discharge, high turbidity, high sediment load and bed load movement. In the remaining time of the year alpine streams are characterized by the same features of groundwater, rainfall, and snowmelt systems. The timing and volume of meltwater production alongside with groundwater inputs widely change the physical and chemical properties of streams (Brittain et al., 2000). This instability is characterizing this environment, influencing the benthonic fauna (Brittain et al., 2000).

1.2.1 Kryal

Glaciar-melt streams are very cold, with temperatures close to 0C near the glacier terminus and with a maximum of four degrees Celsius (Füreder et al., 1999) (Brown et al., 2003) (Ward, 1994). Solar heating influences the temperature seasonally and on daily time.
The diel cycles of solar radiation influence the ice and snow melting, leading the streams to show a peak flow during the summer's afternoons. In these periods of ice melting alpine streams often carry high concentrations of silt and clay, with peaks reaching 500 mgL-1 (Brown et al., 2003), resulting generally in high turbidity (Ward, 1994).
Alpine streams possess a very low channel stability, with frequent disturbances due to sediment and rock moving.
Chemically, Ca^{2+}, HCO_3^- and SO_4^{2-}usually stand out between ions proportions in proglacial streams, along with lesser quantities of Mg_2^+, Na^+, and K^+. These features are to be attributed predominantly to bedrock type and subglacial drainage processes (Brown et al., 2003).
In these streams, plankton, angiosperms, and fishes are absent. Bryophytes are generally not found, leaving few algae and dyatoms species as the only photoheterotrophic eucaryotic organisms. *Hydrurus foetidus* (Villars) is often the predominant algae. The zoobenthos is present but highly restricted, showing longitudinal patterns, mainly composed by Chironomids, of which Diamesa species are the only organisms who can live near the glacier's tongue, characterizing this habitat (Ward, 1994), (Debiasi et al., 2022) (Lencioni, 2018). In the study conducted by Lencioni et al., 2022 it has been assessed that *Diamesa* species go extinct when summer temperatures exceed 6 Celsius degrees, which means that a kryal stream type is only present when the temperatures are lower.

1.2.2 Rhithral

Generally, snowmelt regimes show peak discharge in spring before the maximum glacier runoff happens. In some alpine contexts, due to high altitude, the peak could be delayed to July. Temperatures typically possess a wide range, generally from 5 to 10 Celsius degrees. Turbidity in rhithral streams is usually low, giving water a clear aspect, however during high flows the turbidity may be higher, caused by the resuspension of sediment. The preferential elution of ions from the snowpack gives the water specific chemical attributes, which varies throughout the year, such as high Nitrogen concentration during spring snowmelt runoff and more diluted samples in winter (Brown et al., 2003).

Snowmelt streams are characterized by the presence of bryophytes, a relatively high algae richness, and a macrozoobenthos which includes Ephemeroptera, Trichoptera, Plecoptera and Diptera (Ward, 1994).

1.2.3 Krenal

Groundwater fed systems possess a constant water temperature, as it is correlated to mean annual air temperature, which varies by a couple of degrees in an annual cycle, giving those streams a regular temperature throughout the year (Brown et al., 2003). As groundwater fed streams, they are not affected by ice and snow melt, so they don't show dial or seasonal fluctuations, nor in discharge and turbidity, nor in water chemistry (Ward, 1994) (Brown et al., 2003). Krenal systems possess marked ionic enrichment, typically with high silica concentrations. However not every spring-fed system will possess similar physiochemical characteristics, as those varies with geologic traits and groundwater residence time (Brown et al., 2003).
The constant flow, minimal bed and soil erosion, plus a stable substratum favor life presence. In addition, inhabitants are not subjected to freezing or desiccation due to the constant temperatures above 0C. Algae and diatoms are abundant and macrozoobenthos orders are like kryal streams, although species differ (Ward, 1994)

1.3 Macroinvertebrates

Macroinvertebrates are all invertebrates bigger than 1mm living for at least one stage of their life inside a water stream and are considered good bioindicators, as they occupy many roles in the trophic chain and are sensitive to pollution in different scale. Also, they are not very mobile, and the limited spatial movement is another requirement for a good bioindicator, because if a change occurs the population will be surely affected and cannot avoid it by moving away. Macroinvertebrates generally possess a long lifecycle, which can be used to address also smaller impacts on the long term. Another advantage of studying those organisms is that they are easy to sample and determine. The most frequent groups are insects, shellfishes, mollusks, nematodes, and annelids. From the year 2000, UE with the Directive 2000/60/CE has introduced in the parameters to assess water streams quality the biotic component, which includes macroinvertebrates, determining their importance[1].
Alpine streams are a difficult environment for organisms to establish, as low temperatures, low channel stability, seasonal high discharge and turbidity and high slope degrees preclude this habitat to many species (Ward, 1994).

[1] https://www.arpa.vda.it/en/ambiente-naturale/acqua/ambacqa04macroinvertebrati-e-indice-star-icmi-descrizione-della-comunita-e-del-metodo/1056-metodi-biologici/1361-1361-macroinvertebrati-e-indice-staricmi-descrizione-della-comunita-e-del-metodo 23/06/2022

Temperature and channel stability, and in part inputs of allochthonous organic matter (Brown et al., 2003), have the major role in influencing the distribution and abundance of organisms (Brown et al., 2003) (Milner & Petts, 1994).

Inputs of vegetation in streams are one of the primary sources of food for macroinvertebrates, but if in other contexts the inputs are consistent, in alpine streams those are scarce, as the riparian vegetation is absent, and the surroundings don't provide many organic debris (Milner & Petts, 1994) (Füreder et al., 1999). So, the primary source of food derives from the few algae present in the streams, and in proximity of glacier's tongue from pollen, seeds, spores transported by wind (Ward, 1994).

The model proposed by ward 1994 which identifies the three types of streams by temperatures and water source, does not provide an understanding of the hydrologic mechanisms which influence the macroinvertebrates communities. These concepts were taken in account in the model proposed by Milner & Petts, 1994, which identifies temperature and channel stability as the major drivers. Temperature is surely a determining factor, which impedes many organisms to colonize a water stream. However, even if the temperatures can be optimal, channel stability precludes or delay the colonization from specific taxa (Milner & Petts, 1994). The gradient of channel stability is longitudinal, the closer to the glacier the more unstable it is, and further from it the riverbed becomes more stable, caused by a less variability in water flux. Temperature also has a vertical gradient. Given the longitudinal type of gradient of the influencing factors, macroinvertebrates share the same gradient, with less but more specialized species close to glacier's terminus, principally *Diamesa* sp., and then, moving away we can encounter Diamesiini and Orthocladiini. When the temperatures rises more than 2C ephemeropterans (Baetidae), plecopterans (Nemuridae and Chloroperlidae) can be found. The reaching of a riverbed more stable let trichopteran and others ephemeropterans populations establish (Milner & Petts, 1994).
Glacier fed water streams are having an evolution, from an initial and significant rise in discharge, due to the still existing glacier and its high melting rates, to the complete disappearing of the glacier and the transition from the kryal to rhithral type of the stream. Glacial biodiversity faces extinction, as glaciers retreat and the kryal habitat disappears. Currently we are observing an ascent of eurythermal species to high elevations, once exclusives of *Diamesa* (Debiasi et al., 2022). Seeing those trends, it's possible to imagine that there will be a reduction of diversity and a taxonomic homogenization in alpine areas (Bressan et al., 2018).

2. Aim of the study

The aim of this study was to study the macroinvertebrate community structure in five habitat types with different origin in the Lazaun glacier catchment (Bolzano Province), in relation to their environmental features. Spatial and temporal patterns were investigated even to select the main environmental drivers of biological differences.

3. Area of study

In the Autonomous province of Bolzano-South Tyrol are present 212 glaciers, contributing to the 23% of the whole Italian number. The area covered by them is 84.58 km2, making this region the third in Italy for glacierized zones, after Aosta Valley and Lombardy. Within the Autonomous province of Bolzano can be identified 7 mountain groups (from West to East): Ortles-Cevedale, Venoste Orientali, Venoste Orientali-Passirie, Breonie Occidentali, Breonie Orientali and Aurine, Alti Tauri-Tre Signori, Vedrette di Ries (Smiraglia & Diolaiuti, 2015).

Half of Bolzano glaciers cover less than 0,04 km2 each. (Smiraglia & Diolaiuti, 2015).

In this context we analyzed seven different water streams originating from Lazaun glaciers, in the Venoste Orientali group, right under the peaks Lagaunspitze and Saldurspitze. The glaciers, as catalogued by Smiraglia & Diolaiuti, 2015, are West Lazaun (n 808 in catalogue) and the East Lazaun (n 807), both facing North-East. Respectively they cover 0.13 km2 and 0.08 km2.

A rock glacier is also present, at which the study from Krainer et al. 2017, attributed 10300 years of age.

Figure 1: Lazaun glaciers location (Map generated through GIS, shapefiles taken from www.istat.it)

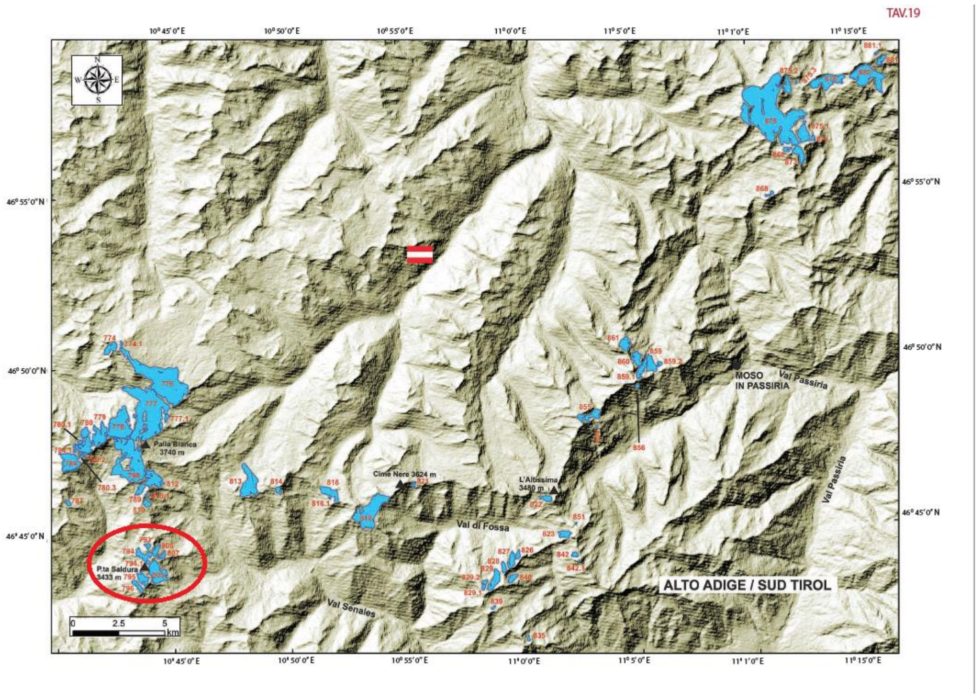


Figure 2: Lazaun Glaciers location, numbers 807 and 808 in Glacier's catalogue by *Smiraglia & Diolaiuti, 2015*

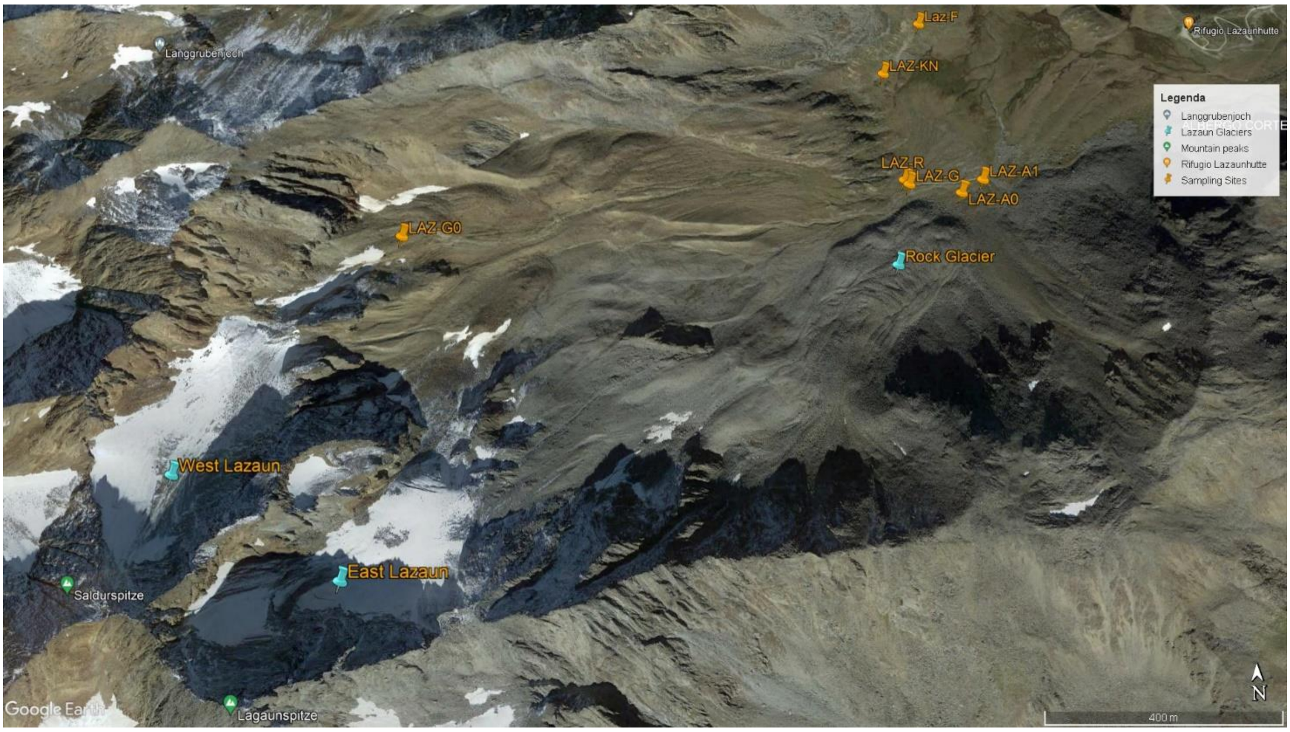


Figure 3: Aerial view of the site from Google Earth. West and East Lazaun are show, as well the rock glacier and the sampling stations.

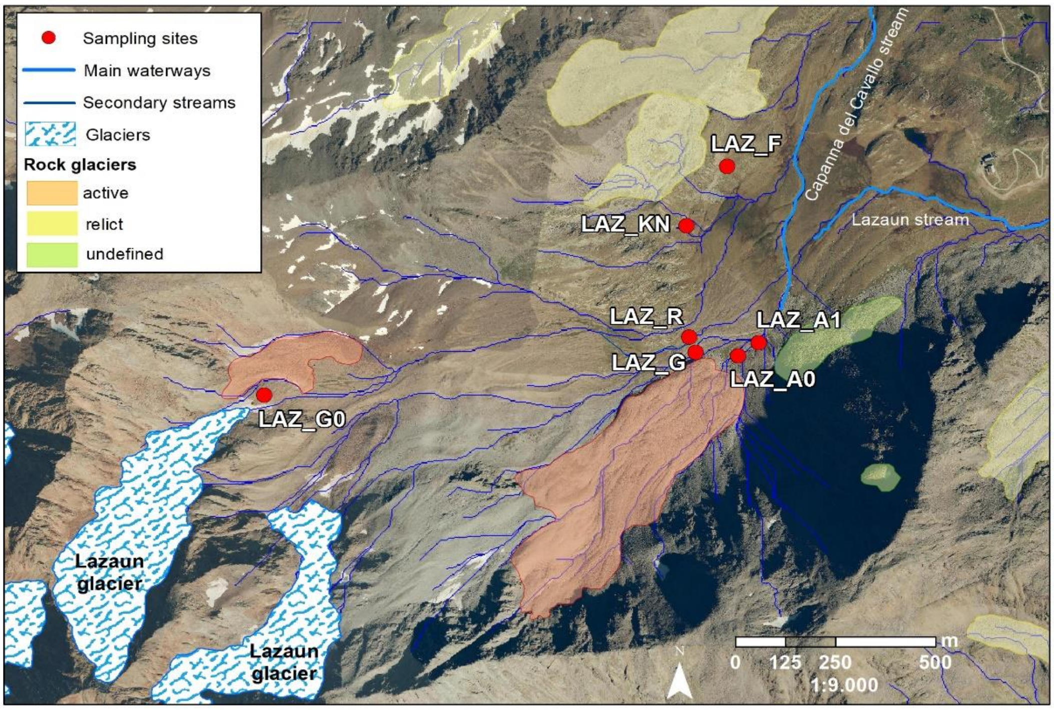


Figure 4: ArcGIS map of the Lazaun glaciers, showing water streams, sampling sites, active and relict glaciers. Shapefiles and WMS-Web Map Service taken from Geoportale of Alto Adige (buergernetz.bz.it). Image kindly granted by Francesca Paoli-MUSE.

4. Materials and methods

4.1 Field activity

Field activities were made in July 2020 and in September 2020, during the maximum ablation period of glaciers. Samplings interested 6 stations in July (LAZ-A1, LAZ-A0, LAZ-R, LAZ-G, LAZ-F, LAZ-KN), and 7 in September (LAZ-G0 was added).

LAZ_A1 and LAZ_A0 are stations positioned on the stream (permafrost fed) originating from the active rock glacier, with LAZ_A0 closest to the glacier, both are considered active RG (Rock Glacier) stations. LAZ_G and LAZ_R receive water from both the Lazaun glaciers and an adjacent glacier and enter the glacio-rhithral category, however they feed on two different water sources. LAZ_KN is krenal, as it is fed by ground water. LAZ_F possess krenal characteristics but, as it is in correspondence of a relict rock glacier, may be influenced by some deep permafrost too, in addition to ground water. LAZ_G0 is meta-kryal.

In the analysis a J was added to identify sites sampled in July and an S for those done in September.

Sampling was made by Valeria Lencioni and colleagues in collaboration with EURAC.

Figure 5: 1. LAZ_A0, with the view of the rock glacier; 2.LAZ_A1, facing the valley. (Photos kindly granted by Francesca Paoli-MUSE)

Figure 6: **1.** LAZ_G in the foreground, in the background LAZ_R **2**. Upper view of the LAZ_G, LAZ_R, LAZ_A0, LAZ_A1 **3.** LAZ_KN in the foreground. (Photos kindly granted by Francesca Paoli and Alessandra Franceschini-MUSE)

Figure 7: **1.** LAZ_F **2.** LAZ_G0, view with the valley on the background **3.** LAZ_G0 with the glacier on the background (photos gently granted di Francesca Paoli and Alessandra Franceschini-MUSE)

4.1.1 Macroinvertebrates communities

In high elevation habitats, the standard methods (IBE= Indice Biotico Esteso, STAR_ICM = Standardisation of River Classification Intercalibration Multimetric Index) described by ISPRA for sampling macroinvertebrates are not viable. This is due to the nature of those habitats, which don't provide so much biodiversity, and would end up in a negative water assessment even for perfectly conserved streams (Lencioni et al., 2001).

The scarce biodiversity, as previously stated, is due to the extreme conditions affecting those habitats, and not by habitat degradation.

In replacement of ISPRA's indexes it was used the method described by Lencioni 2000. The procedure to sample macroinvertebrates provides the usage of a Surber net with mesh net of 250 μm, sampling an area of 0,05m2 in each kick. In each station 5 kicks were sampled, plus a drift sample using a drift net (mesh net of 100 μm).

The Surber net was positioned countercurrent, layed on the streambed and then the sediment ahead of the net was moved for a couple of minutes, with bigger rocks manually washed, and the finer sediment moved with a foot. In this way, macroinvertebrates flow inside the net following the stream direction.

The drift net was put on the surface of the water for an hour to catch the fauna transported on the surface of the water by 'drifting'.

Samples were filtered in field to remove water excess with a mesh net equal to the Surber net (250) and then put inside jars containing alcohol at 75% and moved to the laboratory to be sorted.

This method permits the study of multiple microhabitats in the same stream, and to obtain a representative sample of the whole area. The number of individuals sampled has been related to the sampling area, resulting in a data of individuals per square meter for each site (square cube for drift).

However, sampling in remote and endangered areas aiming to characterize the community composition, species phenology and distribution, may lead to an endangering of already threatened species. Although these studies are necessary for a better comprehension of these habitats, it's important to note that the impacts are unsure and possibly relevant (Lencioni & Gobbi, 2021).

Figure 8: Kick sampling (left), preparation of the field base before the sampling (right) (photos kindly granted by Francesca Paoli and Alessandra Franceschini-MUSE)

4.1.2 Granulometric analysis

In each site the percentual of grain size of the riverbed was visually estimated for each kick, following the table 1. Secondly, the percentual repartition of sediment mean in each class was calculated, to get a characterization of each site.

Microhabitat	**Dimension**	**Code**	**Description**
Silt/clay	< 6 µm	ARG	Silty substrates, also with an important organic component, and/or clay substrates, of thin size which gives an adhesive behavior to particles, that can form a solid substrate
Sand	6 µm -2 mm	SAB	Fine or coarse sand
Gravel	0.2-2 cm	GHI	Gravel and coarse sand (with prevailing gravel)
Microlithal	2-6 cm	MIC	Small rocks
Mesolithal	6-20 cm	MES	Medium sized rocks
Macrolithal	20-40 cm	MAC	Coarse rocks, not bigger than a rugby ball
Megalithal	> 40 cm	MGL	Bigger rocks, boulders, of which only the surface is sampled
Artificial		ART	Concrete, and all the substrates artificially entered in the river
Hygropretic		IGR	Shallow water layer on a solid substrate generally covered with moss

Table 1: Granulometric classification following ISPRA directives[2].

[2] http://www.isprambiente.gov.it/files/pubblicazioni/manuali-lineeguida/metodi-biologici-acque/fiumi-macroinvertebrati.pdf

4.1.3 Water speed and depth

Water speed and depth were calculated using a current meter in each kick. This item is constituted by a metered metal bar, which measure the depth, and a small helix, connected to a tachometer. Leaving the helix in water for a determined time, 1 minute, we know the turns per minute. Using the following converting formula (Lencioni 2000), we can calculate the speed of the water in meters per second:

V (m/s) = [0,2546 _ v (turns/min)] + 0,004

For each site, the mean from each kick was calculated, both for depth and speed.

4.1.4 Suspended solids

To estimate the quantity of suspended solids, water samples were collected from each station (250 mL), to be later filtered and analyzed in the laboratory.

4.1.5 Temperature

During field sampling, temperature point measurements were made, using a multiparametric probe (model HI 9829, Hanna Instruments). An exception was made for September measurements, in which data on temperature was obtained from dataloggers Onset HOBO TidbiTv2 (for LAZ A0, LAZ G, LAZ R), Tiny Talk TK0040 (for LAZ A1), and Tiny Talk GEMINI (to measure air temperature in LAZ A0). The dataloggers measure the temperature of water or air, depending on where they have been placed, at regular intervals of 1h.

4.1.6 Physiochemical analysis

To measure pH and the conductivity (μs/cm) of the water it's been used a multiparametric probe (model HI 9829, Hanna Instruments) with an exception made for September where data were calculated in lab by APPA. Both is July and in September for each station a liter (1L) plus 250 mL (for metals) of stream water was picked up for further analysis, which were made by the laboratory of APPA (Agenzia Provinciale per la Protezione dell'Ambiente di Trento). Measurements regarded: pH (at 20 degrees Celsius), conductivity, alkalinity, hardness, total alkalinity, nitrogen, fluorides, chlorides, nitric nitrogen, nitrates, sulphates, orthophosphate, total phosphorus, total nitrogen, silica, sodium, potassium, calcium, magnesium, and metals.

4.1.7 Primary production analysis

Primary production analysis is calculated from the concentration of chlorophyll a in periphyton. The method used for field sampling is described in Lencioni et al. 2007. In this method 3 rocks covered in algae were casually chosen in the riverbed. At this point, with a toothbrush, an area of the rock was brushed, using a small plastic foil with a 3x3cm square hole to delimit the area. The toothbrush was immersed in a small quantity of distilled water after each brush, to concentrate all the biofilm in few waters. All the biofilm of the site finishes in the same Falcon, to have an average biofilm concentration.

The water with periphyton is then filtered in field using a vacuum flask, connected to a filter holder and to a manual aspiring pump. The sample is poured out in a funnel fixed to the vacuum flask, and manually, using the aspiring pump, void is created inside the flask. In doing so the water passes through the filter, which is retained in the filter holder, and the film deposits on the filter. At the end of the process all the biofilm is deposited in a filter of 47mm of diameter, with 0.7 μm of nominal porosity. All this process must be done in the dark because light damages the photosynthetic pigments. To avoid light each filter has been inserted in a dark jar, conserved at cool temperatures and soon as possible put in a freezer at -18°C, for no more than 3 months.

4.1.8 Biomass

The procedure to sample biofilm which will be used for biomass analysis is the same used for chlorophyll a, with the only difference in the type of filters used (Whatman GF/C, 1.2 micron). The squares of 3x3cm are preferably sampled on the same rocks as the chlorophyll, if not possible nearby rocks are used, to have similar samples. All the filtering is done at dark too, and the filter must be put inside a dark jar and put in a refrigerator as soon as possible.

4.1.9 Seston

Seston can be identified as the sum of particles suspended in the water column, including plankton, organic matter, and detritus. The field procedure expects the collection of 250mL of water from the column of water. This sample is then filtered using Whatman GF/C, 1.2 µm filters to collect the seston.

4.1.10 Organic breakdown rates

To assess the organic breakdown rates, the Gessner & Chauvet (2002) method was used. This system provides the usage of 15 x 1,8 x 0,2 cm untreated wooden tongue depressors. Series of 5 sticks were placed inside the water in 4 sites.

Before positioning, the sticks were conditioned inside an oven (UML400 Memmert) at 70 degrees for 72 hours, then put in a dryer for thirty minutes and finally weighed in a precision balance to obtain the tare (dry weight 0). After that, sticks were numbered with a unique code and put in groups of five, using fishing wire.

During field work each series was immersed in water and anchored to stable supports using the wire. Only in a few sites the sticks were positioned: in LAZ-A0, LAZ-G, LAZ-R and LAZ-A1 in July and in LAZ-G, LAZ-R, LAZ-G0, LAZ-A1 in September. The first series of sticks have remained in water for 70 days, from 09/07/2020 to 17/09/2020. The second one for 356 days, from 17/09/2020 to 08/09/2021 (of which LAZ-R sticks were never been found). After removal, sticks were taken to be processed in laboratory.

4.2 Lab work

4.2.1 Macroinvertebrate sorting and ID

Field samples were sorted and determined in lab under a stereomicroscope (MZ 7.5, Leica Microsystems). For certain taxa, as Oligochaeta, temporary slides were prepared and put under an optic microscope (Nikon Eclipse E600).

In some stations, some kicks were sub-sorted (1/2, 1/4) since the organisms or the debris present were too much, and it would have taken too much time to be sorted.

For macroinvertebrates, the levels of identification were the following:

- Genus (Plecoptera, Ephemeroptera, Pediciidae, Limoniidae)
- Family (Trichoptera, Diptera (partim), Oligochaeta, Coleoptera)
- Order (Tricladida)
- Subclass (Acari)
- Phylum (Nematoda)

The following guides were used for the identification:

- Campaioli S., Ghetti P. F., Minelli A., Ruffo S., (1994): Manuale per il riconoscimento dei macroinvertebrati delle acque dolci italiane", Provincia Autonoma di Trento, (volume 1)
- Campaioli S., Ghetti P. F., Minelli A., Ruffo S., (1999): Manuale per il riconoscimento dei macroinvertebrati delle acque dolci italiane", Provincia Autonoma di Trento, (volume 2)
- Faasch Heide (2015): Identification Guide to aquatic and semiaquatic Diptera Larvae, DGL-Arbeitshilfe 1-2015

The organisms were differentiated in 6 categories: larvae (L), larval exuvia (LE), pupae (P), pupae exuvia (PE), adult (A), juveniles (juv.).

The individuals sampled with the Surber net were rapported to m2, those sampled with the drift net to m^3 according to Marziali (2009) using the following formula:

$N\_ind/m^3 = (n \cdot 100) / (v \cdot At)$

Where n = number of individuals, v = current speed
(m/s) (average speed in the 5 kicks of a site), A = drift net area e t = time of exposure.

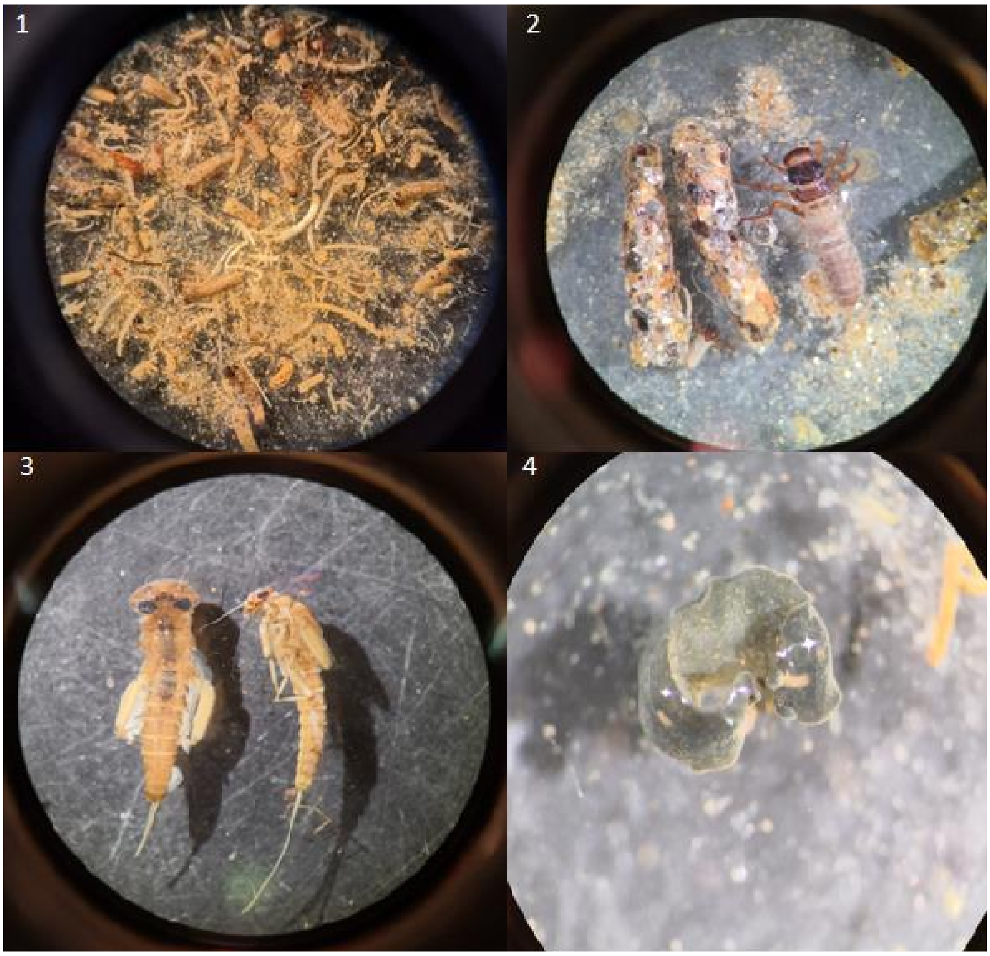


Figure 9: Examples of macroinvertebrates under the stereoscope. Mix of Chironomidae, Plecoptera, Trichoptera **1.** Unsorted sample **2.** Limnephilidae (Trichoptera) **3.** Heptageniidae and Baetidae (Ephemeroptera) **4.** *Crenobia* sp. (Tricladida)

4.2.2 Suspended solids measurements

The water samples for the purpose, were filtered with a vacuum pump (N86 KN.18 6L/min, Laboport) on circular filters (porosity 0,45 μm). In this way particulate dissolved in water deposits on the filter.

Before filtering each filter has been put in an oven (UML400 Memmert) for an hour at 105 °C, put to rest in a dryer for 1h and then weighted with a precision balance (Ohaus Corporation E11140) to obtain the tare (DW0). This same process has been made also after the filtration, obtaining the gross weight of the filter (DW1).

The net weight of total suspended solids was calculated according to the procedure IRSA-CNR, A. P. A. T., 2003:

$DWnetweight = (DW1 - DW0) * 1000/vol$

Figure 10: **1.** Each bottle contains one kick-sample from a site **2.** Sorted individuals divided per taxa for each kick **3.** Each sample gets its label, with locality, coordinates, date, site, kick number, taxon, sampler.

4.2.3 Primary production measurements

The analysis of primary production has been done following IRSA-CNR, A. P. A. T. (2003) protocol, adapting it at the peculiar characteristics of alpine streams. The spectrophotometer method was followed.

The filters, conserved in a freezer at -18°C for no more than 3 months, were subjected to a pigment extraction with 95% acetone. The acetone destroys cellular walls and thylacoidal membranes, so the pigments can exit. A quantitative of 10-15mL of acetone was used. The filters were put in 15 mL Falcon tubes and retained in fridge for 18-24h before processing. After the time has passed, the filters were chopped, and then centrifugated (2800 rpm for 30 minutes). The centrifuge helps to divide the filter from the pigments, which will be found on top of the falcon, inside the supernatant. Using a spectrophotometer (Lambda XLS/XLS+ Perkin Elmer) the absorbance of the 3 mL of supernatant was measured at three diverse wave lengths (664, 665, 750 nm). The spectrophotometer was calibrated using a 'white' containing 95% acetone. The measurements at 664 and 665 nm were to assess the real absorbance, the 750 nm measure was made to calculate the turbidity of the sample. The protocol also requires the measurement of pheophytin, which is the degradation of chlorophyll. For doing so 3 μL of HCL 0.3 M were added to the cuvette and it was put to rest for 10 minutes, then the measurements were made again at the same wavelengths. The concentration of chlorophyll a was calculated with the formula:

Chlorophyll a (mg/m^3) = {26,7 [(664 – 750) – (665a – 750a)] v}/V·L

With 664 and 750 as the absorbance values at the corresponding wavelength, 665a and 750a as the absorbance values of the acidified version at the correspondent wavelength, v as the volume (mL), V as the surface investigated (cm^2) and L the optical path of the cuvette (cm).

4.2.4 Biomass

The filters from the field, previously pre-conditionated (1h in the oven) and weighted (DW0), were put in the oven (UML400 Memmert) for 1h at 110°C to dry. The filters were then let dry in a desiccator for 30 minutes and then weighted again (DW1). The difference between DW0 and DW1 gives the net weight present. The last step was putting the filters in marked cradles (with a known weight) to be burnt in the muffle (A024, Matest) for an hour at 500°C. After cooling off the cradles were weighted and their weight was subtracted to the value, obtaining an Ash Dry Mass (ADM). The weight of the remaining ash (ADM) is then subtracted to DW1, to have the value of the organic matter (the effective biomass, AFDW) of the sample. Having sampled 27 cm^2 of biofilm, the following formula was used to calculate the biomass per area:

Biomass (mg/cm^2) = AFDW/ 27 cm^2

4.2.5 Seston

As for biomass filters, also seston filters have to be conditioned before field work, passing an hour at 110°C in the oven, to be weighted after a period in the desiccator (obtaining DW). After the field filtrations, the filters were put in dark jars and transported inside freezer bags to the lab. There all the filters were put again in the oven, for an hour at 110°C, let desiccate, and weighted (DW1). Then they've been associated with a marked cradle with known weight and put in the muffle for 1h at 500°C. After the further desiccation the cradles were weighted again. The weight of the cradles, which was known, is subtracted to the value obtained, to calculate the Ash dry mass (ADM). DW1 minus ADM gives the ash free dry weight (AFDM) which is related to the volume of water filtered (250mL) by the following formula:

Tot seston (mg/L) = AFDM*1000 mL /250 mL

4.2.6 Organic breakdown rate

Organic breakdown rate relies on many variables, with factors intrinsic to organic matter, as nutrient content or presence of particular molecules, as lignin, and extrinsic factors, as temperature, O_2 and nutrients concentration (Gessner and Chauvet, 2002). Previsions on the breakdown rates are usually difficult, as some factors speed up the rate, but others slow it (Aristi et al., 2012).

Once the sticks were removed from the field they were enveloped in paper and conserved inside plastic bags. In the lab, the sticks were separated and were washed with clean water and brushed with a toothbrush to remove the dirt. Once clean the sticks were put in the oven (UML400 Memmert) at 70 degrees Celsius for 96hrs, to remove all the water inside and then weighted in a precision balance (Ohaus Corporation E11140) to measure DW1 (Dry Weight 1). The sticks were then cut in smaller pieces and associated and put in numbered ceramic crucibles of known tare.

The simple subtraction DW0-DW1 is giving us the mass loss of the sticks, but there is no certainty that all the losses are from organic matter. To get that information the sticks had to be burned off completely, leaving only inorganic matter, and with some calculations later explained we can determine the organic loss and breakdown rate (Petersen and Cummins, 1974)

At this point the crucibles were transferred inside a muffle (A024, Matest) at 500°C for 5 hours, process at the end of which only inorganic matter remains. After the removal of the crucibles from the muffle, a drying time was needed until the burnt sticks cooled off. Once they were ready, each crucible has been weighed, calculating the Ash Dry Weight (ADW). This ADW is subtracted to DW1, which calculates the organic matter that has been burnt in the muffle, which was all the organic matter remaining in the sticks giving us the Ash Free Dry Mass (AFDM).

The breakdown rate (k), has been calculated using the formula used by Petersen and Cummins, 1974:

$Mt = M0e - kt$

where Mt is the AFDM at time t (number of days of exposure), M0 is DW0, and t is again the permanence time (in days) of sticks in water.

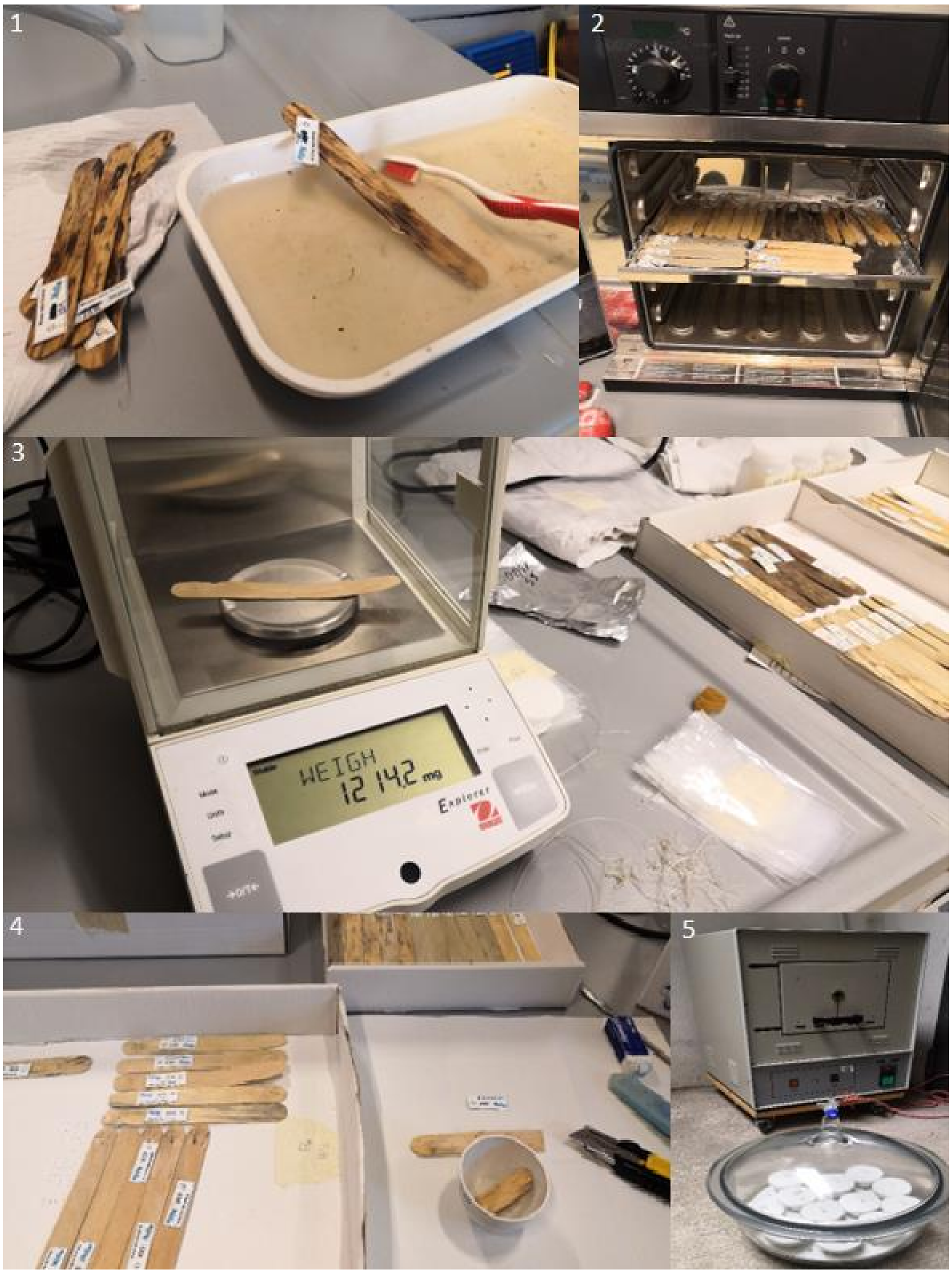


Figure 11: Process of cleaning, weighting and incinerate the sticks to calculate the degradation rate. **1.** The dirt is removed carefully from the sticks with a toothbrush **2.** Oven process **3.** Weighting **4.** The sticks are cut in smaller pieces to fit in the crucibles **5.** Crucibles removed from the muffle and put to cool off.

4.2.7 BPOM and FPOM

Benthic Particulate Organic Matter (BPOM) and Fine Particulate Organic Matter (FPOM) are respectively the quantity of organic matter bigger than 1mm and the one included between 1mm and 1.2 micron. To calculate this quantity the particulate and debris present in samples must be separated and then burnt off, to eliminate the organic matter. Having weighted the samples before and after the burning process we get to know the BPOM and FPOM by subtracting the after-burning value to the initial net weight. The following process has been made.

From each sample sorted for macroinvertebrates, all the remaining material was filtered with a vacuum pump (N86 KN.18 6L/min, Laboport) with 2 filters, one with mesh net of 1mm, to separate BPOM, and the other with 1.2 micrometer to retain FPOM. The FPOM filters were previously conditioned and weighted and will be treated with FPOM. At the end of this procedure, for each kick sample, two new samples were created (one with BPOM and another with FPOM). BPOM and FPOM are put in marked foil, so each sample could be recognized from the others. All the samples were put in the oven (UML400 Memmert) for an hour at 110 °C, to eliminate the water in excess, then put in a dryer and finally weighted calculating DW1 (with the foil, and for FPOM samples also with the filter) using a precision balance (Ohaus Corporation E11140).

After this procedure all the foils have been associated with marked crucibles, which were put in a muffle (A024, Matest) for 1hr at 500 Celsius degrees. The crucibles were removed from the muffle after the set time and put in a dryer for 24hrs, to let the samples cool off. After this time the samples were weighted again (ADW= Ash Dry Weight). The subtraction between DW1 and ADW calculates the organic matter present in each sample (AFDM= Ash Free Dry Mass), which burned off.

If a subsampling of a kick was made, using a proportion correlated to the fraction subsampled, we calculate the AFDM on total surface (if we analyzed ½ of a sample, we multiplied the AFDM result by 2 to get a value who resembles the total surface).

The final step was to correlate the AFDM, in grams, to the sampled area, to obtain a value in g/m^2 to have comparable data with other works.

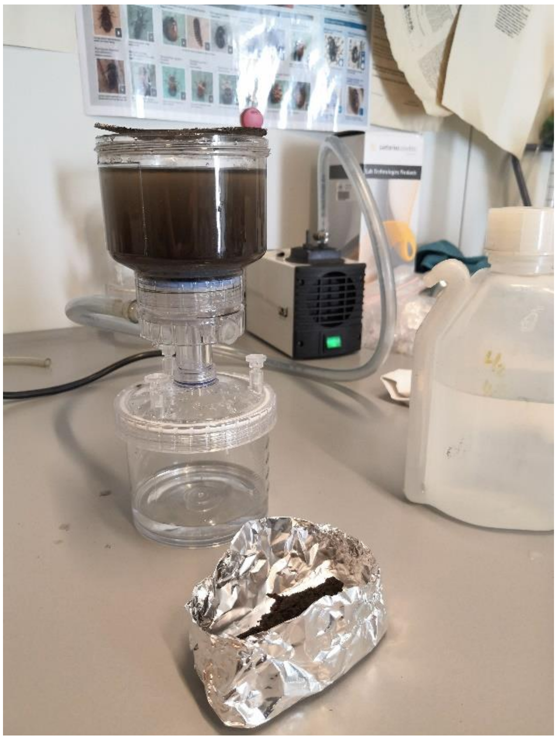


Figure 12: Filtering machine, foil with a sample on the foreground

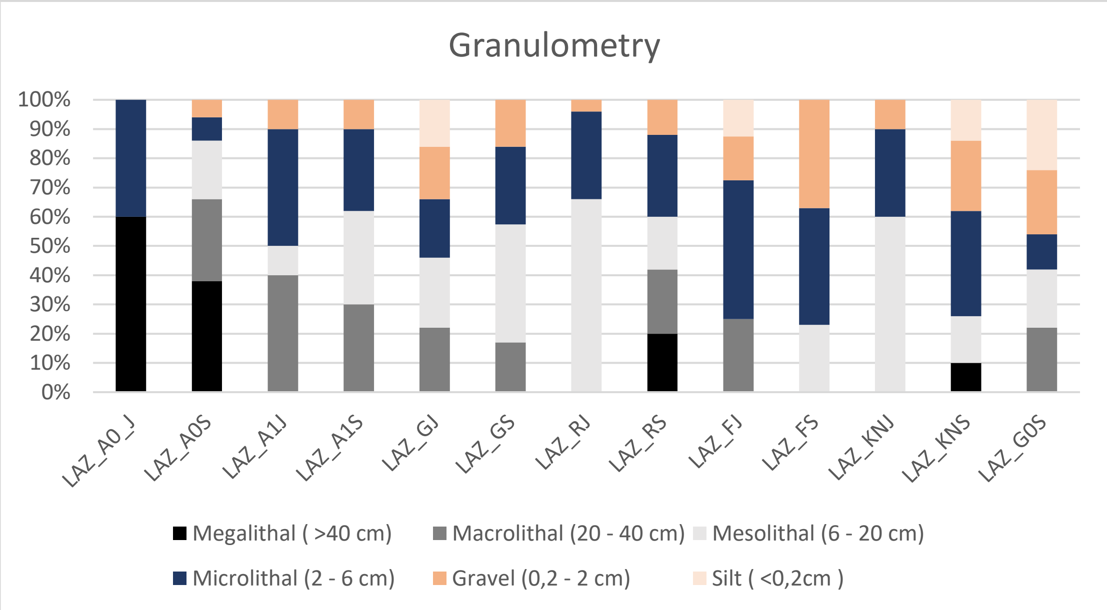
Figure 13: Foils containing samples ready to be weighted. Crucible samples cooling off in the background

4.2.8 Statistical analysis

Using the software Past4.03 the Bray-Curtis cluster analysis and the Canonic Correspondence Analysis (CCA) were done. The Principal Component Analysis (PCA) was instead done with CANOCO software. CCA taken in consideration the sites mean of 13 environmental variables and of biotic components. The variables selected were Temperature, Suspended solids, Biomass, Chla + pheophytin, SiO_2, pH, Mn, Al, Ptot, Ni, Hardness, conductibility, SiO_4.
For these analyses the number of individuals was converted to [log(x+1)]

5. Results

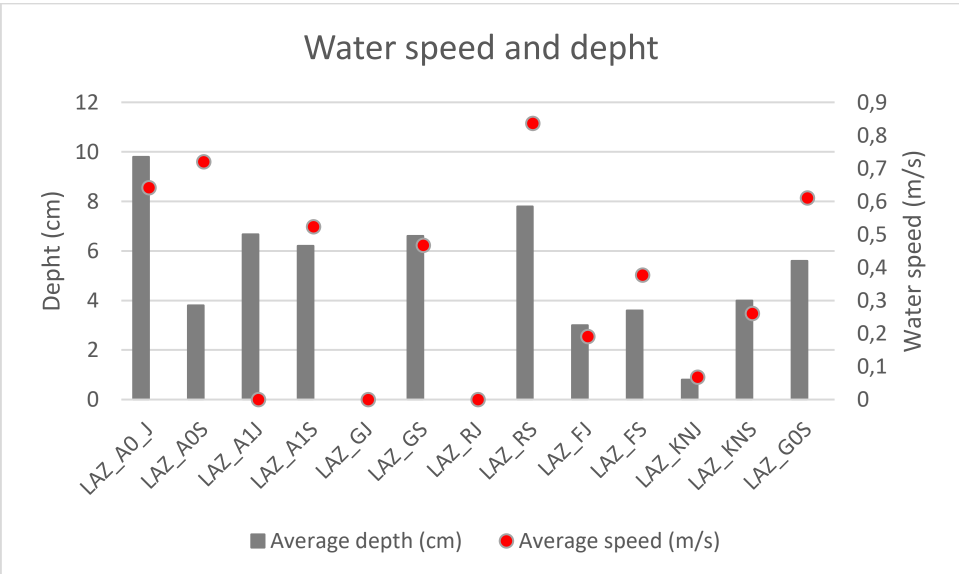
5.1 Granulometric analysis



Graphic 1: Granulometric percentages in each site each date.

Alpine streams are characterized with low channel stability (Ward, 1994) (Brown et al., 2003), so the same riverbed can change granulometric composition during time. All stations presented variations during time, with changes more evident in LAZ_A0, LAZ_R and LAZ_F, with high variations in the percentage composition and with the adding of new granulometric categories. The other stations had changes too but fainter.

5.2 Water speed and depth

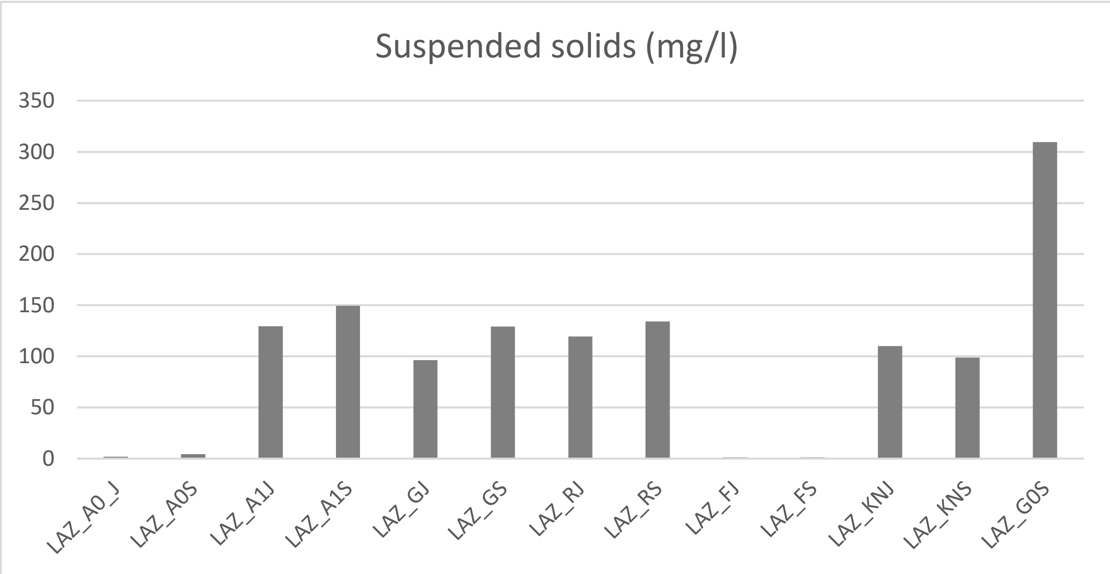


Graphic 2: Depht (column) and water speed (dot) measured in each station.

The channel depth is correlated with channel stability, so it's reasonable to assume temporal changes in depth too. From data sampled it's evident that there is no general trend about depth in the stations taken in consideration, as in some stations, like LAZ_A0, LAZ_A1 the mean depth is reduced, and in LAZ_F and LAZ_KN it increases. Depth data are missing from station LAZ_GJ and LAZ_RJ, accompanied with water speed data, of which misses also for LAZ_A1J. This is due to a malfunctioning of the currentmeter used.

If depth has no apparent trend, the opposite can be said regarding water speed, as an increase is seen in all the sites that had data for both temporal dates.

5.3 Suspended solids

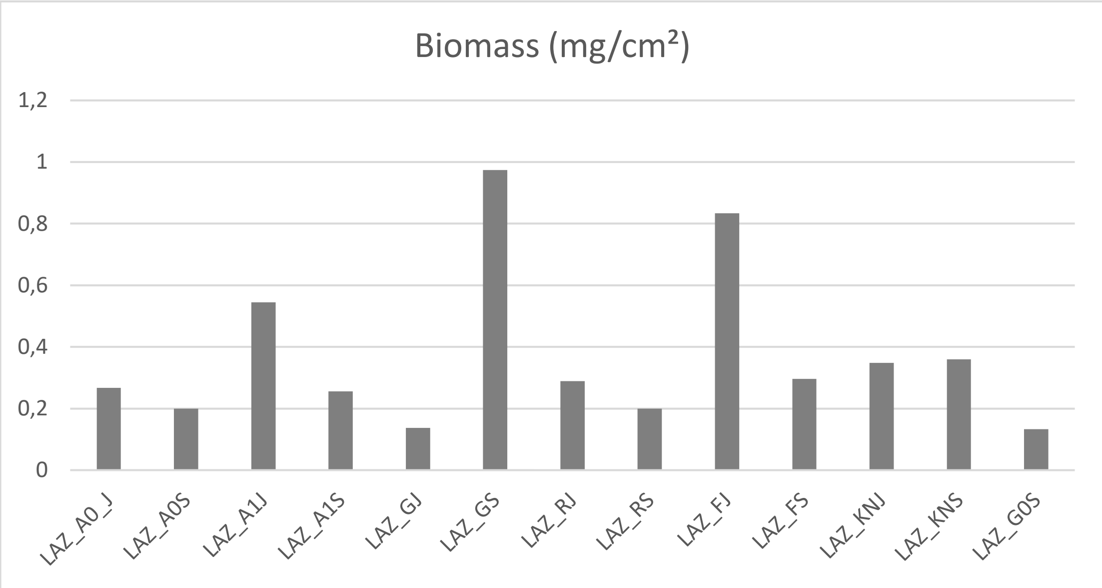


Graphic 3: Quantity of suspended solids (mg/l) measured in each site.

The suspended solids analysis showed an increase in SS in all stations except LAZ_KN, that had a faint decrease. LAZ_G0S has the most quantity of suspended solid, probably due to the proximity to the glacier and its ablation, which is higher in September, a characteristic of kryal stream types (Milner & Petts, 1994) (Brown et al., 2003).

LAZ_A0 and LAZ_F show a significant low amount of suspended solids in both dates. The case of LAZ_A0 it's peculiar as LAZ_A1, which is positioned further from the glacier but in the same stream, shows dozen times more the quantity of SS.

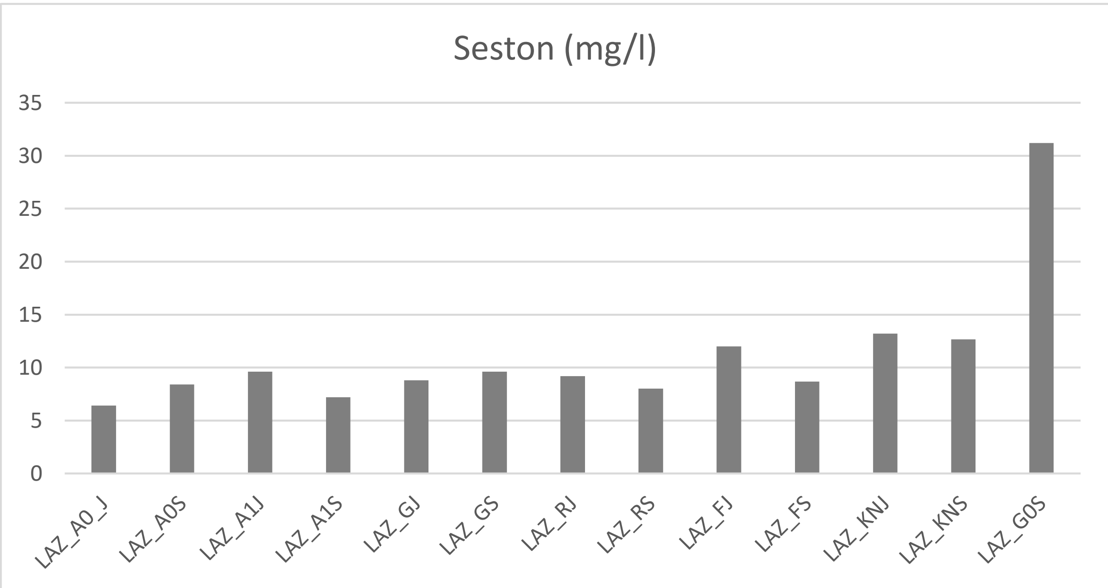
5.4 Biomass



Graphic 4: Biomass (mg/cm²) in each site for each date.

Biomass for alpine streams represents the total of microorganisms present in a determined area of the film which envelops the surface of streambed's rocks. The majority of sites presents a temporal decrease in biomass quantity, of which LAZ_A1 and LAZ_F have the bigger decrease. LAZ_KN has a little increase. A different pattern is showed by LAZ_G during September, with a massive increase in biomass, probably correlated to the explosion of Plecoptera juv present in that site in that period. LAZ_G0 has the lowest biomass, in accordance with its lower temperature.

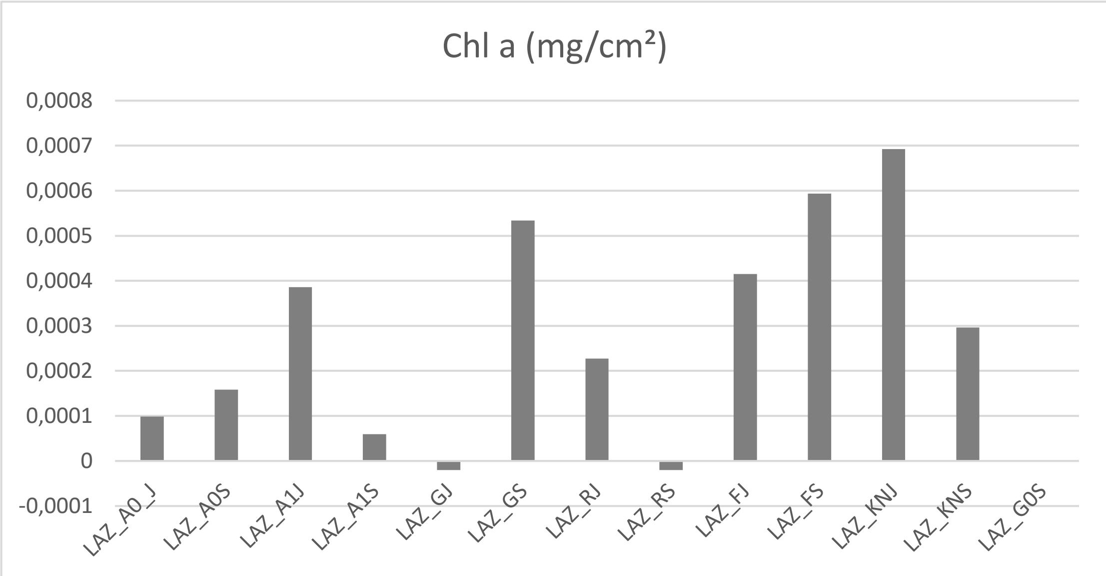
5.5 Seston



Graphic 5: Seston quantities (mg/l) for each sampling date and site.

Seston values differ slightly in each station spatially and temporally, with some sites presenting a faint increase and others a small decrease. The exception is LAZ_G0, with double the seston of the other sites, which is in accordance with the suspended solids data of the station.

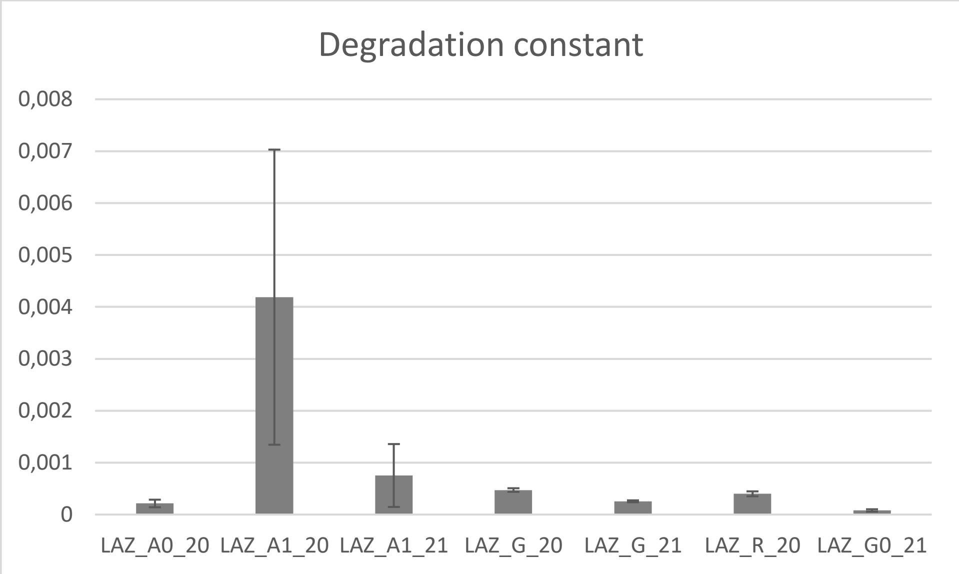
5.6 Chlorophyll a



Graphic 6: Punctual data for Chorophyll a concentration in each site and date.

Chlorophyll a concentrations are showed in the Graphic 6, showing how kryal and RG stations tend to have the lower values (LAZ_A0, LAZ_A1, LAZ_G0) (Ward, 1994) in contrast to krenal stations (LAZ_F, LAZ_KN) which have the highest. Two negative values occur (LAZ_GJ, LAZ_RS), probably due to the really low quantity of chlorophyll which could have been degraded before the analysis.

5.7 Degradation



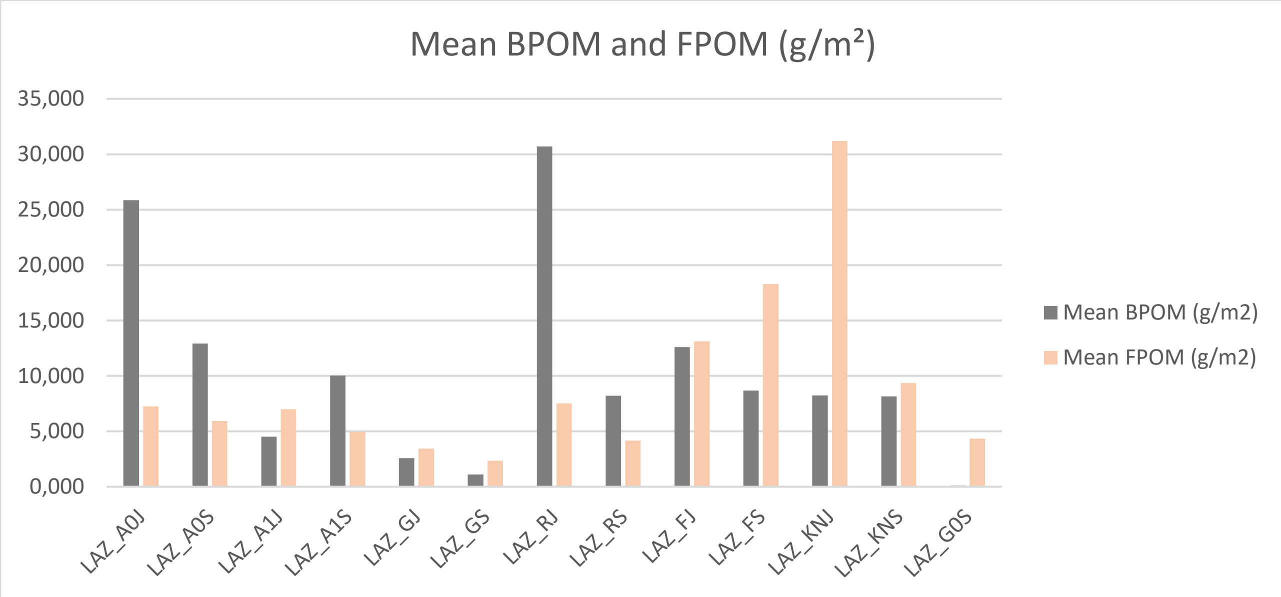
Graphic 7: Degradation constant *k* values and standard deviation for the sites in which the sticks were placed and removed.

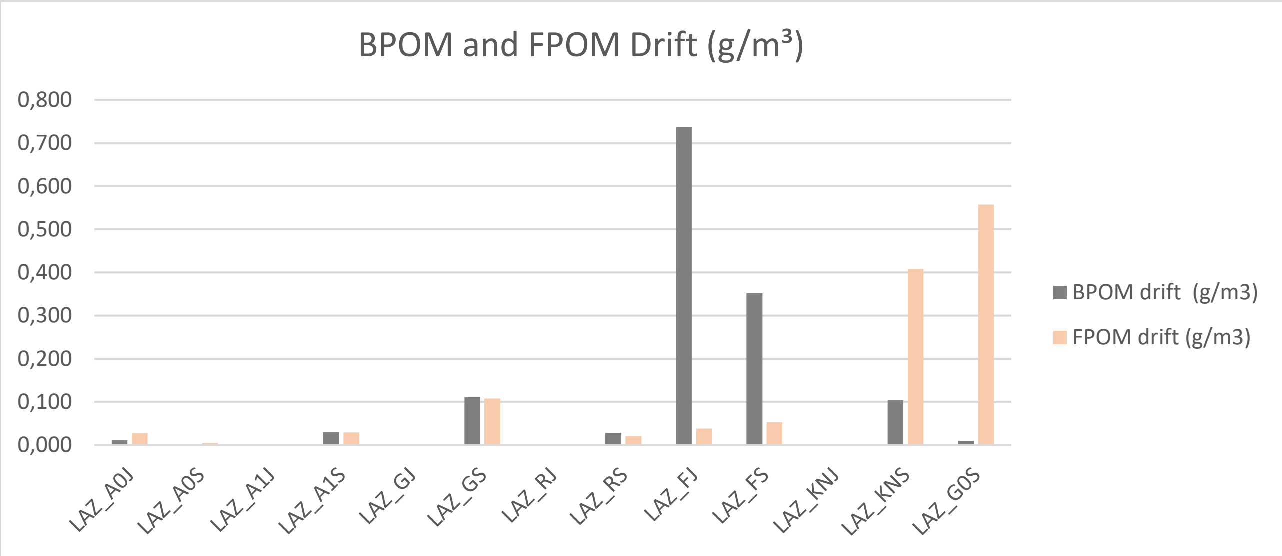
The degradation constant is low in all the sites, with kryal and RG sites having it a little lower. The exception is LAZ_A1_20. In this station the sticks have been immerged for only 70 days (July-September 2020) but show the biggest degradation, even greater than the following sticks series put in the same site for a year.

5.8 BPOM and FPOM

BPOM and FPOM values change over time in all stations. LAZ_A0 shows a decrease in both the variables between July and September, LAZ_A1 instead increase the BPOM and decrease the FPOM. LAZ_G has the lowest values for FPOM and low values for BPOM, having also FPOM higher than BPOM in each month. The highest Benthic Particulate Organic Matter is shown by LAZ_R during July, with a steady decrease in September. LAZ_F increases the FPOM and decreases the BPOM over time, having moderate values for both. LAZ_KNJ presents the highest FPOM value, which decreases over September. LAZ_G0 has the lowest BPOM quantity of all sites.

Regarding organic matter in drift the values are low in the majority of sites. Exception made for LAZ_F, with high BPOM drift concentration in both months, LAZ_KN which in September has high values of FPOM drift and LAZ_G0 showing a high quantity of FPOM too.





Graphic 8: BPOM and FPOM values of the kick samples (g/m²) and of the drift samples (g/m³).

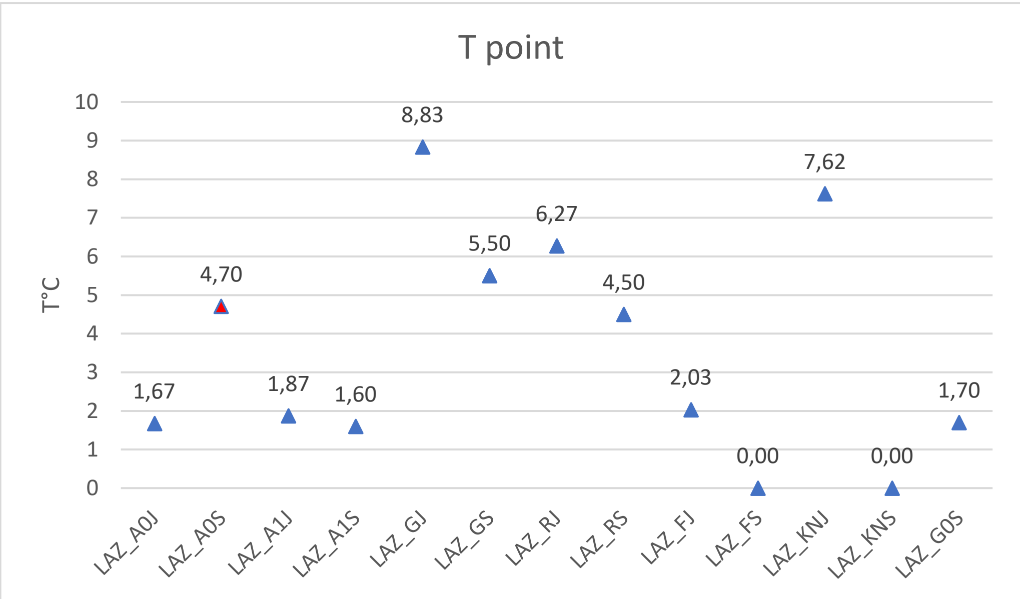
5.9 Physiochemical analysis

Temperature for the month of July was calculated by a multiparamentric probe, and by dataloggers for September, with an exception for LAZ_FS and LAZ_KNS, of which the data is missing. LAZ_A0, LAZ_A1 and LAZ_G0 present temperature below 2°C, which are comparable respectively to the active rock glacier and kryal water stream type (the value of T of LAZ_A0S is odd, probably the logger was out of the water, as the water temperature logger shows the same trend over time as the air temperature one as shown in Graphic 10). In the other stations the temperatures lower in September as it could be expected. For krenal stations, LAZ_F, LAZ_KN, it’s possible to imagine that the temperature remains constant as the literature says (Ward, 1994) (Brown et al., 2003), but we cannot be sure.

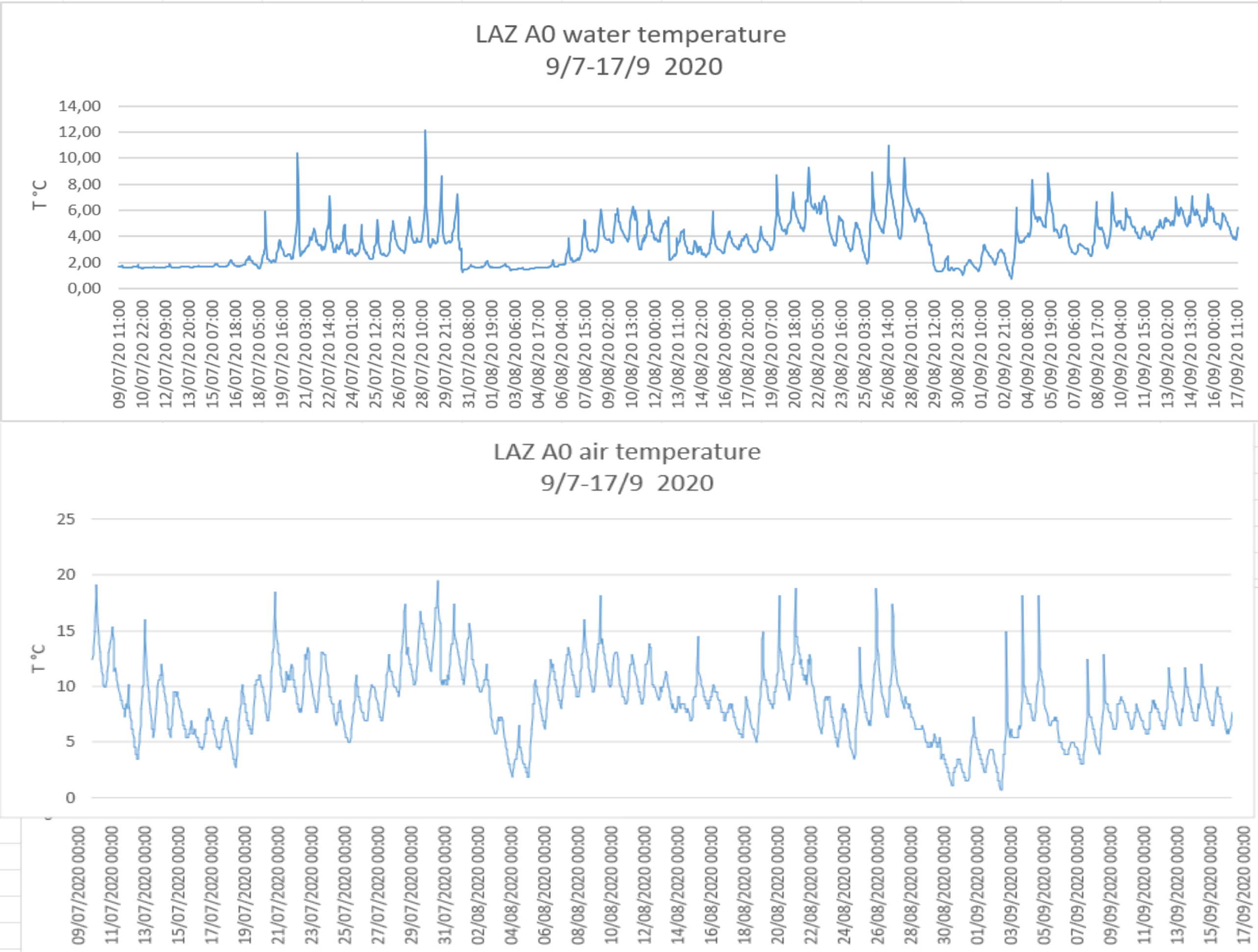
Conductivity shows a positive trend in every station, with increased values in September. As the ablation of the glacier goes further a bigger quantity of ions is released in the waters so the conductibility rises (Brittain et al., 2000). The highest values are present in LAZ_A1, LAZ_A0, LAZ_G, LAZ_G0 and LAZ_R respectively. LAZ_KN has the lowest, probably for its krenal nature, but differs from LAZ_F, which is a stream originated from a relict rock glacier which present a higher value. The highest variation between dates is seen in LAZ_G (Graphic 11).

pH remains constant in every station beside the stream originated from the active rock glacier, in which both the stations LAZ_A0 and LAZ_A1 show an increase of one point (Graphic 12).

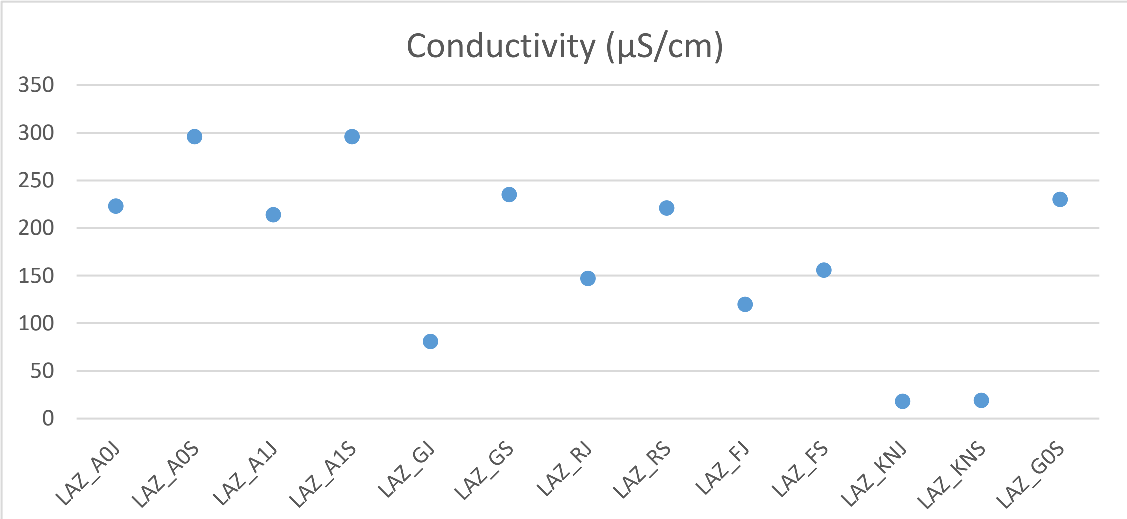
SiO2 values are low and similar for every station in each month. On contrast SO4 values increase across late summer in each station, with higher values shown by LAZ_A0 and LAZ_A1, in accordance with rock glacier streams.
Between Ptot values LAZ_G0 stands out, probably due to the nature and chemical behavior of the rocks in proximity of the glacier (Graphic 13).



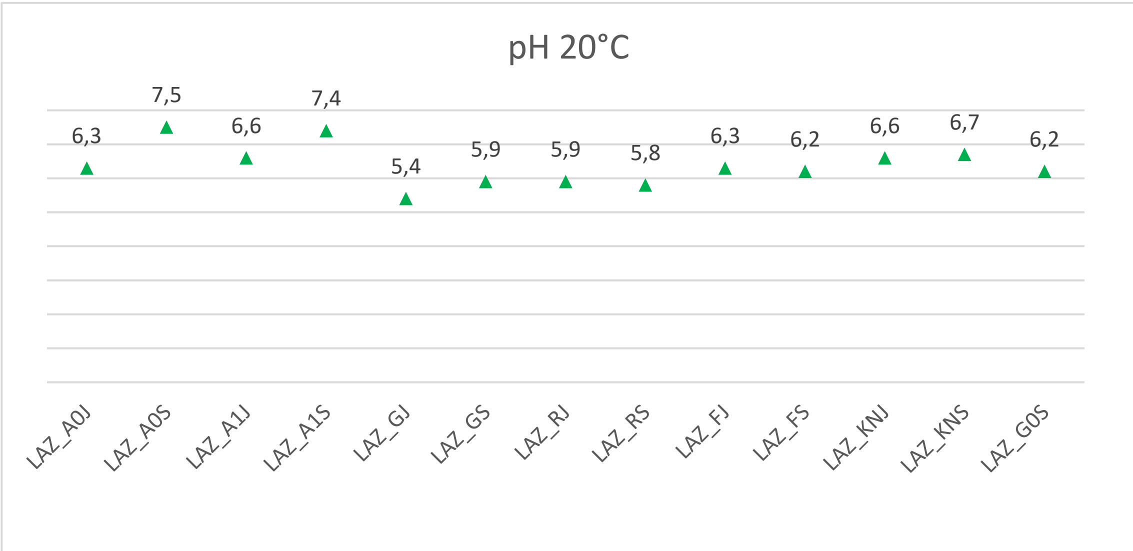
Graphic 9 :Punctual values for water stream temperature.



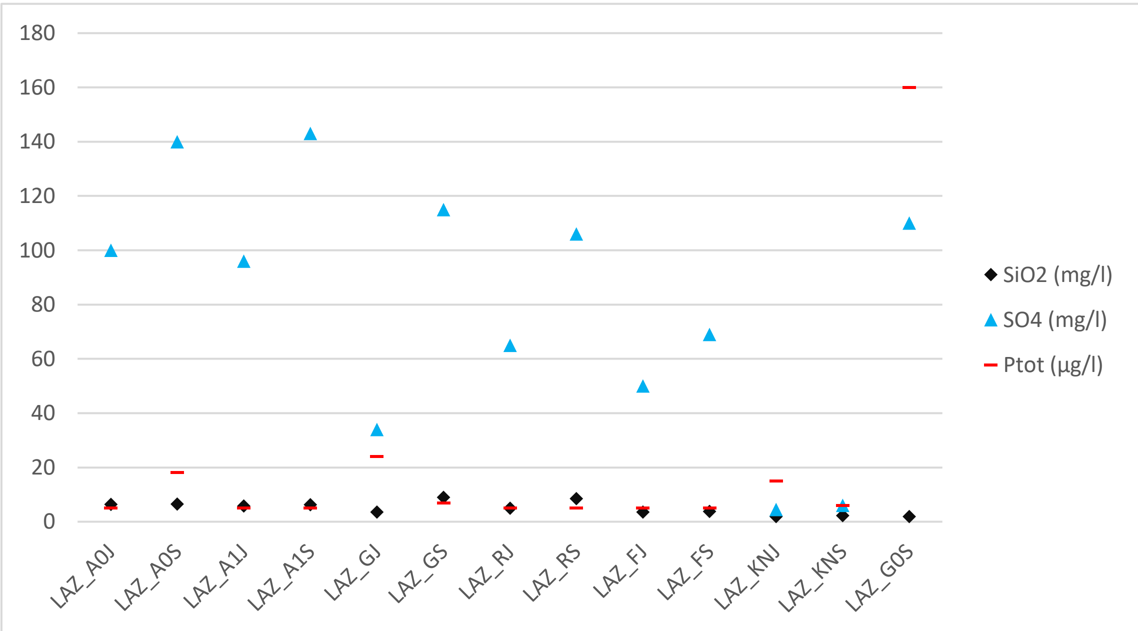
Graphic 10: Water and air Temperature of LAZ_A0 between 9 July 2020 and 17 September 2020, measured by dataloggers Onset HOBO TidbiTv2. The pattern shown in the majority of the water T graphic is similar to the air one, except for the first week of July and the first of August. In the rest of the graphic, as well for the 17th of September (day of sampling), the dial air temperatures fluctuation is comparable with the fluctuations perceived by the logger that should have measured water temperature. The logger presumably stayed properly inside water only for two weeks, this is due to the instability of the stream, which can change its course, and the terrain instability, as falling rocks can change the water flux.



Graphic 12: Conductivity point measurements.



Graphic 13: pH 20°C point values calculated in lab from water samples by APPA.



Graphic 11: SiO_2, SO_4 and Ptot punctual values.

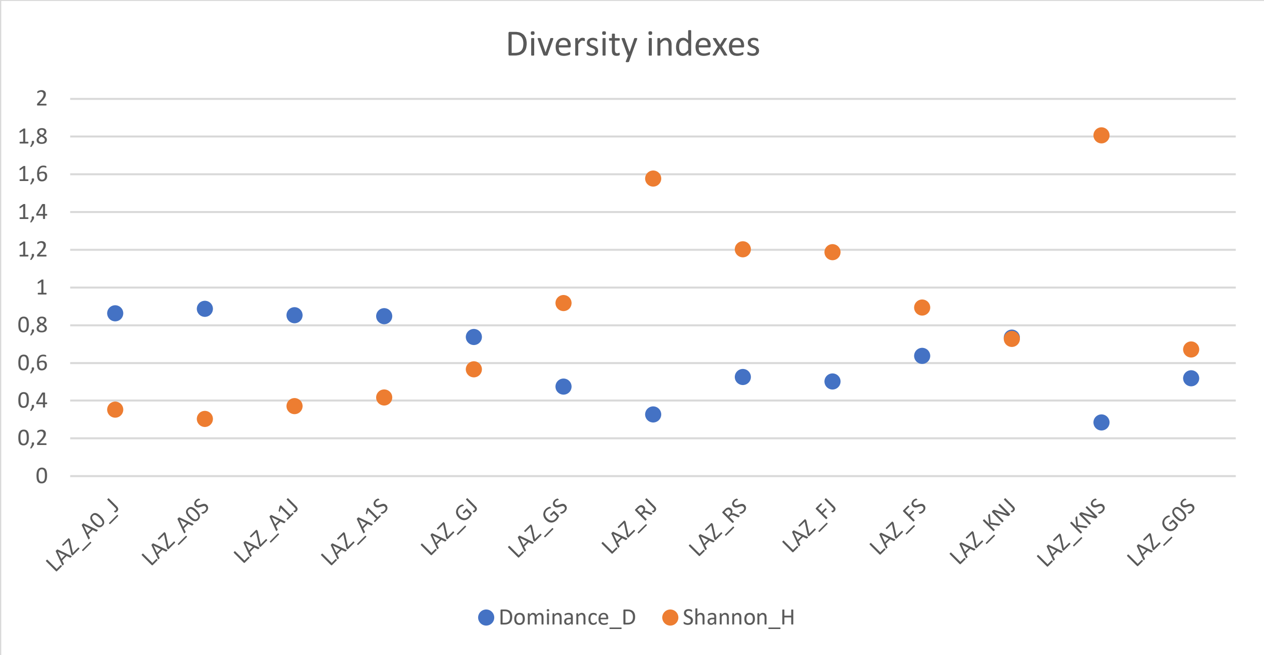
5.10 Biological analysis

During field sampling a total of 21310 individuals was kick-sampled, of which 16250 were Chironomids. In the drift sampling 1082 individuals were collected, of which 907 were Chironomids. This data alone shows how the majority of the alpine stream communities is composed mainly by this Diptera family.

The graphic n.15 shows the total of individuals per m² for each site and date of sampling and the total number of taxa. Chironomids in total were estimated to be around 30.000, other Diptera at 1500, Trichoptera at 1100, Plecoptera at 2500, Ephemeroptera at 50, Anellida at 1660.

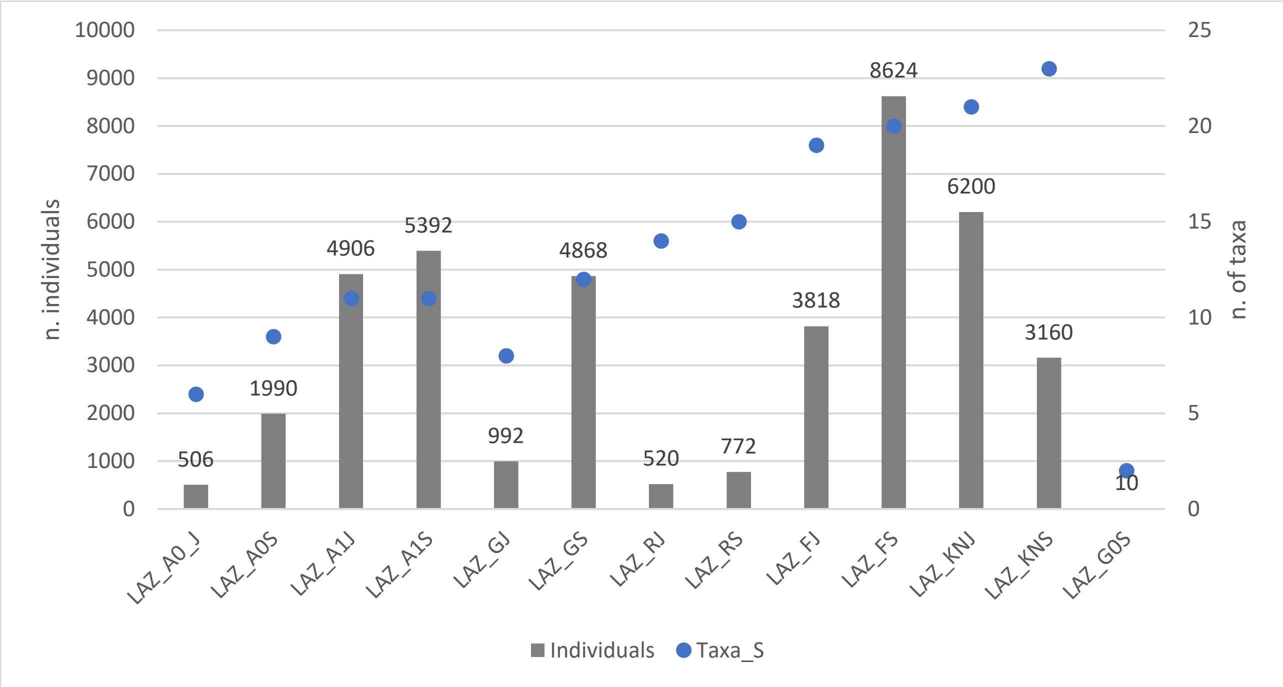
Shannon-Wiener and Simpson indexes were calculated using the tot.indiv/m² for each site at each date, as shown in graphic n.14 and n.16.

Shannon index is used to assess the evenness of a community, the more diverse and balanced the higher is the value. In a community with the dominance of a single taxon its value will be low. Simpson index measures the dominance of taxa inside a community, with values going from 0 to 1. If a taxon is completely dominant, it's value will be 1, otherwise lower according to the dominance. Rock glacier sites (LAZ_A0, LAZ_A1), show the higher Dominance index, meaning that one taxon, in this case the Chironomids, is the most present in these environments (also shown in graphic n.17). Glacio-rhithral and krenal sites have a lower Simpson index and a higher Shannon-Wiener, with LAZ_RJ and LAZ_KNS having the highest values. From the graphic n.15 is also notable the difference in number of taxa between sites. If the number of taxa remains overall similar in each site between dates, it is clear that it gets significantly higher from kryal stations to rhithral and krenal ones. The difference between LAZ_A1, which is RG type, and LAZ_G, which is rhithral, is not high, especially if we consider only the number of taxa. If we use indexes, it is clear that overall the dominance index of LAZ_G is always lower (in September consistently lower) and the evenness is higher. But the evenness and the dominance for LAZ_GS are influenced by a massive Plecoptera egg-hatching, which lowers the dominance and heightens the evenness.

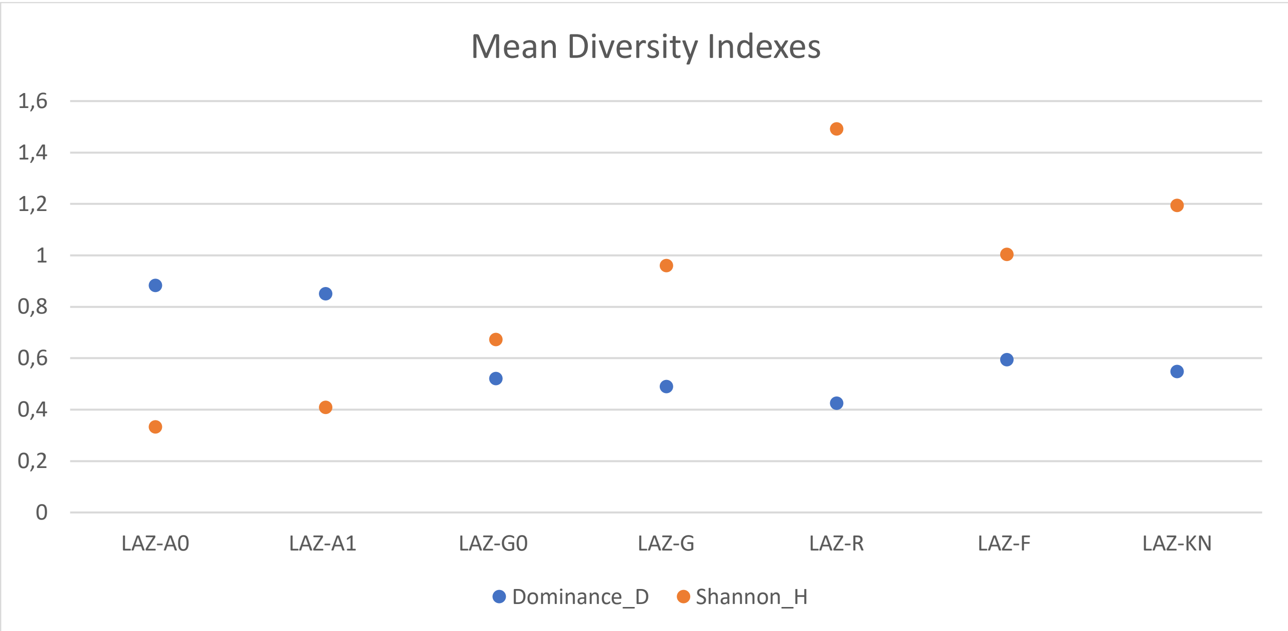


Graphic 14 : Shannon-Wiener and Simpson index for each site and date.

LAZ_G0 is the site with less individuals sampled, as it is the closest to the glacier and furthermore the one with the more extreme environment. The stations under the rock glacier, LAZ_A0 and LAZ_A1, showed an increase in individuals the further from the glacier, with a mean of 5000 individuals per square meter in LAZ_A1, despite having mean temperatures under 2°C. LAZ_G in September had a notable increase in individuals, with, as we will discuss later, a consistent hatching of Plecoptera. The most individuals were present in LAZ_F during September, with an esteem of 8624. All sites have an increase in abundance, LAZ_KN is the only site where a decrease in number of arthropods is present.



Graphic 15: Total individuals per square meter and number of taxa.



Graphic 16: Mean Diversity indexes.

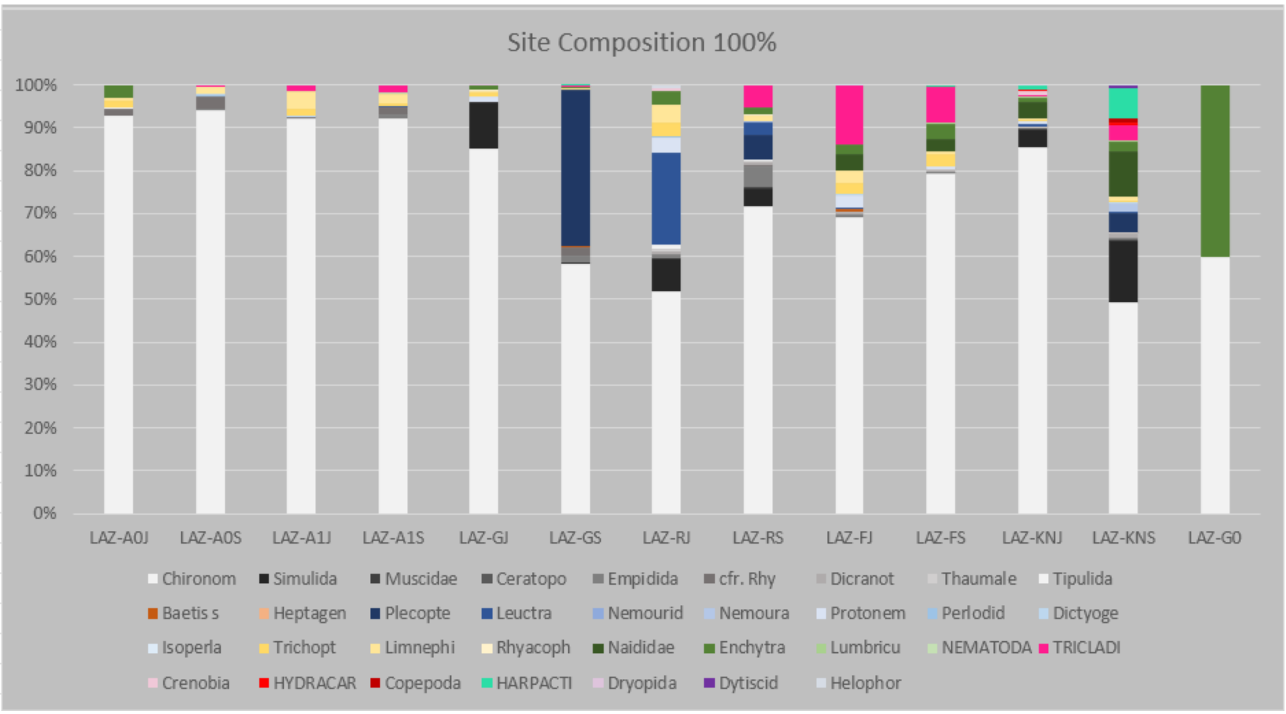
As previously stated, Chironomids dominate the sampling, and it is clearer in the graphic n.16 which respectively show the taxa composition of each site, reported to the total of individuals and to the percentage. All sites are composed more than 50% by Chironomids, with 7 sites out of 13 above 80%.

LAZ_A0 and LAZ_A1, despite the different abundance in total individuals, show both a composition of Chironomids above 90%, in similarity with a kryal habitat as Ward (1994) stated that kryal habitats were populated mostly by chironomids. LAZ_G0 which is metakryal has 6 Chironomids and 4 Enchytraeidae. In these three sites taxa belonging to Plecoptera and Ephemetoptera are nearly absent.

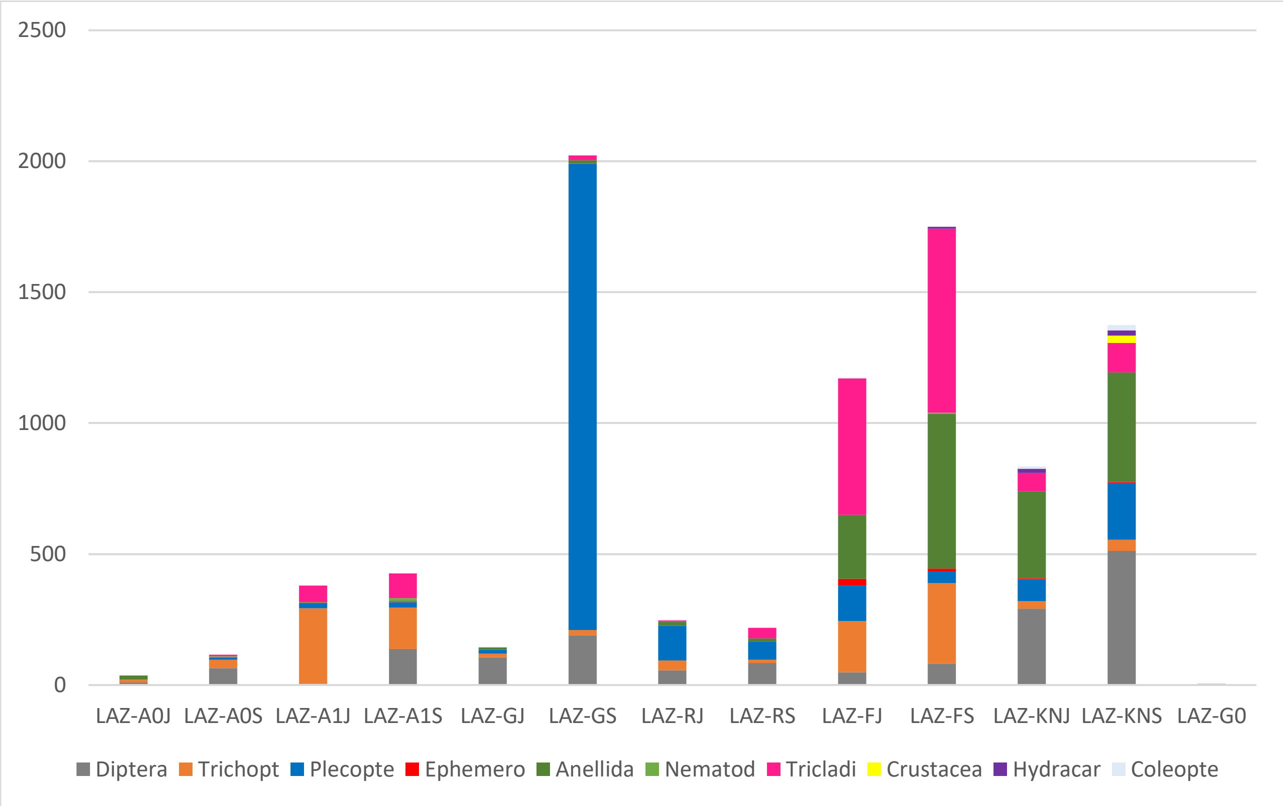
LAZ_G and LAZ_R, which are rhithral show different composition with each other, reflecting the different water sources. LAZ_G in July shows a predominance of Chironomids followed by Simuliidae, and in September shows a boom in Plecoptera with around 1700 juveniles, which redistributes the abundance percentages and increases the total number of individuals. LAZ_R between July and September does not have a steady increase in numbers instead has a decrease in Trichoptera and *Leuctra* sp. and an increase in Tricladida.

LAZ_F doubles the number of individuals between the dates from 3818 to 8624 still maintaining the taxa percentages constant, having hundreds of Tricladida, Enchytraeidae and Naididae, and Trichoptera. This site also shows a lesser abundance in other Diptera, in contrast to LAZ_KN which has hundreds of Simuliidae. LAZ_KN in September shows half the numbers of July, but with a more mixed community, including Plecoptera, Anellida, and Harpacticoida.

The graphic n.18 enlightens the composition of the sites taking out of consideration Chironomids. In a longitudinal perspective of the sites (from LAZ_G0 to LAZ_A0, LAZ_A1 and finally LAZ_G and LAZ_R) the proportion of Plecoptera and Trichoptera along with other taxa increases, with LAZ_A1 having more Trichoptera than other stations, and LAZ_G and LAZ_R with more Plecoptera. Ephemeroptera in these environments are scarce. Krenal sites abound with Anellida, with LAZ_F with more Tricladida and Trichoptera than LAZ_KN, which has more Diptera, Plecoptera and Trichoptera. These differences are in line with Ward 1994, Brown et al.,2003, Debiasi et al 2022.

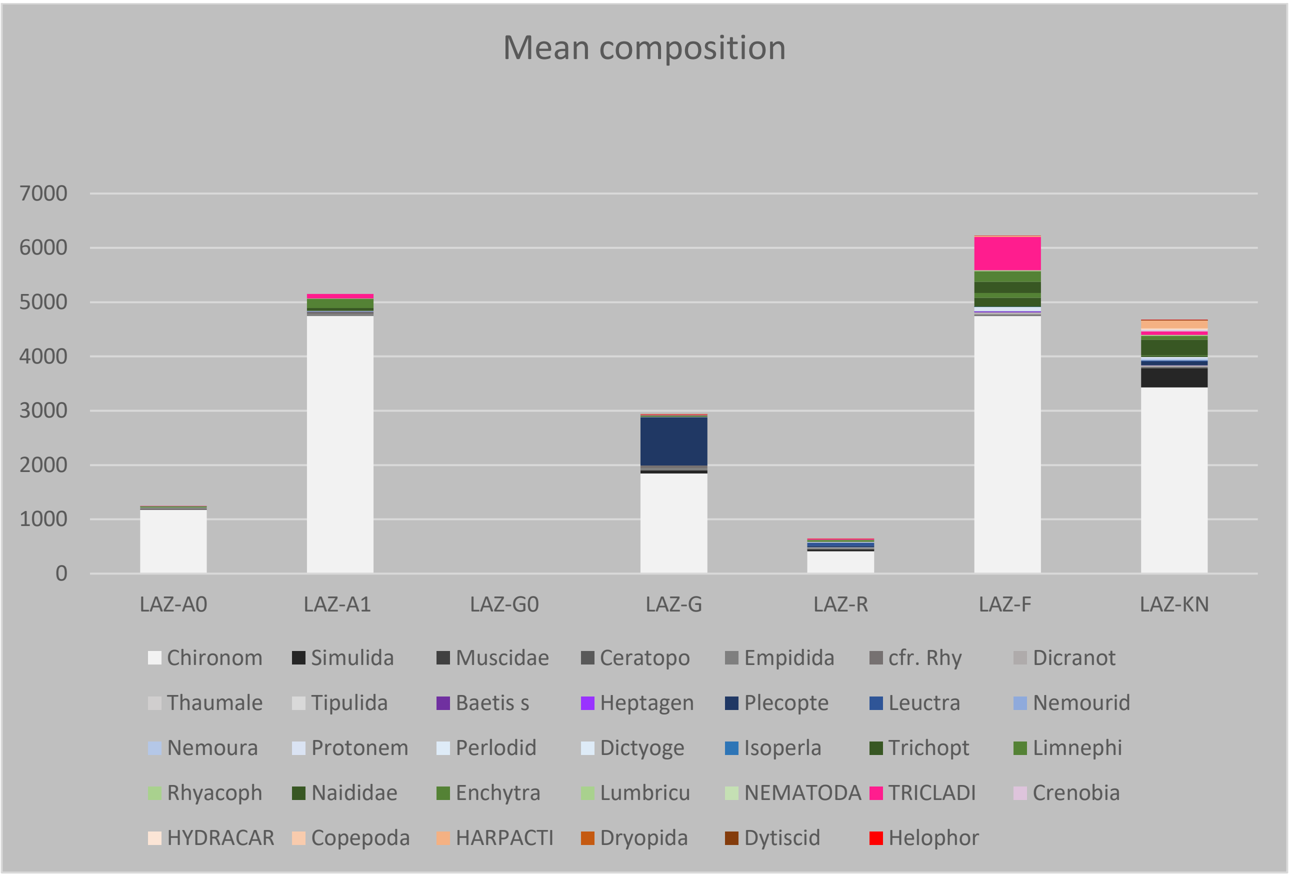


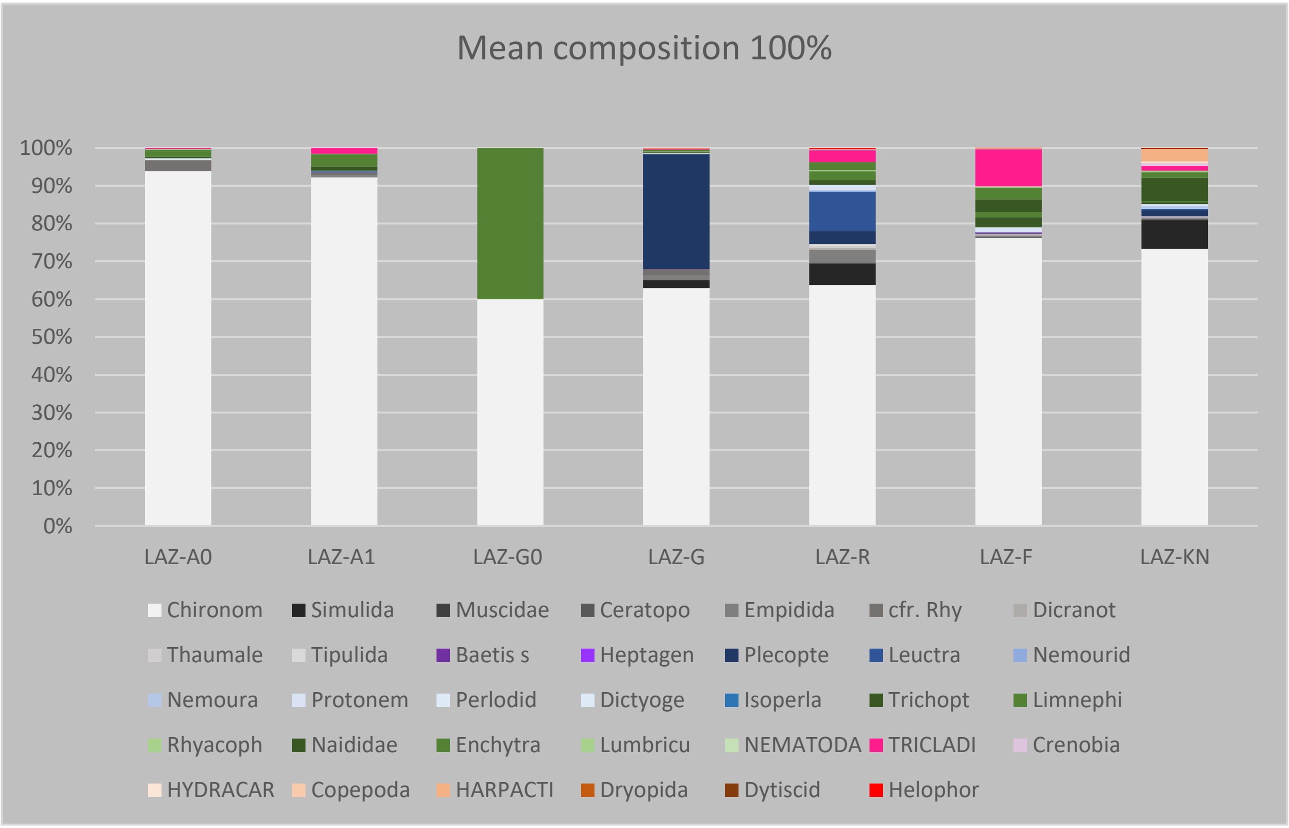
Graphic 17: In order: community composition repartitions on total individuals per site and composition percentage for each site.



Graphic 18: Site composition without Chironomidae.

The mean composition of the sites (considering both dates to enlighten the spatial differences) is show in the graphic n.19. LAZ_F results in having the more individuals, followed by LAZ_A1, which although is an active RG station has a high abundance. LAZ_KN as another krenal station has high abundance and a high biodiversity characterized by a high presence of Simuliidae, Crustacea, and aquatic Coleoptera. LAZ_F is characterized by having lots of Tricladida and Anellida, both Naididae and Enchytreaidae. Plecoptera is the taxa which characterize LAZ_G.





Graphic 19 : Mean site community composition and mean site percentual composition.

5.11 Spatial and temporal gradients of distribution

The cluster analysis divides groups following their similarity in variables, which in this case are given by the community composition (34 taxa). The graphic produced shows a branched tree with a site at each end of the branches. The lower the branches separate the lower is the affinity between those groups, and so their differences are greater (Similarity=0). For example, LAZ_G0, which separates immediately, is the most different compared to the others.

Considering the cluster with mean values, apart LAZ_G0, the branched tree separates at 0.5 similarity to divide the 'krenal group' (LAZ_KN and LAZ_F) from the rest. LAZ_F, still behaving like a krenal site, has peculiar features which separates it from the only ground water fed krenal stream (LAZ_KN). The differences may be conditioned by some deep permafrost of the relict glacier. The other branch, soon divides again, separating the group with LAZ_A1, and LAZ_A0 (active RG) from LAZ_G and LAZ_R (glacio-rhithral). The cluster shown in graphic n.22 take in consideration each site for each date of sampling. LAZ_G0 separates immediately, followed at 0.45 similarity by the 'krenal group', dividing it from the rest. The tree then divides again separating the July samples of LAZ_G, LAZ_R and LAZ_A0, from the branch which soon divides in the glacio-rhithral September samples and the rest of the active rock glacier samples. This separation suggests that in krenal sites and in LAZ_A1 there is no temporal difference, in contrast to glacio-rhithral sites and to LAZ_A0.

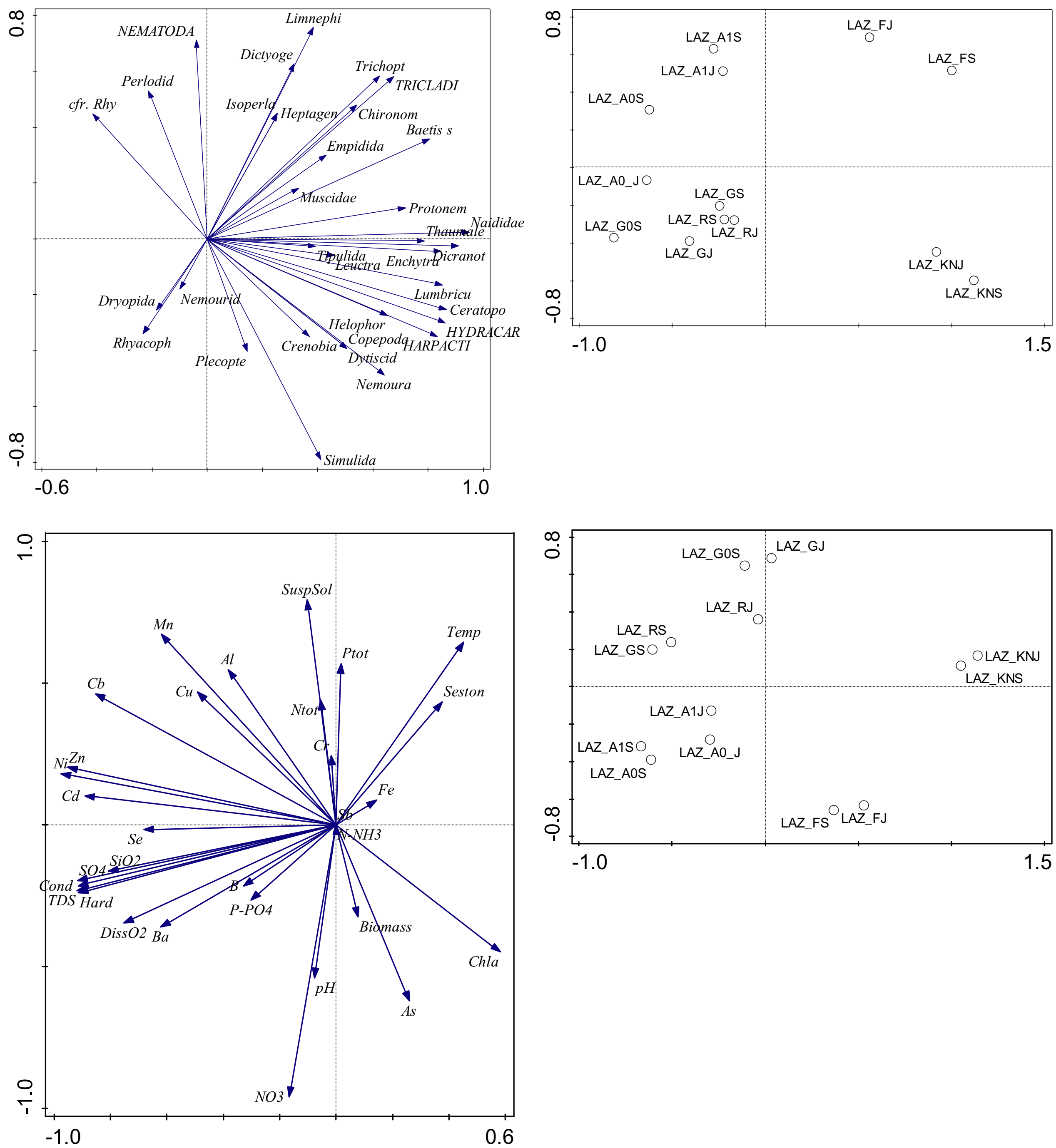
The PCA linearly transforms the variables given into a new coordinate system, in which the new formed axes display the variance. The first axis explains the most variance, the second one will display a lower variance and so on. The sites with the most distance in the first axis will be the most diverse.

PCA has been calculated both for the abiotic and the biotic component. The PCA for the abiotic component calculated four eigenvalues (PCA1=0,3416, PCA2=0,1803, PCA3=0,1269, PCA4=0,0823) explaining the 73.1% variance for abiotic factors overall. Active rock glacier sites (LAZ_A0, LAZ_A1) are positioned in the III quadrant, characterized by high conductivity, dissolved O_2, SiO_2, SO_4, P and low temperatures and seston quantities. The July samples are closer to the center of the graph suggesting a temporal variation, in accordance with the clustering results. Glacio-rhithral sites (LAZ_G, LAZ_R) and LAZ_G0, which is metakryal, are positioned in the II quadrant, with LAZ_G0 closer to July values of glacio-rhithral stations. High suspended solids, Ptot, Ntot characterize the meta-kryal and the July glacio-rhithral stations. As for September data the rhithral sites show a particular ionic footprint, with Mn, Cu, Cb, Al. Krenal stations position in the first quadrant, with LAZ_KN, and in IV quadrant with LAZ_F. LAZ_KN has opposite characteristic respect active RG sites, as low dissolved oxygen, low conductivity, low P and SiO_2 ad SO_4^{2-}, and has higher temperatures and seston. LAZ_F is characterized by having high biomass and Chla and As concentrations. Also for the biotic component four eigenvalues were calculated (PCA1=0,407, PCA2=0,1955, PCA3=0,1568, PCA4=0,0651) explaining 82.43% of the variance. In this analysis both krenal sites occupy a quadrant each, with LAZ_F in the first one and LAZ_KN in the IV. *Dicranota* sp., *Protonemura* sp., Naididae, Enchytraeidae, Thaumaleidae and Tipulidae are taxa common to krenal sites, as the arrows are pointing in the mean of the two groups. LAZ_F is characterized by the presence of Chironomidae, *Dyctiogenus* sp., Empididae, Heptagaenidae, *Baetis* sp., *Isoperla* sp., Tricladida and Trichoptera. On contrast LAZ_KN has a high presence of *Crenobia* sp., Simuliidae, *Nemoura* sp, Hydracarina, Harpacticoida, Coleoptera and Lumbriculidae.

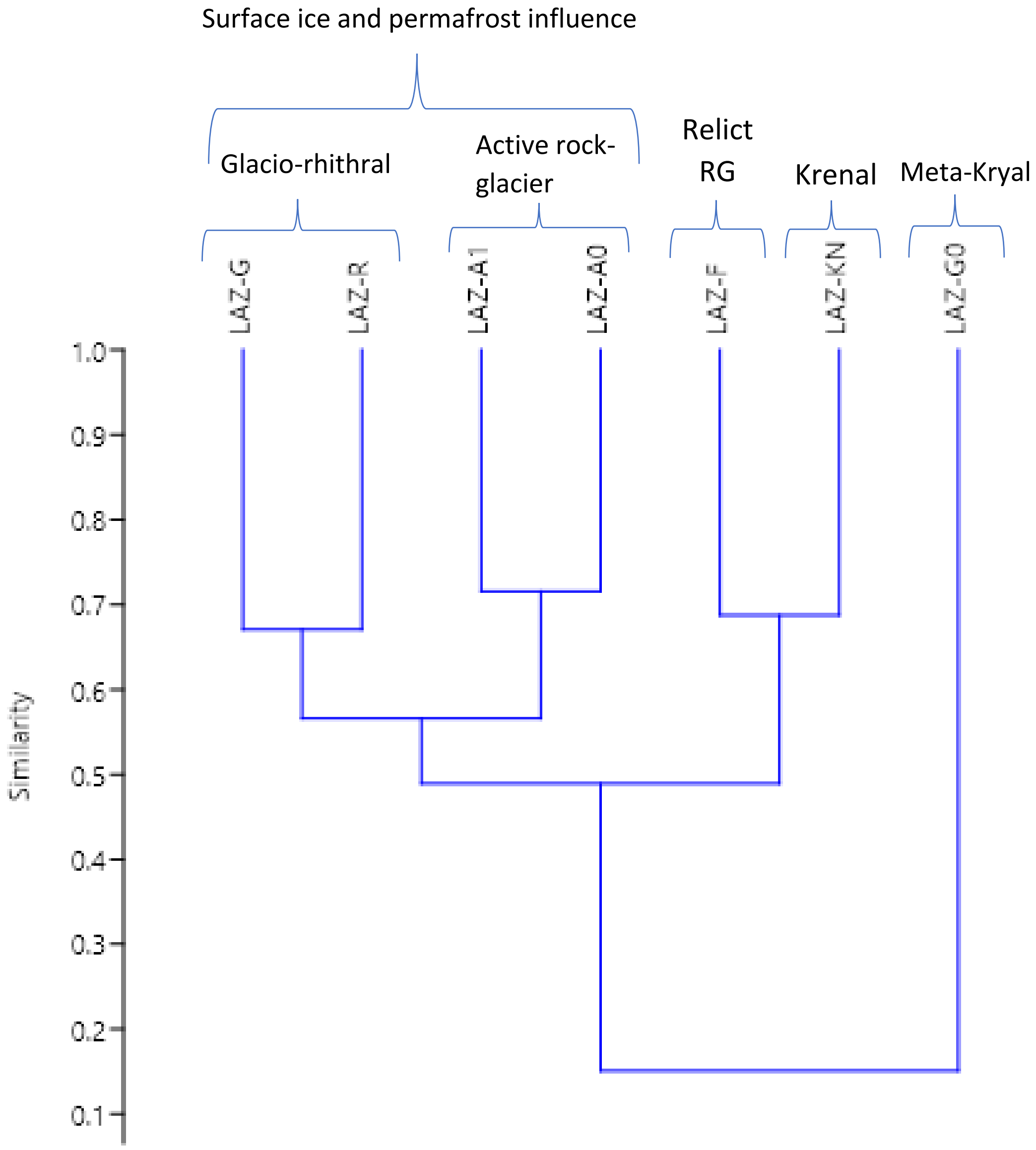
Active rock glacier sites are in the II quadrant except for LAZ_A0J, which is in the III. The RG group is distinguished by Nematoda, Perlodidae and crf. *Rhypholophus* sp.

LAZ_G and LAZ_R are characterized by Dryopidae, *Rhyacophila* sp., and Nemouridae.

Overall, the sites, and so the habitats, separate in the same way in each PCA, with LAZ_KN and LAZ_F always having a separate quadrant meaning that despite being both krenal-type stations there are some major differences in the biologic community and in the abiotic components. Glacio-rhithral sites always share the same quadrant with the adding of LAZ_G0 and considering the abiotic component they also show a temporal difference between them, with a more present ionic footprint in September. Sites related to the active rock glacier share the same abiotic features, with more conductivity, dissolved oxygen, hardness, SiO_2 and SO_4 concentrations in September, due to the higher ablation of the glacier. Considering biotic factors, the sites share the same quadrant with the exception of LAZ_A0J which is similar to glacio-rhithral communities.



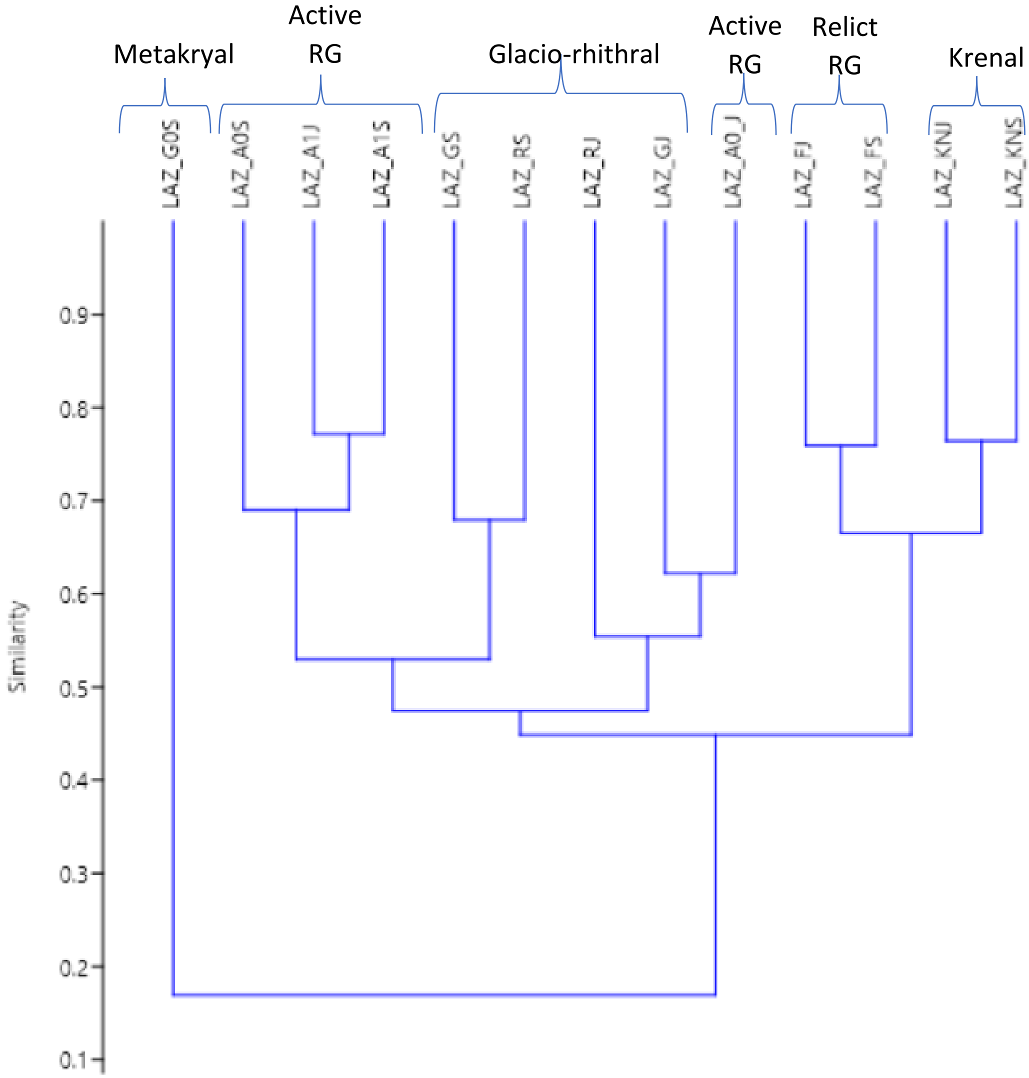
Graphic 20: In order: PCA for biological components and PCA for abiotic components (Elaborated with CANOCO).



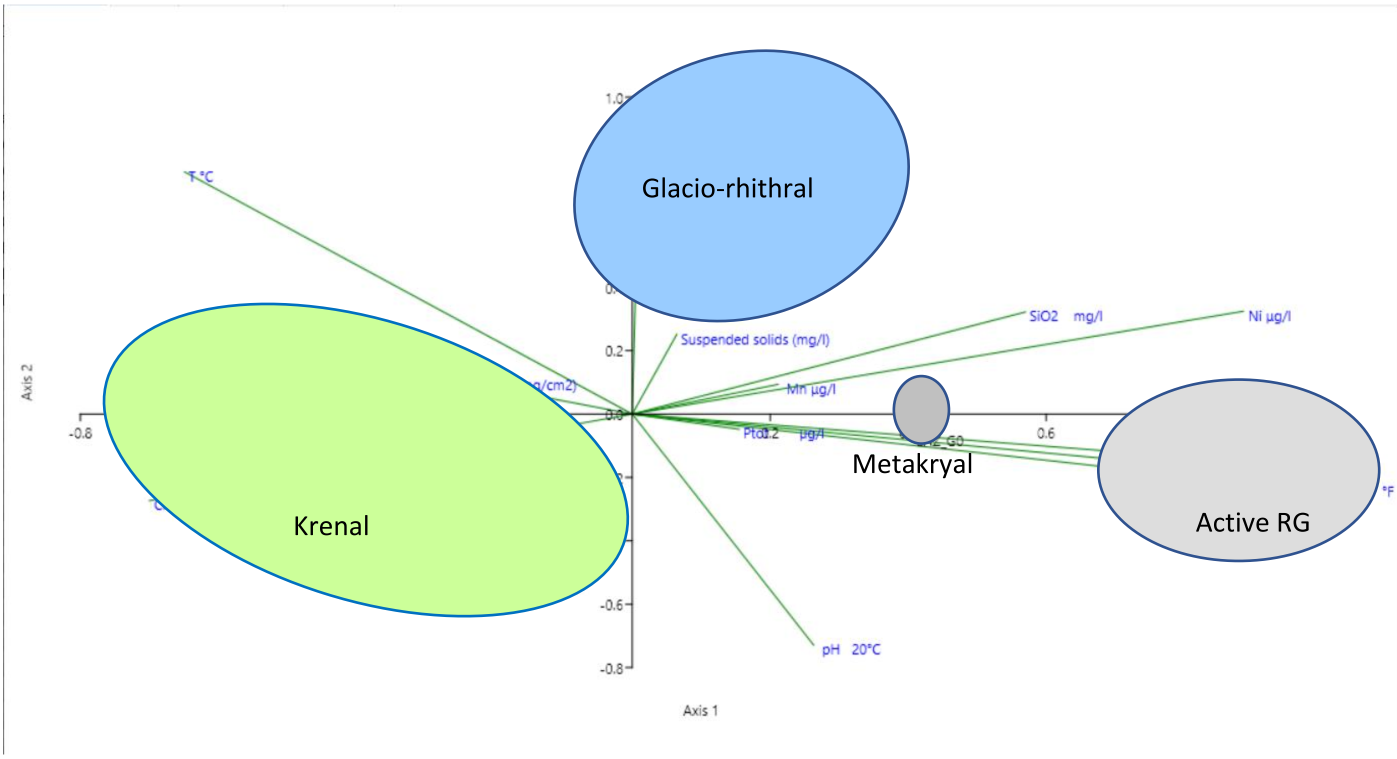
Graphic 21 : UPGMA Clustering Bray-Curtis model elaborated with Past 4.03 software.

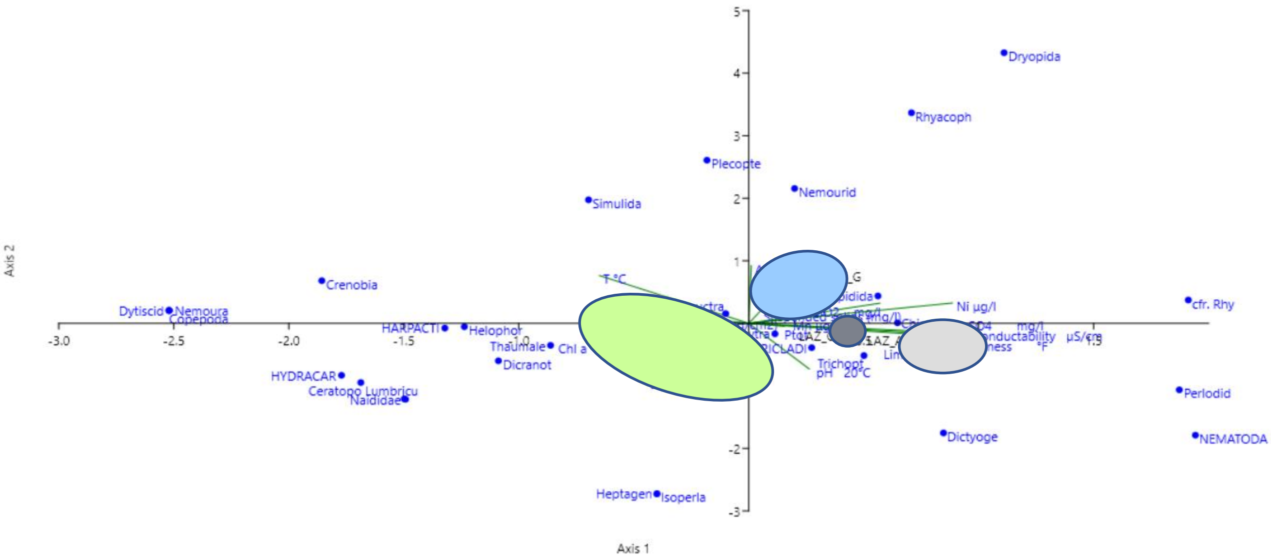
The results of the Canonic Correspondence Analysis (CCA) are shown in graphic n.22. The sites are displayed in the graphic in relation to thirteen habitat variables and to community composition. The division shows glacio-rhithral sites in the first quadrant, krenal sites in the second and third quadrant and active RG types in the fourth as well LAZ_G0, which is metakryal. If in the PCA LAZ_G0 was more related to the rhithral-type, in this analysis, which comprehends at the same time abiotic and biotic components, is shown more in relation to the active rock glacier type, with low temperatures (below 2°C), moderate conductivity, hardness and SO_4^{2-}.

Krenal sites maintain their diversity from other types and maintain an inner difference, as LAZ_F and LAZ_KN are always in different quadrants.



Graphic 22: UPGMA clustering Bray-Curtis model, with each site for month of sampling.





Graphic 23: In order: CCA graphic showing only the thirteen abiotic variables; CCA complete with biotic variables (taxa), elaborated with Past 4.03 software.

6. Discussion

The spatial difference between sites is highlighted by clustering and PCA, which divide the sites between metakryal, active rock glacier, glacio-rhithral and krenal types.

The knowledge we have about rock glaciers is concentrated to their geological and physiochemical properties more than their macroinvertebrate community composition, as works on this argument are scarce. With this study we want to add information regarding this topic. With temperatures below 2°C, high SO_4 (100-140 mg/l), high conductivity (220-300 µS/cm), LAZ_A0 and LAZ_A1 share the same characteristics of an active rock glacier type alpine water streams (Brighenti et al., 2019). The conductivity, pH and SiO_2 concentration are higher than in kryal streams (LAZ_G0) in line with Fegel et al., (2016). The presence of Chironomidae, Simuliidae, Plecoptera (Perlodidae, *Leuctra* sp., *Protonemura* sp.) as well other Diptera is in line with Sertić Perić et al. (2015) where a Swiss rock glacier stream's community was analyzed. In Lazaun the abundance of Plecoptera and Ephemeroptera is far lower (mean indiv/m² Ephem=307, mean indiv/m² Pleco=1162, in Sertić Perić et al. (2015); mean indiv/m² Ephem=1, mean indiv/m² Pleco=22, in Lazaun). The distance from the glacier could be the major responsible for the difference between the Swiss RG stream (sampled at 1400 altitude meters below the RG stream origin) and Lazaun stream (LAZ_A0 at the origin of RG, LAZ_A1 a hundred meters away).

The active rock glacier community shows a dominance superior of 90% of Chironomids. The presence of Trichoptera, Simuliidae and Plecoptera makes LAZ_A1 more similar to a glacio-rhithral habitat, as EPT (Ephemeroptera, Plecoptera, Trichoptera) in kryal habitats should not be present (Ward, 1994) (Lencioni, 2018). It is then assumable that an active rock glacier water stream possesses intermediate characteristics between kryal and rhithral habitat. In this situation LAZ_A0 is more similar to a kryal water stream, as the Trichoptera and Plecoptera are present but in lesser quantities, and LAZ_A1 to a rhithral. A possible explanation for this higher diversity in LAZ_A1 is the bigger distance from the rock glacier, meaning that the rock glacier's influence is lower than in LAZ_A0 and, as in ice glaciers (Debiasi et al., 2022) (Lencioni et al., 2021), permits to more taxa to be present.

Seasonal changes in the active rock glacier sites occurs mainly in the abiotic component, as seen in PCA, with rises in SiO_2, SO_4^{2-} and dissolved O_2 concentrations and conductivity, which is in line with literature, as in September with a higher outflow from the glacier these parameters tend to rise (Brown et al., 2003). Furthermore, the biotic PCA shows a separation between dates, especially in LAZ_A0 with the appearance of *Rhypholophus sp.* and Limnephilidae.

Krenal water streams, are characterized by a constant temperature, a specific ion imprint, the presence of algae and diatoms and by a conspicuous number of taxa, which derives by constant temperature always above 0 °C and the presence of more sources of food (Ward, 1994) (Brown et al., 2003) (Milner et al., 2001) (Scotti et al., 2019). These characteristics are all seen in LAZ_KN and

LAZ_F, which have the highest number in taxa (25 total taxa each), the highest chlorophyll a quantity and overall show small changes over time (as shown by PCA and Clustering). The constant temperature could not be seen through the dates as the data is missing, but we assume it was, as all the other variables indicated before are coinciding with the krenal habitat description by Ward (1994), Brown et al. (2003) Debasi et al., (2022). The Chironomids abundance ranges from 70% to 80% in LAZ_F and from 50% to 85% in LAZ_KN. LAZ_F is characterized by a high presence of Tricladida, Enchytraeidae, Naididae, and Trichoptera. LAZ_KN by Anellidae as well, Harpacticoida, Hydracarina, Coleoptera, *Protonemura* sp., *Leutra* sp., Simuliidae and other Diptera taxa, as Tipulidae, Thaumaleidae. Another difference between these sites is the quantity of suspended solids, which is basically zero in LAZ_F. The differences between these stations, which also include diverse ionic footprints, may suggest that there still could be some influence by deep permafrost belonging to the fossil rock glacier to LAZ_F stream.

LAZ_R and LAZ_G show higher temperatures (5-8°C LAZ_G; 4,5- 6,5°C LAZ_R) and an ionic footprint higher in September, in line with Ward (1994) and Brown et al., (2003). Intermediate conditions between kryal-active RG and krenal ecosystems are shown, such as chlorophyll a and biomass concentrations.

EPT, Diptera and Anellida are the taxa which characterize these glacio-rhithral streams (Ward, 1994), and except Anellida all of these taxa are well present in LAZ_G and LAZ_R. They show a seasonal change, which for the biota is more evident in the clustering and for the abiotic factors in the PCA. This suggest that the variation in water characteristics, caused by a higher runoff, affect the macroinvertebrates communities, which change between July and September, with taxa as Simuliidae present only in July and Plecoptera, *Rhypholophus* sp. and Empididae characterizing September.

LAZ_G0, which is classified as metakryal, as expected showed low biodiversity, low quantity of individuals (10) however the composition of the community did not have the abundance of Chironomids expected, as 40% was composed by Enchytraeidae. Anellida taxa should not be present in kryal habitats, so their presence is an anomaly (Ward, 1994) (Lencioni et al., 2021) (Debiasi et al., 2022).

In conclusion, we demonstrated that in Lazaun water streams the typologies differentiate in line with Ward (1994), Scotti et al., (2019), Lencioni et al., (2021) and Debiasi et al., (2022), with the addition of a macroinvertebrate community description of active rock glacier streams and confirming previous abiotic characteristics regarding these habitats. Habitats that, in the optic of the glaciers decline may serve as a refuge area (Brighenti et al., 2019) to kryal species as *Diamesa* sp., but this will be clear only once the Chironomidae ID process will be made. As for a seasonal change in communities in krenal stations only the individual abundances vary, in RG sites is low but not negligible, and in glacio-rhithral is higher as shown in the cluster analysis. This could lead to a diminishing in number of samplings in krenal habitats as one sampling could suffice to the understanding of the macroinvertebrate community present, even though it would not work if the individual’s abundance was the target.

The sampling and sorting of Lazaun water streams also increased the consistency of the collections of MUSE – Museo delle Scienze di Trento.

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