UNIVERSITÀ DEGLI STUDI DI PADOVA

Dip. Agronomia Animali Alimenti Risorse Naturali e Ambiente
Dip. di Biologia

Corso di laurea magistrale in
Scienze e Tecnologie per l'Ambiente e il Territorio

Temporal pattern of insect emergence in two alpine streams: an experimental approach

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A mamma e papà,
per il loro esempio

Contents

Abstract

In alpine areas, streams fed by glaciers and permanent snowfields are “extreme” for life due to harsh environmental conditions mainly in terms of hydrological and thermal pattern. Thus they are naturally colonized by few invertebrate species belonging to few taxonomical groups.

Two sites, in two different stream types (glacial and non glacial), in the NE Italian Alps, were investigated using an experimental approach mainly to study (1) the structure invertebrate community and (2) the emergence pattern of insects in the two streams. To this purpose, six emergence traps, one malaise trap, pond and drift nets were used during summer 2015 (July-September) with two different temporal patterns, hourly and biweekly.

As expected, the two benthic and drift communities resulted significantly different from each other, with higher taxa richness and abundance in the less stressful non glacial stream. These results was not confirmed by adults captured with emergence traps, with which we collected few specimens mainly belonging to Diptera families, without significant differences between the two stream types. A higher frequency of captures and/or a higher number of traps/site might improve the efficiency of sampling with these traps so difficult to manage in alpine streams especially in glacier-fed streams. Furthermore, bad weather conditions (fog, low temperature, etc.) probably reduced the emergence rate in insects living here during our sampling season.

Malaise trap worked very well even if it is not selective for aquatic insect adults. Notwithstanding, some interesting correlations have been highlighted such as the concomitant increase in adult specimens in the Malaise trap and decrease in aquatic stages in the benthos samples and the daily patter of Chironomidae emergence (preferably from 9 am to 3 pm) in both streams.

1. Introduction

High altitude and latitudes comprise two important boundaries for life in alpine environments. Ecosystems at these situations experience extremely harsh conditions such as low temperature, daily and annual extremes in wind, extended periods of snow cover, precipitation, and low humidity (Franz, 1979). The combination of these factors demands special physiological adaptation for survival in winter and to allow development and reproduction in a short time window in summer (Füreder et al., 2001). Since 2000s, this has been the subject of numerous studies of aquatic systems (e.g Füreder, 1999; Lencioni, 2000; Lencioni et al., 2000).

In alpine areas, some rivers are fed by glacier and permanent snowfields. The seasonally fluctuation in discharge and suspended solids, the low channel stability, and the year-around low temperatures together with season, distance from the glacial snout, and the availability of water from non–glacial sources represent harsh environmental conditions for the stream fauna (Füreder et al, 2005). The glacial stream fauna composition, which is therefore conditioned by all these factors, has been illustrated from several studies (Milner and Petts, 1994; Ward, 1994; Tockner et al., 1997; Schütz, 1999; Lencioni, 2000; Brittain and Milner, 2001; Burgherr and Ward, 2001; Füreder et al., 2001; Schütz et al., 2001).

In these environments, aquatic insects face those harsh conditions both during their aquatic and terrestrial stages.

Insects in alpine streams must be able to grow and develop at temperatures usually between 4 and 8°C (Gibberson and Rosenberg, 1992; Newbold et al., 1994) with some *Diamesa* species (Diptera, Chironomidae) that are able to complete an entire life cycle at 0-2 °C (Milner and Petts, 1994). These values are lower than the minimum temperature usually experienced by most temperate aquatic insects (Füreder et al., 2005).

The flying forms (terrestrial stage) of high altitude aquatic insects has to overcome to the most extreme condition during the majority of the year. Only 2 or 3 months per year present warmer and more stable condition (Füreder et al., 2005).

During this season the rate of insects' emergence is higher than in the rest of the year thanks to the increased probability of experiencing favourable climate conditions on land (Füreder et al., 2005).

The main goals of this study were to (1) implement knowledge on benthic and drift community in alpine streams with different origin and (2) better understand the patterns of insects' emergence in both glacial and in non-glacial streams by applying two different sampling strategies: the biweekly and hourly sampling. In particular, we focused on diurnal pattern from 6 am to 9 pm, rarely assessed in previous similar studies.

1.1. The stream: a simple ecosystem?

An ecosystem is a system, or a group of interconnected components, formed by the interaction of abiotic and biotic factors. The ecosystem concept is extended to all networks that are set on the biosphere. The river ecosystem is, therefore, the totality of abiotic factors that correspond to river's habitat (water temperature, water chemicals, riverbed substrate, etc.) in relation with the river communities (fauna, flora and humankind).

The abiotic components have a profound effect on an ecosystem structure and composition. Early studies in the 19th century suggested huge diversity between terrestrial environments when comparing the biological richness (in term of species richness) between temperate and tropical areas. However, the analysis of the river systems provided a rather different picture: in 1874, the entomologist and botanic Thomas Belt wrote in *The Naturalist in Nicaragua* that "*All the land fauna was strikingly different from that of other region but, the water fauna was strikingly similar*". Very different environmental factors shape the evolution of life in the forest, while life in rivers is affected by one, unique, factor, that is the water flow.

Another fundamental difference between river ecosystems and terrestrial ecosystems lies in the structure of the trophic web. If described as a biomass pyramid a typical food web includes a large base constituted of primary producers followed by primary consumers and finally at the last trophic level, it ends with a limited amount of apex predators. If we observe a typical alpine river ecosystem, however, we find many apex predators among vertebrates (e.g. the trout) and invertebrates (e.g. shredders, scrapers, filterers, collectors and gatherers) but very few primary producers (e.g. moss and unicellular algae). Therefore, the trophic pyramid is strongly biased due to the almost complete absence of autotrophic organisms. In particular, one of the macroscopic difference between terrestrial and freshwater environments is that in the first ecosystem the

higher plants cover the 99 % of the available surface, while, in the latter their presence is significantly close to zero (Hynes, 1970). The major source of primary production in the river ecosystem is provided by leafs, branches, and organic debris originated by the terrestrial ecosystems and carried to the river thanks to gravity and precipitation. Once deposited on riverbed, those elements are processed and metabolized by the primary producers.
The lotic environment, therefore, represents the typical open ecological system where the habitat is strongly influenced by the upstream energy flux.

1.2. The theory of River Continuum

A unique feature of the river ecology is the deep interconnection from the spring to the outlet of the stream, from which is derived the River Continuum Concept (RCC) (Vannote et al., 1980). According to RCC the whole fluvial extension is in a state of dynamic balance. It means that a minimal parameter variation in any river point involves a variation in the longitudinal features of the stream.
RCC implies that the biological communities are connected with organic matter availability, in every single river point: with a continuous and integrated series of physical and chemical parameters, it is associated in a continue transformation of biotic components (Salmoiraghi, 1992). In particular, this means that the macrobenthonic communities change along the river, from upstream to downstream (Battegazzore et al., 1992; Mascolo et al., 1999; Fenoglio, 2000).
As reported in the previous paragraph, the external organic matter input is a fundamental pattern for the stream ecology (Cummins, 1974; Cummins et al.,1981: 1989). In this sense, we can find 3 different situations along the river channel (see Figure 1).
Upstream, near to the stream's spring, we found a low internal productivity. Actually, the internal productivity (P) is minor than the community metabolic consumption or respiration (R) so the P/R ratio is lower than 1. This means that the river ecosystem is in energy deficit, therefore the organic matter depends on external input. In this environment, the macrobenthic communities mainly consist of shredders, collectors and gatherers (Figure 1, upper set).
Downstream, when the riverbed is bigger, the turbulence decreases and also the direct solar radiation increases, vegetation and periphyton become more relevant in the river ecosystems. Here, the P/R ratio is bigger than 1 so the external energy input decreases and the internal energy

production increases. Scrapers become dominant in the macrobenthonic communities (Figure 1, middle set).

Close to the river outlet, the turbidity increases with the decreased penetration of solar radiation. In this situation the P/R ratio reverts to values lower than 1 and the river becomes heterotrophic. In this section collector's gatherers are the dominant trophic group (Figure 1, lower set).

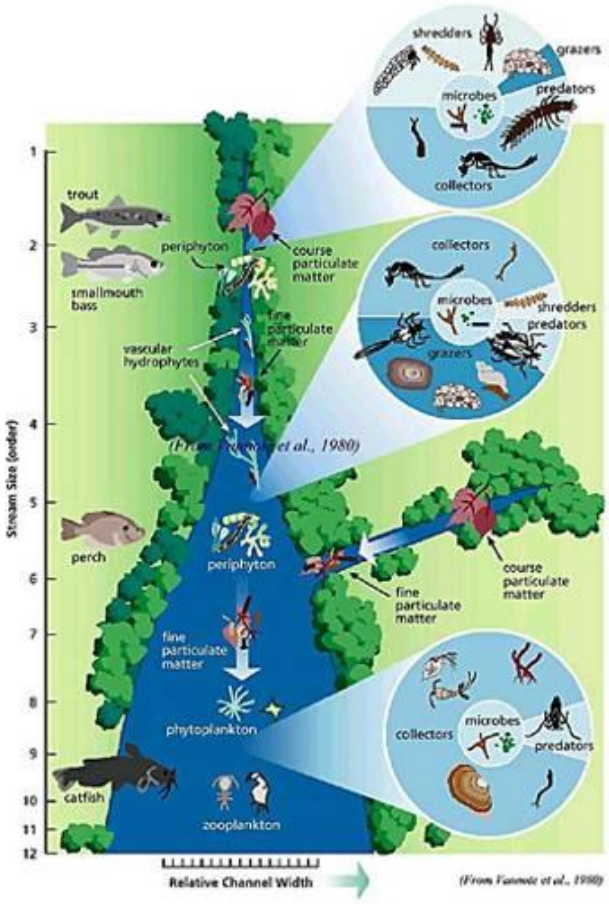

Figure 1. Deep interconnection from the spring to the estuary of the stream(source: http://www.stroudcenter.org/).

1.3. The high altitude streams

High altitude streams play an important role in the world river systems (Füreder, 1999) and may be more affected by anthropogenic impacts than mountain streams at lower altitude (Chapin and Körner, 1994; McGregor et al., 1995).

Another unique factor of the alpine streams is that above the tree line, the abiotic factors are particularly extreme and this makes the life of animal and plant communities, in the streams, more difficult, or even impossible.

The three principal stream ecosystem that can be distinguished in these areas are (Ward, 1994): kryal (glacier – melt dominated), krenal (groundwater – fed), and rhithral (seasonal snow – melt dominated).

Kryal

The kryal streams are fed by glacial melting, with temperature below 4 °C (Ward, 1994), high turbidity and high solid transportation depending on the season (Milner and Petts, 1994). These streams are characterized by a remarkable daily and seasonal discharge variation.

Flora is composed mainly by *Hydudus foetidus*, especially in winter or late summer (Ward, 1994) while the fauna is composed mainly by *Diamesa* (Diptera Chironomidae) often together with Simuliidae (Diptera) (Lencioni, 2004).

Figure 2. A typical kryal stream (Sarca d'Amola sx orographic ,Amola Valley)(source: D. Frizzera).

Krenal

Krenal streams are fed by high altitude groundwaters, and have an elevated chemical and thermal stability.

The temperature ranges between 1 and 2 °C (Ward, 1994; Fureder, 1999) with maxima of 4 °C (Fureder et al., 2001) and is independent from air temperature.

Other physical and biological features are: a constant hydraulic regime, a high water transparency with a Diatomea and bryophytes abundance, and a high ions concentration compared with kryal and rhithral streams (Milner and Petts,1994: Ward, 1994).

The macrobenthos species composition is intermediate between the kryal and rhitral communities.

Rhithral

These streams are characterized by the snow melting or, by a mixture of glacial, spring and snow water. These streams can extend for a considerable distance, with temporal changes in discharge and temperature reflecting the relative proportion of glacial influence but usually, the mean temperature range from 5 to 10 °C (Burgherr and Ward, 2001) (Milner and Petts, 1994).

The macrobenthic community is well diversified with the presence of Plecoptera, Ephemeroptera, Trichoptera and Diptera. Other invertebrates are Turbellaria, Acarina, Oligochaeta and Nematoda.

The plant component is composed by a good variability of Bryophyta, Crysophyta, Chlorophyta, Cyanophyta, Rhodophyta and Diatomee (Milner et al., 2001).

The chemical compounds are related to those present in the snow, in the glacier and in the rain.

Figure 3. A rhithral stream (Sarca d'Amola dx orographic , Amola valley)(source: D. Frizzera).

1.4. Macrobenthic fauna of glacial streams

Insects represent the 75% of all animal species and are therefore the majority of existing biodiversity (Resh and Cardè, 2009).

Only the 3% of the whole insect diversity is found in freshwater. However, these species belong to the five biggest orders of Hexapoda: Diptera, Ephemeroptera, Plecoptera, Trichoptera, and Coleoptera.

These species initially evolved as terrestrial and only later moved to freshwater, where several adaptive strategies were developed (e.g. gills, siphon, cutaneous respiration) that allowed them to thrive in the new environment (Resh and Cardè, 2009).

According to the RCC, upstream, near the river spring, the ecosystem is heterotrophic. Especially above the tree line, the physical and biological condition become really harsh with extended periods of snow cover. Daily and annual extreme variations in wind speed, temperature, substrate instability occur and important daily and seasonal discharge variation is recorded.

Highly specialized plant and animal species bearing unique adaptations were able to colonize such an extreme environment.

In 1994, Milner and Petts developed a model to explain the succession of macrobenthic communities from upstream to downstream in glacial streams (Figure 4).

They mainly took two parameters into account: the water temperature and the substrate stability. According to this model, revised in the AASER project by Brittain and Milner (2001), *Diamesa* (Diptera, Chironomidae) is the only insect genus able to survive in stream waters close to the glacial front, where the temperature ranges between 0 °C and 2 °C. Chironomidae abundance decreases with the distance from the glacier. The opposite trend is observed in the abundance of Ephemeroptera, Plecoptera and Trichoptera orders (Milner and Petts, 1994).

The main sources of information for the following group description are the *Encyclopedia of insects* (Resh and Cardè, 2009) and *Encyclopedia of entomology* (Capinera, 2008).

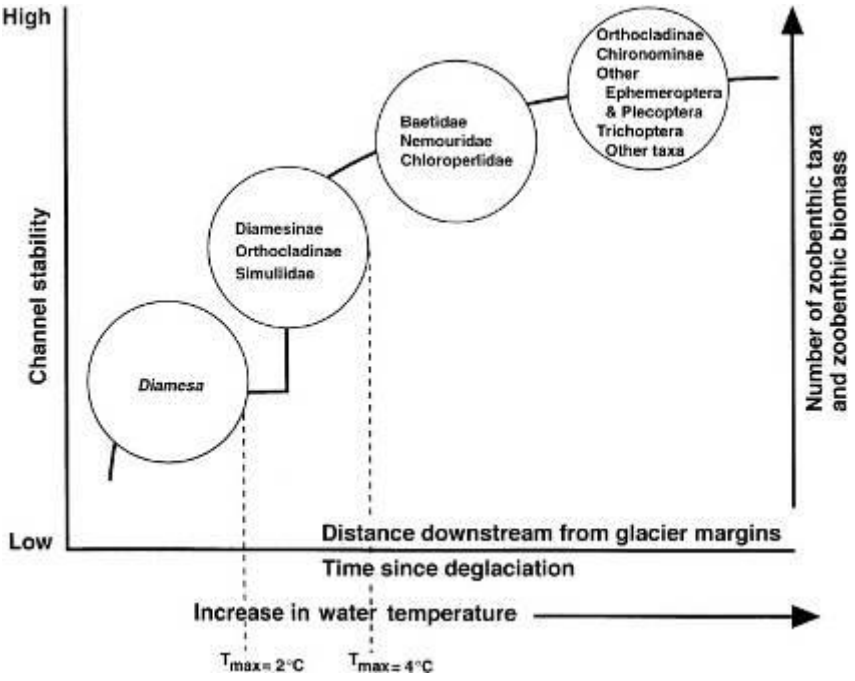

Figure 4. Conceptual model proposed by Milner and Petts (1994)(source: Milner A.M., Petts G. E. 1999).

Ephemeroptera

Ephemeroptera's names (mayflies) derives from Greek (*ephemeros* = short-lived and *ptera* = wings). These insects are hemimetabolous (incomplete metamorphosis) and differently from the other insects, they have two winged adult stages, the subimago and imago and are named after their short adult's life. They usually survive from 1-2 hours to a few days. Therefore, mayflies spend most of their life cycle in the aquatic environment either as eggs or nymphs.

Nymphs have an elongated, cylindrical or, to a certain extent, flattened body, which undergoes a number of stages of size increase.

Ephemeroptera nymphs differ from other benthic organism for what concerns the abdomen operculate gills even if up to seven pairs of gills arise from the top or sides of the abdomen in most taxa. In some species they are under the abdomen.

The majority of mayfly nymphs are herbivorous, feeding on detritus and peryphiton (scrapers and collectors).

Ephemeroptera, especially Baetidae, are one of the major components of invertebrate drift in stream water. In addition, they are successful colonizers of new habitats thanks to their winged stage.

In temperate and arctic areas, mayflies have a distinct and finite emergence periods depending on water temperature (Lencioni and Maiolini, 2002).

Plecoptera

Plecoptera's name (stoneflies) derives from Greek (*pleco* = folded *pteros* = wings) and is due to the hind wings, which expand posteriorly in a lobe that folds longitudinally under the main wing.

Stoneflies are hemimetabolous, this means that they pass, step by step, from nymphs stage to adult stage through about 10 – 24 sizes (instars).

Plecoptera nymphs are primarily associated with running waters, while winged adults rest in the streamside microhabitats such as rocks, debris, leaf packs and riparian vegetation.

They are divided into two trophic groups. The Nemouridae family, shredders species, has mandibles with molariform surfaces or scraping ridges. The Perlolidae are predators mainly feeding on other aquatic insects such as midge larvae (Chironomidae), mayfly nymphs (Ephemeroptera) caddysifly larvae (Trichoptera) and, occasionally on the smaller nymphs of other stoneflies.

Adult stoneflies usually emerge during the night and live from one to a few weeks.

Trichoptera

Trichoptera (from Greek *trichos* = hair and *ptera* = wings), or caddisflies, are holometabolous insects closely related to Lepidoptera. Caddisflies are tightly connected with lotic environment. In fact, eggs, larvae and pupae are usually found in or in the proximity of freshwaters, while adults are flying insects but always connected with aquatic habitats.

In many Trichoptera, larvae construct a portable case of various material that serves as a physical protection. Only few families have free larvae.

In terms of species diversity and density, Trichoptera are one of the major groups of macroinvertebrates living in freshwater ecosystems.

Because of their population are so developed, the trophic regime of caddiesflies is extremely various: there are carnivorous species (Rhyacophilidae), filterers (Hydropsychidae), periphyton scrapers (Psychomyidae) and leaf shredders (Limnephilidae).

Trichoptera species are sensitive to changes in environmental condition; therefore, they are commonly used as a bioindicators.

Diptera

Diptera (from Greek *di* = two and *ptera* = wings) or true flies, are holometabolous insects, with legless larvae and two–winged adults. They have a pair of fight wings on the mesothorax and a pair of halters on the metathorax. Due to the structural variability, especially among larvae, it is difficult to generalize about morphology and ecology.

Diptera inhabit lots of environments especially lotic systems. In these systems, Diptera have colonized almost the totality of trophic regimes with scrapers, shredders, predators and collectors.

Diptera larvae show an extreme structural variety but they can be distinguished from the larvae of most other insect by the absence of jointed thoracic legs. Larvae are morphologically divided as eucephalic, characterized by a complete, fully exposed and sclerotized head capsule, hemicephalic with a retracted head, and acephalic with no head.

The dipterian pupa also varies considerably in form.

Chironomidae is the most important Diptera family in terms of number of species and biomass. They have eucephalic larvae and live in the glacial streams, in the estuary zone and in brackish waters (Bazzanti, 2000; Rossaro et al., 2006a; b).

Simuliidae

Simuliidae or black flies, is a family of Diptera order. They feed on the blood of mammals, including humans. Males feed also on nectar and frequently considered as pest insects.

Simuliidae eggs are laid in running waters. Larvae hatch in water and use tiny abdominal hooks to hold on the substrate together with silk threads to move or hold at their place. They are able to move downstream on these threads without being swept away by the current.

Adults have short antennae and a small and grayish - black body. Some species can swarm in a large number of individuals.

Chironomidae

The family of Chironomidae, are one of the most important group of insects in freshwater ecology. They are known as “non-biting midges” but can still transmit infectious diseases to humans (Figure 7).

Chironomidae larvae (Figure 5) represent a big portion of aquatic zoobenthos in terms of biomass and number species. They colonize every kind of freshwater environment.

Figure 5. Chironomidae larva (source: V. Lencioni).

Some species have developed a haemoglobin analogue and thanks to this adaptation we can find these larvae also in highly polluted waters with low concentration of oxygen (O_2). These Chironomidae are known as “bloodworms” because of their red colour. Their ability to capture oxygen is further increased by their waving movements.

Some Chironomidae larvae can survive in anhydrobiosis. These larvae can survive in a prolonged complete desiccation state or when exposed to extreme conditions, such as ionizing radiation.

Some Chironomidae species are adapted to survive at low temperature and became the dominant taxon in glacier waters streams (Lindergard 1995).

Figure 6. Pupa of Chironomidae (source: V. Lencioni).

In particular, Diamesinae larvae can survive without compromising their metabolism, growth rate and feeding activity, at temperatures close to 0 °C (Lencioni 2004).

During the coldest periods of the year, especially in winter, when the flow speed is low and the water freezes, Diamesinae larvae enter a dormancy phase.

This ability to endure at low temperature is due to the cryoprotective substance present in hemolymph that prevents freezing damage maintaining to the fluidity of cell membranes.

Polyunsaturated lipids and metabolites, with a low molecular mass and high solubility in water (carbohydrates and lipids) decrease the freezing point by increasing the hemolymph density. Notably, species from the *Diamesa* genus, are able to synthesise antifreeze proteins (AFP). These molecules can hinder the growth of ice crystals within body fluids and avoid the damaging of cell membranes (Lencioni et al., 2015).

Figure 7. Adult of Chironomidae (source: V. Lencioni).

1.5. Colonization cycle

Invertebrates in lotic environments are distributed in a continue redistribution along the longitudinal axes of river channel (Townsend and Hildrew, 1976). There are four kinds of movements according to Williams and Hynes (1976) to colonization new bare stream. All four movements are important in recruiting new areas, and many invertebrate groups have a preferred direction of colonization (Lencioini et al., 2006)

1. Downstream: this relocation is deeply associated with the drift. This is a passive transport.
2. Upstream: this is an active counter-current movement observed in several species (Söderström, 1987) and it is aimed at reducing of inter- and intra-specific competition, escaping from adverse environmental conditions, searching for a good emergence or reproduction point or of a new trophic habitat.
3. Substrate vertical movement: this movement significantly contributes to overall biodiversity of rivers with a role of migratory upstream corridor, nursery in some phases of life cycle, and refuge area from hydrological events such as drought and freezing.
4. Air movement: many aquatic insects have an adult winged phase. Among these, the most important are: Ephemeroptera, Plecoptera, Trichoptera, Coleoptera and Diptera.

Before several studies on insects air movement provided a solution, the main problem was the *Drift paradox*. According to this theory the constant flux of organisms, from the spring to the river's estuary, brings the zoobenthos community to a drastic impoverishment caused by the downstream movement.

Müller (1982) highlighted that the air upstream counter - current movement is higher than the downstream, with a percentage of 75 – 95%.

This shows a complex cycle with the immature insect stage stationed downstream of the water flow and the adults that return upstream in order to lay eggs.

2. Materials and Methods

2.1. The study area

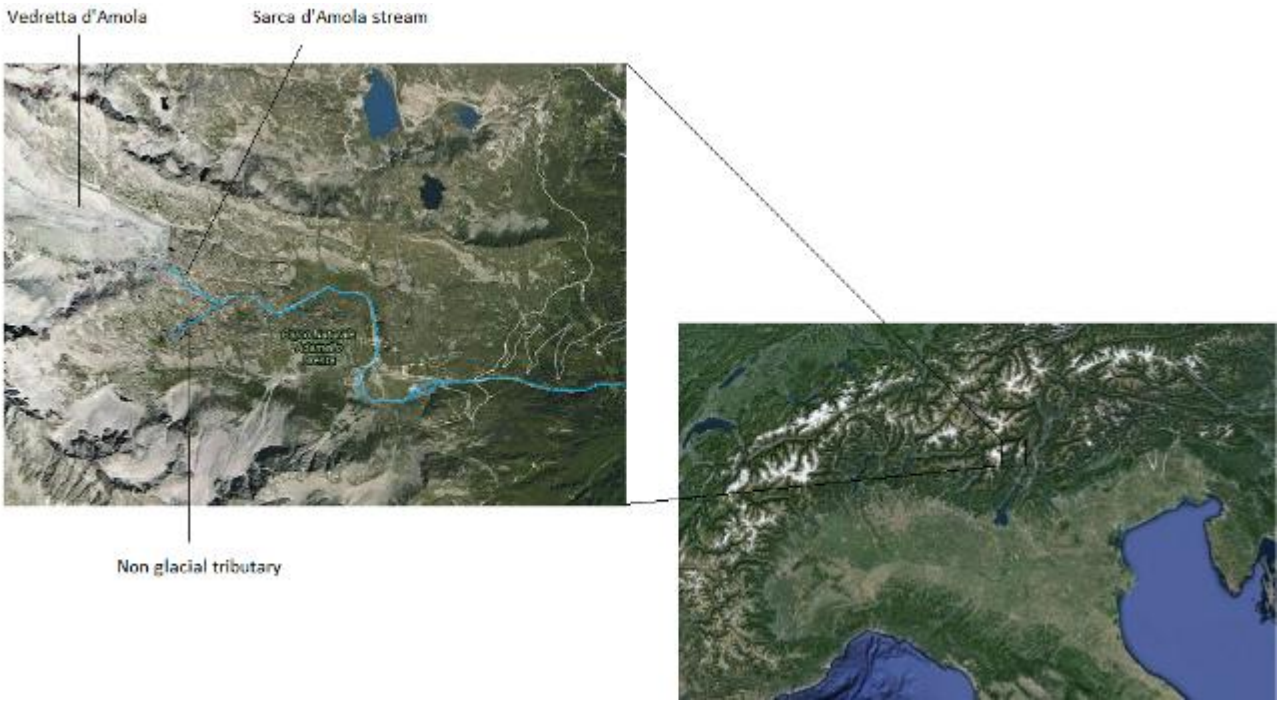

Figure 8. The Sarca d'Amola valley, located in the west Trentino.

The study area (N 46° 12.776' E 010° 42.404') is located in the Natural Park Adamello-Brenta (west Trentino, Italy) in the Adamello Brenta mountain massif.

The Adamello- Brenta Natural Park is the largest protected area in Trentino. It is 620 Km^2 wide and entails two mountain groups: the Adamello-Presanella, an intrusive-metamorphic massif and the Brenta sedimentary carbonate massif. This two groups are separated by the Rendena Valley and are surrounded by three valleys: Non, Sole and Giudicarie (Figure 8). It is endowed with 80 lakes and with Adamello glacier, one of the largest glaciers in Europe.

The Sarca d'Amola is a tributary of the Sarca-Nambrone river. It has a total extension of 41675 Km^2 and is composed by three sub-basins: 1) the Amola Valley, located at the right side of the main valley (1340 m above sea level - asl), 2) the Corosinello Valley, in sub-parallel position of Amola Valley and 3) the Nambrone Valley, the main valley.

The stream of Sarca d'Amola origins from the Vedretta d'Amola glacier (2580 m asl). A parallel tributary stream of non-glacier origin flows into Sarca d'Amola at 2408 m asl. The Amola stream flows in Amola Valley, the main side valley of Nambrone Valley, which stretches for 6 kilometers from east to west into the heart of Presanella mountain massif.

In the table below (Table 2) the main physico – chemical features of the alpine's part of Sarca d'Amola river are shown, as recorded in the 2014 season (the records refers to A1 station).

2.2. Geomorphology of the study area

The Amola Valley represents the typical lateral hanging valley created by the advancement and retreat of the glacier, which has also determined a series of terminal and lateral moraines with a main, central flat ground moraine (Figure 9).

Figure 9. The Amola Valley (source: D.Frizzera).

The Vedretta d'Amola glacier is part of Adamello-Brenta glaciers and belongs to the catchment of Sarca – Mincio–Po.

It is a typical mountain glacier, located at the base of the Monte Nero's crossing in the north-west of the valley. Due to the glacial melting processes, which took place during last Pleistocenic glacial

and interglacial fluctuations, Vedretta d'Amola comes up like a debris covered glacier, whose ablation basin was covered by rocky debris of varying size and thickness by more than 50%.

The glacier is mainly supplied by direct precipitation, which contributes to increase the mass and avalanche descending from the steep cliff surrounding. The glacier has been retreating since the end of Little Ice Aged (Comitato Glaciologico Italiano 1962's land register). The process is not constant: there is evidence of high retreat rate opposed with positive phases. This pattern could be the result of the protective action of the debris (Trentino's civil protection personal communication).

Two kinds of streams spring from the flat ground moraine, which extends for 1,5 km through the Amola Valley: The first one is a glacier-fed stream, with waters coming directly from the Vedretta d'Amola glacier and springing at an altitude of 2580m asl. The second one is a spring- fed stream, and its waters come from a spring located on the south-west side of the valley.

The glacial streams flows over a bed of debris originated by the retreat of the glacier. It often changes its course due to periodic variation in discharge and energy while its spring-fed tributary flows in a wide variety of environments with a stable and constant flow.

2.3. Climate

To better understand the climate state of the project area we used a meteorological station (which belongs to "Meteotrentino") near the Presanella mountain massif since a specific meteorological station in Amola Valley is missing. We collected the information about historical monthly precipitation (from 01/01/1975 to 09/05/2012) (Figure 10) from the T0166 "Val di Genova O.P ENEL" (900 m asl) station records while the temperatures were obtained from the T0167 "Pradalago" station located at 2048 m asl, a comparable altitude for our two sampling stations (Figure 11).

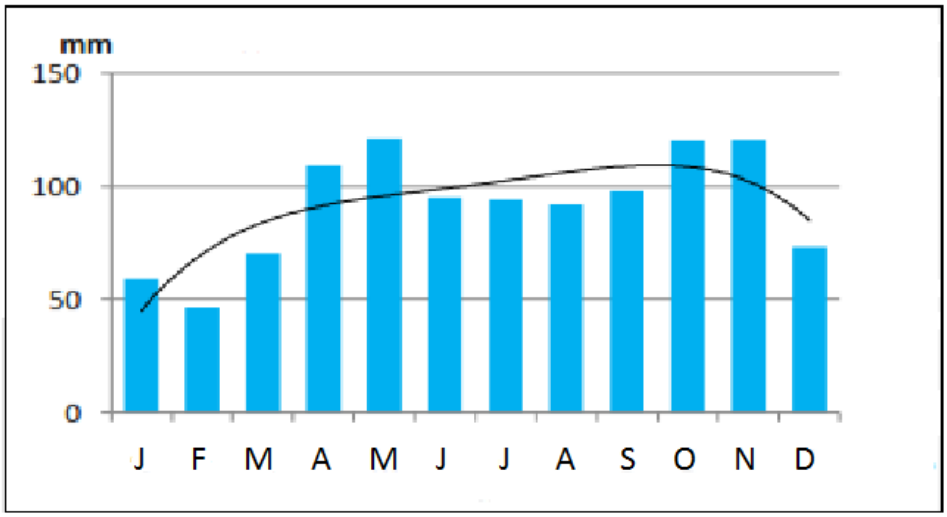

Figure 10. Precipitation (monthly average) "Val di Genova" (T0166) (from 01/01/1975 to 09/05/2012). (X axis months, Y axis millimetres of rain)

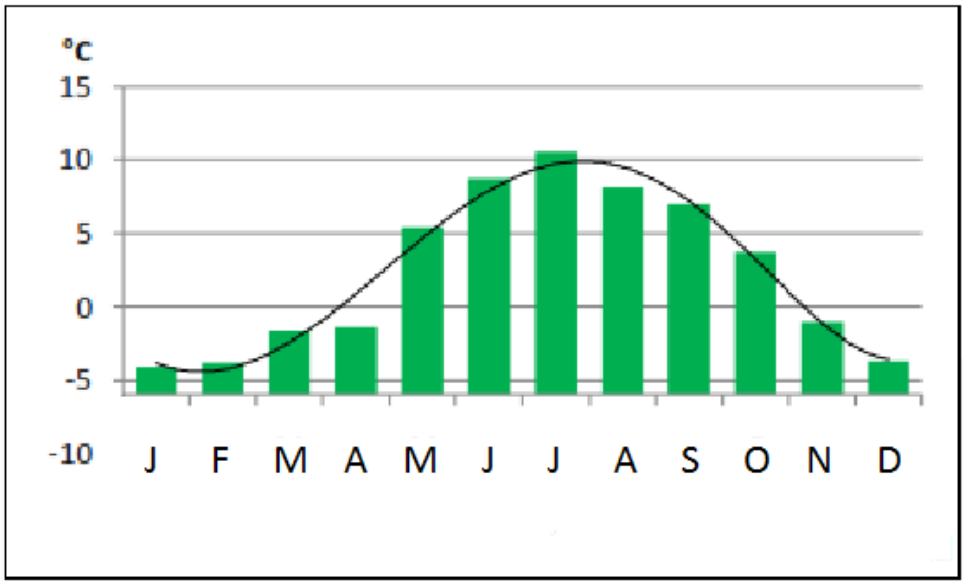

Figure 11. Air temperature (monthly average) "Pradalago"(T0167) (from 01/01/1975 to 09/05/2012). (X axis months, Y axis temperature in °C).

All records were taken from the historical archive of "Meteotrentino (*www.meteotrentino.it*)" from 1974 to 2012 for precipitations, 1991 to 2011 for the temperature.

During the sampling collection air temperatures data were recorded with a data logger located at 2544 m asl in AP-I station, an historical station of *"Museo delle Scienze di Trento (MUSE)"*, close to

the glacier front, while for precipitation, temperature, and solar radiation we used the Meteotrentino station T0175 “Pinzolo”: the nearest, still active, station to the project area.

2.4. Sampling sites

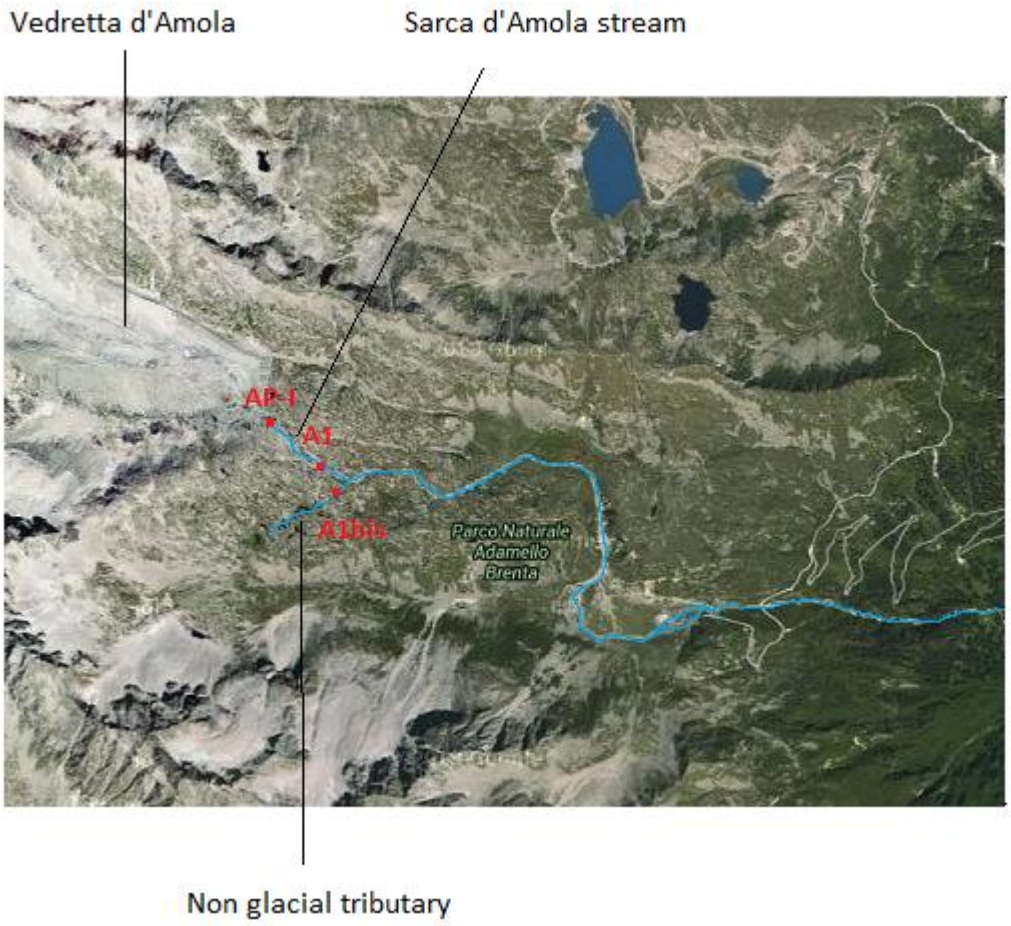

Figure 12. The Amola Valley with the position of A1, A1bis, and AP-I station (AP-I station was used only for the data logger)

This study was developed at two sites on two streams localized in the flat ground moraine in the Amola Valley. The two collection sites were chosen according to their accessibility and representativeness of the river main environment. Accessibility is crucial for a successful sampling: the sites must be easily accessible in order to ensure the possibility of traps retrieval even during night time, with low visibility (fog) and harsh weather conditions. The sites must be representative of the macroinvertebrate communities without strong fluctuations in climate and altitude

conditions. Thus, in this project two stations that meet these requirements were located in A1 and A1bis, which were barely above the merge point of the two streams (Table 1).

A1 STATION is located in the glacier-fed stream. It is 1153 m far from the glacier mouth, at an altitude of 2421 m asl (Figure 12). A1 is located at the edge of the glacier moraine of the Little Glacial Age (LIA), in a small plain, characterized by a high variable riverbed with little granulometry and high water speed and turbulence.

A1bis STATION is located on the non glacial stream. It originates from a spring and from a small glacier residual. It is located at 2414 m asl, similar to A1 station (Figure 12). This choice was made to abide at the principle of the representativeness and to ensure a direct comparison with its glacial counterpart. This station is characterized by slow and transparent waters that flow in a meandrific zone with long puddles and sand and limo sediments.

Table 1. Table with stations' features.

Station	Stream type	Coordinates	Altitude	Picture
A1	Glacier fed	N 46°12.776' E 010°42,404'	2421 m asl	
A1bis	Non glacial	N 46°12.740' E 010°42.402'	2414 m asl	

The sampling site of Amola Valley is a historical study area of the "*Invertebrate zoology and Hydrobiology"* section of *"Museo delle Scienze di Trento (MUSE)"*. Physico and chemical parameters were recorded in the 2014.

The water analysis of Sarca d'Amola stream was carried out in the 2014 by the *Laboratorio dell'Unità Operativa Chimica delle Acque della Fondazione E. Mach (Trento)* with the Standard Methods (APHA, 1992).

All parameters were determined according to the “Metodi Analitici per le acque”(I.R.S.A.-C.N.R.,1994):

- Conductibility at 20 °C;
- pH at 20°C;
- Hardness, based on calcium and magnesium ions concentration;
- Calcium ion, with atomic absorption spectroscopy with air-acetylene flame (plus 0,2% Sr);
- Magnesium ion, with atomic absorption spectroscopy with air-acetylene flame;
- Sodium ion, with atomic absorption spectroscopy;
- Potassium ion, with atomic absorption spectroscopy;
- Sulfate, with ionic chromatography and turbidimetry;
- Bicarbonate ion, for alkalinity calculation;
- Chlorides, with Mohr methods and ionic chromatography;
- Reactive silica, with ultraviolet-visible spectrophotometry on filtered water;
- Nitrogen ammonium with ultraviolet-visible spectrophotometry on filtered water after sodium silicate nitroprussidereaction (Verdouw et al., 1978)
- Nitrous nitrogen with ultraviolet-visible spectrophotometry after sulfanilamide and N-(1-naftil) ethylenediaminereaction;
- Nitric nitrogen with ultraviolet-visible spectrophotometry after sodium silicate reaction and ionic chromatography;
- Reactive soluble phosphorus, with ultraviolet-visible spectrophotometry on filtered water (ascorbic acid method)
- Total phosphorus, as reactive phosphorus.

The following chemical and physical parameter were also analyzed in the 2014:

Granulometry composition

Most of the water organisms are benthonic, in other words these organisms live on, in, or in the proximity of the riverbed. Substrate represents therefore, one of the principal aspects of the life cycle of organisms. It is extremely variable both on small scale and on large scale. The upstream river substrate is characterized by big rocks with remarkable granulometry while, downstream, when water lose its energy, the substrate is represented by clay and silts sediments. On small scale we can find a big heterogeneity of microhabitats such as riffles (relatively shallow and coarse-

bedded length of stream over which the stream flows at slower velocity and heightened turbulence) and pools (a quiet slow-moving portion of a stream).
Usually heterogeneous substrate hosts a most diverse variety of biotic communities than homogeneous substrate (Ciutti et al., 2004).

Pfankuch Index (Pfankuch, 1975)

The Pfankuch channel assessment was developed to rate the relative stability of a channel, and is based on eleven parameters. Every parameter has a score that increases with the channel stability. The value of this index is made up by the sum of every single score parameter. Pfankuch rating allows an easy determination of the overall stream bed physical conditions.

Discharge

The discharge represents the volume of fluid per unit time. The discharge variation influences the morphology of the riverbed, the physical and chemical properties and, hence, the macroinvertebrate community. In the present study, the "salt method" by Hongve (1987) was applied. The "salt method" is used to study the high turbulence streams discharge. The salt concentration should be, in the injection point, high enough to measure the increase in electrical conductivity, the NaCl ions in waters cause effectively an electric conductivity variation, proportional to the stream discharge.
The salt concentration must be low enough to avoid raising the salt concentration above any threshold associated with negative ecological influences (Moore, 2004).
Knowing the electrical conductivity variation, the water temperature and the salt mass makes possible to calculate the stream discharge.

Chlorophyll *a*

The chlorophyll *a* concentration is measured as a proxy for autotrophic biomass index since it represents the necessary pigment for the photosynthesis. Chlorophyll represents the 1- 2% dry mass of organic algae matter. Knowing the chlorophyll content allows to infer the total organic mass of the sample, which in turn gives an estimate of the environment primary production. To measure chlorophyll concentration: one rock was collected from the stream; a toothbrush was

used in order to rub 27 cm^2 of the rock surface. The toothbrush was rinsed with 10 mL distilled water in order to transfer the algae content. The operation was repeated with other two rocks.

The water with algae solution was strained through a fiberglass filter, which was subsequently folded and kept in total darkness at –18°C to avoid the chlorophyll degradation. The chlorophyll extraction was performed by submerging the filter in 17 mL of acetone 95% for 12 hours at – 18 °C, then the extract was centrifuged in order to remove the impurities. The chlorophyll *a* concentration was measured by difference, before and after the degradation, with a spectrophotometer at 665 and 750 nm (APHA, 1992).

In the table below (Table 2) are shown the main physico – chemical features of the alpine's part of Sarca d'Amola river, recorded in the 2014 season (the records refers to A1 station).

Table 2. Physico – chemical features of the alpine's part of Sarca d'Amola river (A1 station).

	16/07/2014	**11/08/2014**	**24/09/2014**
Discharge (l/s)	0.48	0.57	0.31
Pfankuch index	35	35	35
Chlorophyll *a* (g/l)	0.0981	N.D	0.4008
Suspended solids (ml/l)	124.0	198.4	36.0
Conductivity µS/cm	12	13	20
Alkalinity mg CaCO3/l	6.2	6.4	15.9
Hardness °F	0.4	0.7	0.9
N-NO3 µg/l	197	77	173
N-NH3 µg/l	5	11	15
Ntot µg/l	268	1663	1152
P-PO4 µg/l	4	4	5
Ptot µg/l	94	112	59
SiO2 mg/l	1.3	0.9	1.5
HCO3 mg/l	7.5	7.8	19.4
SO4 mg/l	0.7	0.2	0.3
Cl mg/l	0.4	0.2	0.2
F µg/l	157	107	95
Ca mg/l	1.4	2.0	3.4
Mg mg/l	<0.05	<0.05	<0.05
Na mg/l	1.1	1.6	1.9
K mg/l	0.4	0.7	0.9

2.5. Sampling schedule

For this study samples were collected biweekly and hourly during the summer season in 2015 in the Sarca d'Amola stream. The sampling schedule was:

- Biweekly sampling from 07/07/2015 to 09/26/2015
- Hourly sampling occurred twice and lasted 4 days each time: 07/23/2015 to 07/26/2015, and 09/10/2015 to 09/13/2015. The samples were collected every 3 hours from 6 am to 9 pm for a total of 6 samples per day, 18 for each campaign.

During biweekly collection, both biological and physical sampling were carried out.
The physico–chemical variables consisted in verifying pH, water temperature, electrical conductivity and water hardness.

The biological sampling consisted in emptying the malaise and emergence traps, making a kick semi-quantitative sampling and recovering the drift sample.
In the data processing, the biweekly samplings were standardized to 15 days to permit a correct data correlation.
During the hourly collection, only biological sampling was performed with the drift net, malaise and emergence traps.
For this study, water temperature was measured every one hour by two data loggers placed in A1 and A1bis station.

2.6. The physico – chemical parameters

Water temperature, conductivity and pH were recorded on the field with a portable pH/EC/TDS/Temperature meter instrument (models HI991301 and HI1288, Hanna instruments) and two data logger. Additional chemical parameters, such as air and water temperature and suspended solids concentration, were analysed for each station.

Suspended solids

Suspended solids are particles smaller than 0.2 mm, so called because they are suspended by the water turbulence. Often, they are silt and clay particles. The suspended solid concentration exerts a relevant influence on the river ecology, such as transparency and thermal output. Water samples were filtrated on a fiberglass filter and their dry mass weight was measured.

pH

pH measures the concentration of hydrogen ions, and indicates the acidity or alkalinity of a solution. Pure water has a pH of 7; a pH value greater than 7 is defined as alkaline, while if it is less than 7 the solution is considered acidic. In non-contaminated waters, pH is strictly dependent on carbon dioxide, bicarbonate and carbonate equilibrium. Usually, high pH values are linked to slow waters flow with advanced autotrophic communities. Generally, the pH of stream waters ranges between 6,5 – 8,5 and depends on the chemical composition of rainfall. pH also influences the decomposition of organic matter.

Total dissolved solids (TDS)

Total dissolved solids are the total amount of all inorganic and organic contained in a given volume of water. Generally, the operational definition is that the solids must be small enough to survive filtration through a filter with two-micrometer pores. TDS is directly related to the purity of water and the quality of water purification systems.

Electrical conductivity

The electrical conductivity measures a material's ability to conduct an electric current while in waters estimates the total amount of dissolved salts.

The electrical conductivity depends by the total ionized salts and by water temperature: the lower the temperature the lower the electrical conductivity would be. In low altitude streams the electrical conductivity will be higher than high altitude due to the high temperature and the mineralization process that enriches the water. Streams at low altitude, sometimes, could have unexpected reduction of conductivity due to introduction of rainy or glacial waters.

Organic matter

Organic matter in freshwater can be classified in multiple ways, according to different *criteria*. The origin-based classification distinguishes between *exogenous organic matter*, if it derives from the hydrographic basin and it is funnelled in the water flow by gravity, or *endogenous organic matter*, if it is produced directly in the water by algae, water organisms, aquatic plants, vascular and non-vascular plants. The source-based classification defines organic matter as natural or anthropic, according to its origin.

Organic matter can also be classified by its granulometry:

- DOM: dissolved organic matter, its granulometry is < 0,45 µm. The DOM is mainly composed by carbohydrate, amino acid, bacteria, virus colloidal suspension and humic acid. (Volk et al., 1997)
- FPOM: fine particulate organic matter, 0,45 µm – 1,0 mm
- CPOM: coarse particulate organic matter, < 1,00 mm. Mainly composed by leafs and exogenous plants matter.

COD (Chemical Oxygen Demand) and BOD (Biochemical Oxygen Demand) tests are commonly used to indirectly measure the amount of organic matter in water.

Both tests measure the oxygen demand needed to completely oxidize the organic matter in a specific sample.

Air and water temperature

Temperature is one of the major factors influencing freshwater ecosystem. Water temperature mainly depends on the solar radiation and secondarily on the heat soil conduction. This feature enhances the dynamism at the alpine lotic environment where the solar radiation is very marked.

Water temperature exercises direct and indirect effects on biotic compartment. Direct effects occur on the biological life cycle of aquatic invertebrates (Nunn et al., 2003) since the whole community is poikilotherm (their internal temperature varies considerably, and is strictly dependent on the environmental temperature). Indirect effects of temperature on biotic environment involve the amount of dissolved oxygen and the water viscosity, which bring about changes in flow speed and stream capacity.

During the study, water and air temperature were recorded with 3 data logger arranged respectively one in A1 stream station, one in A1bis stream station, and the last one near the glacial front, on the ground in AP-I station (2544 m asl).

2.7. The biological sampling

2.7.1. Kick and drift sampling

The macrobenthic aquatic fauna was collected by two methods: the kick and drift sampling.

The kick sampling method consisted in the use of a hand net (square structure measuring 30 cm per side and 250 µm mesh size, Figure 13) for semi-quantitative sampling. Each sample was taken from 5 different microhabitats for a total of 10 min (2 min per microhabitats) in order to have a good stream representativeness. The collected samples were filtered with specific sorting bucket to remove excess of water and, after that, preserved in ethanol at 75% concentration.

The kick-sampling was applied only during the biweekly sampling.

Figure 13. The hand net (source D. Frizzera).

The drift samples were collected with a specific drift net (Figure 14), formed by a round rigid plastic structure 10 cm diameter with a 1 m net and 100 µm mesh size placed against current.

This kind of sampling allows to collect the macrobenthic organisms drifted by the stream flows.

During the biweekly sampling, the drift net was exposed at the stream flow for 1 hour in each station, and for 30 minutes for the hourly collection.

Figure 14. The drift net (source D. Fizzera).

The collected samples have been taken to laboratory at ambient temperature.

We used a Leica MZ7.5 50 stereo microscope (50x) to sort the samples.

During sorting, the organic matter larger than 1 mm (CPOM) was extracted from the samples and its mass weighted after heating at 60°C for 1 hour. After this process the organic matter was dried in the muffle furnace (500 °C) and the dry mass weighted by difference.

Samples with high organic mass were sub-sampled sorting ¼ of the total amount available.

The aquatic invertebrate community was identified using the following keys: Campaioli et al. (Vol.1, 1994), Campaioli et al. (Vol.2, 1994), Chinery (1987) and Sansoni (1988).

All insects were classified according to order, family, and genus. All individuals were preserved in 75% ethanol and stored at the *Zoologia degli Invertebrati e Idrobiologia* section of the *Museo delle Scienze di Trento.*

2.7.2. Malaise trap

The malaise trap (Figure 16) is a tend structure used for trapping flying insects, and made of a netting material. The insects fly into the tent wall and, thanks to their tendency to move upward, are funnelled into a collecting vessel attached to the highest point. The malaise trap is made by a central vertical wall, from whose extremity two flaps branch off forming a cover. From the shortest vertical side two additional shorts clothes branch off creating a wall. The trap contains a killing solution (usually polyethylene glycol) where the insects fall and are preserved.

The way in which the trap is placed is very important in order to maximize the number of flying insects.

For this study, one malaise trap was set up on the final part of A1 plain (Figure 15), perpendicularly

Figure 15. The A1 station. In blue the location of malaise trap, is marked in red the position of emergence traps (source D. Frizzera).

to the main stream axis. This was made to intercept the highest number of insects moving upstream from A1 and A1bis station.

The malaise trap samples collection was done both during the biweekly and hourly sampling.
For the entire duration of biweekly sampling, polyethylene glycol was used as killing agent while ethanol 95% was used during the hourly sampling. This was made in order to solve the evaporation problem of the ethanol and to give the future opportunity of analysing insects' genetic variability by preserving DNA.

Figure 16. Malaise trap in A1 station (source D. Frizzera).

2.7.3. Emergence trap

Emergence traps are cage-like structures used to capture aquatic insects during their transition from late aquatic instar (nymph, subimage, pupe) to terrestrial adults. The trap consists of a floorless, pyramidal, closed tent (rectangular base 42 cm base length, 100 µm mesh size) with a jar containing a killing agent arranged on the highest point (Figure 20). The emergence trap usually is used to estimate the insect population density.

For this work six emergence traps were located in six different points, three in the A1 station and three in A1bis station (Figures 17, 18, 19).

The emergence trap collection has been done both during the biweekly and hourly sampling.

During biweekly sampling, polyethylene glycol was used as killing agent while ethanol at 95% concentration was used during the hourly sampling.

For the sample sorting we used a Leica MZ7.5 50 stereo microscope (50x).

The whole community of insects was classified according to order, family, genus and species, and conserved in 95% ethanol.

All specimens are stored in the "*Zoologia degli Invertebrati e Idrobiologia*" section of the *Museo delle Scienze di Trento.*

The aquatic adult invertebrates were identified using the following keys: Chinery (1987), Pinder (1978), and Gobbi and Latella (2011).

Figure 17. The emergence traps in A1 station (source D. Frizzera).

Figure 18. The emergence A position in A1bis station (source D. Frizzera).

Figure 19. The emergence B and C in A1bis station (source D. Frizzera).

Figure 20. Emergence trap in A1bis station (source D. Frizzera).

2.8. Statistical analyses

Data were managed and analysed with Microsoft Excel. Three diversity indices were calculated: Shannon (H), Evenness (J), and Dominance (D) at replicate and station level.

Shannon diversity index takes into account the number of individuals as well as number of taxa. It varies from 0 for communities with only a single taxon to 1 for communities with many taxa (Shannon and Weaver, 1948).

It is calculated as:

$$H = -\sum_i \frac{n_i}{n} \ln \frac{n_i}{n}$$

Where n_i is the number of organisms of *i* species and *n* is the total number of organisms .

Evenness is defined as a measure of biodiversity which quantifies how equal the community is numerically (Mulder et al., 2004). Evenness measure is given by:

e^H/S

where H is the Shannon Weaver index and S is the species number.

Dominance is the relative importance of a species related to degree of influence it has on ecosystem components (McNaughton, 1970).

It ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely). It is calculated as:

$$D = \sum_i \left(\frac{n_i}{n} \right)^2$$

where n_i is number of individuals of taxon i.

Differences in time and space were tested by Mann-Whitney U test and Analysis of variance (ANOVA Kruskal Wallis test). The Mann–Whitney *U* test is a non-parametric test of the null hypothesis that two samples come from the same population against an alternative hypothesis, such as that a particular population tends to have larger values than the other. The Kruskal-Wallis test is a non-parametric ANOVA, comparing the medians of several univariate groups (given in columns). It does not assume normal distribution, but does assume equal-shaped distributions for all groups.

Correlations between biological and environmental variables were also calculated.

Results were considered significant for a p-value < 0.05.

All statistical analyses were performed by using the software PAST 3. Past is a free software (http://folk.uio.no/ohammer/past) for scientific data analysis, with functions for data manipulation, plotting, univariate and multivariate statistics, ecological analysis, time series and spatial analysis, morphometrics and stratigraphy.

3. Results

3.1. The physico – chemical parameters

3.1.1 Weather conditions

Data of precipitation, air temperature, and solar radiation during the sampling collection were recorded by the meteorological station T0175 “Pinzolo” of Meteotrentino. Table 3 shows the records during the first and the second hourly sampling.

Table 3. Air Temperature, precipitation and solar radiation recorder by “Pinzolo” (T0175) meteo-station.

Sampling	Date	Air Temperature (°C)	Precipitation (mm)	Solar radiation (Kj/m^2)
First hourly sampling (July)	07/23/2015	10.37°C	0.0	20214.6
	07/24/2015	9.7°C	19.8	14334.3
	07/25/2015	8.80°C	4.6	14525.8
	07/26/2015	8.15°C	0.0	18075.7
Second hourly sampling (September)	09/10/2015	1.78°C	3.2	4060.1
	09/11/2015	1.20°C	38.0	9381.1
	09/12/2015	2.70°C	9.6	841.1
	09/13/2015	4.06°C	10.4	4354.7

3.1.2 Air and water temperature

During the study, water and air temperature were recorded with 3 data logger arranged respectively one in A1 stream station, one in A1bis stream station, and the last one near the glacial front, in AP-I station.

These data loggers recorded every hour the temperature from 07/23/2015 to 09/26/2015.

The table 4 shows minimum, maximum, and mean water temperature for each data logger in A1 and A1bis station during the whole sampling period while table 5 shows the same parameter set per each hourly sampling.

Table 4. Minimum, maximum, and average temperature of the whole sampling period.

Station	Average	Min	Max
A1	1,805 °C	-0,080 °C	5,477 °C
A1bis	3,490 °C	0,275 °C	8,714 °C
AP-I (air)	6,46 °C	-1,8 °C	15,4 °C

Table 5. Minimum, maximum and average of water temperature in A1 and A1bis station during hourly sampling.

Station	Sampling	Mean ± DV	Min	Max
A1	First sampling (07/23-07/26)	2.05 ± 0.87	1.05°C	4.94°C
	Second sampling (09/10-09/13)	1.51 ± 0.81	0.01°C	4.64°C
A1bis	First sampling (07/23-07/26)	4.26 ± 1.13	2.7°C	6.59 °C
	Second sampling (09/10-09/13)	2.17 ± 0.59	1.18°C	3.67°C
AP-I (air)	First sampling (07/23-07/26)	9.23 ± 1.63	6.5°C	12.80°C
	Second sampling (09/10-09/13)	2.40 ± 1.6	0.2°C	4.80°C

It is important to note that the temperature recorded by the AP-I data logger refers to Earth surface, so the temperature in AP-I may result higher than “Pinzolo”(T0175) Meteotrentino station.

To better understand the trend of water temperature in the figure 21 we compared the A1 and A1bis water from 07/23/2015 to 07/26/2015.

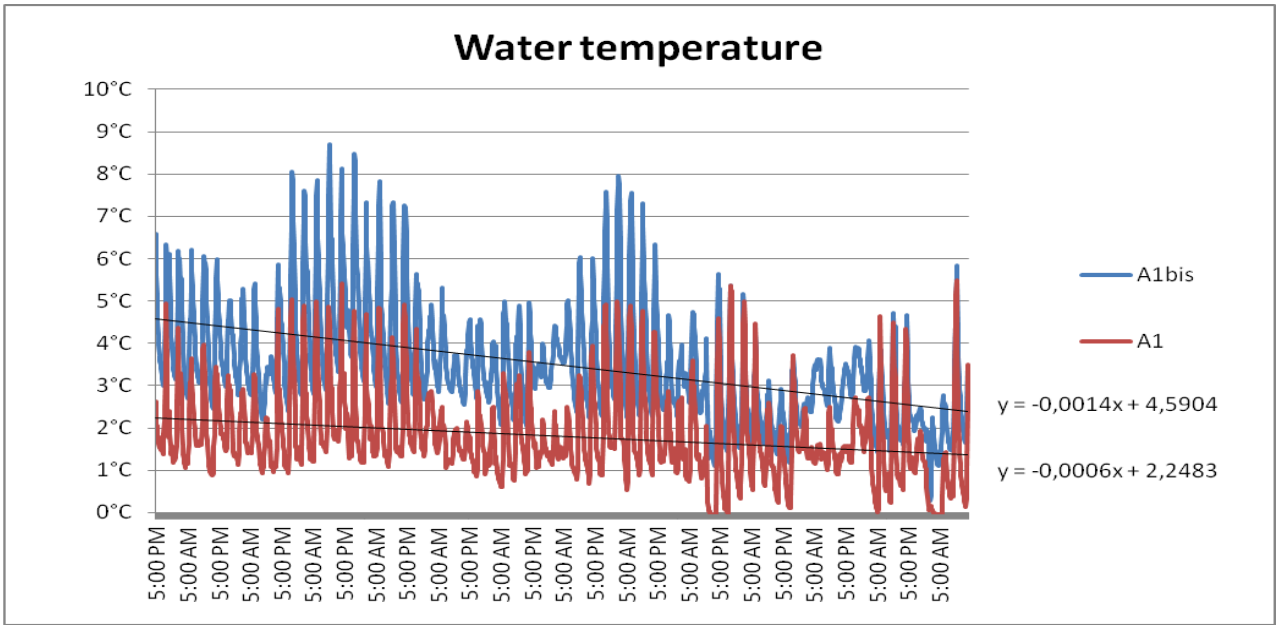

Figure 21. Water temperature trends of A1 and A1bis station from 07/23 to 09/26 taken at 5:00 AM.

3.1.3 TDS, pH, and conductivity

Conductivity, TDS and pH were recorded on the field with a portable pH/EC/TDS/Temperature meter instrument (models HI991301 and HI1288, Hanna instruments).

In the table 6 the results per date and stream are shown.

Table 6. pH, TDS, and conductivity of A1 and A1bis station during sampling period.

Date	Station	pH	Conductivity (µS/cm)	Total Dissolved Solids (ppm)
07/07/2015	A1	7,43	5	4
07/07/2015	A1bis	7,01	4	3
07/23/2015	A1	7,13	6	3
07/23/2015	A1bis	7,06	4	2
08/06/2015	A1	7,09	3	1
08/06/2015	A1bis	7,1	9	5
08/26/2015	A1	6,97	6	3
08/26/2015	A1bis	6,97	12	6
09/10/2015	A1	7,4	14	7
09/10/2015	A1bis	7,43	13	6
09/26/2015	A1	6,96	15	7
09/26/2015	A1bis	6,96	13	6

3.1.4 Coarse particulate organic matter (CPOM)

In the table 7 the records of the organic matter for each sampling technique (kick and drift) are shown.

Table 7. CPOM of the samples.

Station	Sampling	Date	Hour	CPOM (mg)	Station	Sampling	Date	Hour	CPOM (mg)
A1	drift	06/12/2015	9	43,3	A1	drift	09/11/2015	12	58,6
A1bis	drift	06/12/2015	9	47,1	A1bis	drift	09/11/2015	12	61,8
A1	drift	07/23/2015	12	57,9	A1	drift	09/11/2015	15	38,3
A1bis	drift	07/23/2015	12	50,9	A1bis	drift	09/11/2015	15	49,1
A1	drift	07/23/2015	18	44,7	A1	drift	09/11/2015	18	61
A1bis	drift	07/23/2015	18	58,5	A1bis	drift	09/11/2015	18	45,9
A1	drift	07/23/2015	21	48,9	A1	drift	09/11/2015	21	45,8
A1bis	drift	07/23/2015	21	51,6	A1bis	drift	09/11/2015	21	48
A1	drift	07/24/2015	6	39,8	A1	drift	09/12/2015	21	40,1
A1bis	drift	07/24/2015	6	42	A1bis	drift	09/12/2015	21	44,4
A1	drift	07/24/2015	9	53,5	A1	drift	09/12/2015	6	40,4
A1bis	drift	07/24/2015	9	52,7	A1bis	drift	09/12/2015	6	51,4
A1	drift	07/24/2015	12	70,2	A1	drift	09/13/2015	9	38,8
A1bis	drift	07/24/2015	12	55,3	A1bis	drift	09/13/2015	9	32,5
A1	drift	07/24/2015	18	43,7	A1	drift	09/12/2015	12	37,7
A1bis	drift	07/24/2015	18	45,4	A1bis	drift	09/12/2015	12	47,9
A1	drift	07/24/2015	21	56,1	A1	drift	09/12/2015	15	44,9
A1bis	drift	07/24/2015	21	57,8	A1bis	drift	09/12/2015	15	50,5
A1	drift	07/25/2015	6	40,8	A1	drift	09/12/2015	18	48,3
A1bis	drift	07/25/2015	6	42,3	A1bis	drift	09/12/2015	18	41,8
A1	drift	07/25/2015	9	48,4	A1	drift	13/09/2015	6	57,9
A1bis	drift	07/25/2015	9	56,8	A1bis	drift	09/13/2015	6	38
A1	drift	07/25/2015	12	51,3	A1	drift	09/13/2015	12	40
A1bis	drift	07/25/2015	12	53,8	A1bis	drift	09/13/2015	12	42,9
A1	drift	07/25/2015	15	59,4	A1	kick	07/07/2015	14	84,5

Table 7. CPOM of the samples.

Station	Sampling	Date	Hour	CPOM (mg)	Station	Sampling	Date	Hour	CPOM (mg)
A1bis	drift	07/25/2015	15	63,9	A1bis	kick	07/07/2015	15	438,2
A1	drift	07/25/2015	18	54,7	A1	kick	07/23/2015	14	72,9
A1bis	drift	07/25/2015	18	54,7	A1bis	kick	07/23/2015	15	160,6
A1	drift	07/25/2015	21	55,2	A1	kick	08/06/2015	14	79,2
A1bis	drift	07/25/2015	21	55	A1bis	kick	08/06/2015	15	482,5
A1	drift	07/26/2015	6	53,4	A1	drift	08/06/2015	14	45,8
A1bis	drift	07/26/2015	6	51,3	A1bis	drift	08/06/2015	15	65,6
A1	drift	07/26/2015	9	52,5	A1	drift	08/26/2015	15	77,8
A1bis	drift	07/26/2015	9	46,4	A1bis	drift	08/26/2015	16	42,9
A1	drift	07/26/2015	12	46,3	A1	kick	08/26/2015	15	79,1
A1bis	drift	07/26/2015	12	46	A1bis	kick	08/26/2015	16	598,7
A1	drift	07/26/2015	15	58,6	A1	kick	09/10/2015	12	42,2
A1bis	drift	07/26/2015	15	59,1	A1bis	kick	09/10/2015	13	773,1
A1	drift	09/10/2015	18	59,8	A1	drift	09/10/2015	12	33,7
A1bis	drift	09/10/2015	18	56,9	A1bis	drift	09/10/2015	13	41,2
A1	drift	09/10/2015	21	51,8	A1	kick	09/26/2015	13	62,9
A1bis	drift	09/10/2015	21	46,4	A1bis	kick	09/26/2015	14	442,4
A1	drift	09/11/2015	9	47,3	A1	drift	09/26/2015	13	44,2
A1bis	drift	09/11/2015	9	45,7	A1bis	drift	09/26/2015	14	49,5

3.2. Biological results

3.2.1 Aquatic invertebrates: total community

A total of 28170 aquatic invertebrates were collected in 171 samples (51 from biweekly sampling and 120 from hourly sampling). The majority of invertebrates, the 98.11% of the total, belonged to the order of Diptera. The family of Chironomidae dominated the Diptera order with a percentage of 89.88% (Table 8). The remaining individuals belonged to the orders of Plecoptera (0.65%), Ephemeroptera (0.55%), and Trichoptera (0.61%) (Figure 22).

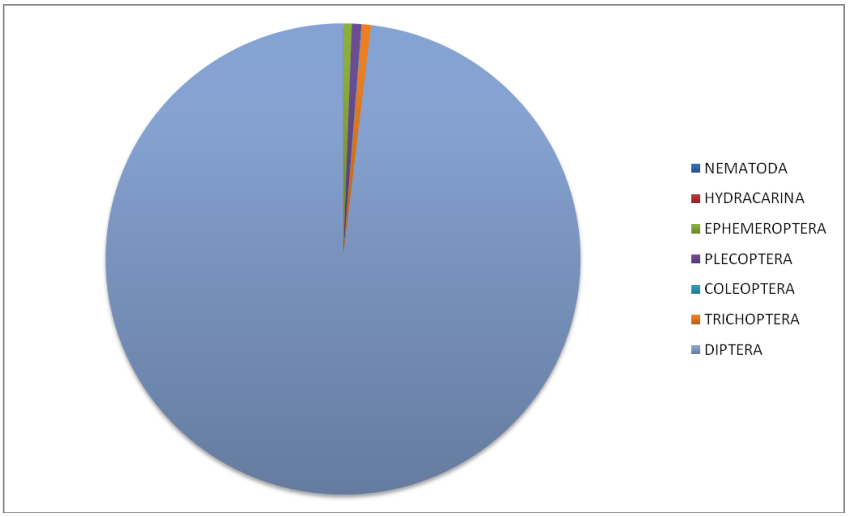

Figure 22. Total aquatic insect collected

Kick samples

In the 12 kick samples, 3830 specimens were collected from glacier-fed (A1 station) and 17584 from non glacial stream (A1bis station). The majority of insects in both streams belonged to the dipteran family of Chironomidae (abundance ≥ 50%). In A1 station, aquatic invertebrates, with a relative abundance included between 0.1 and 0.9%, belonged to the order Hydracarina and the families Limoniidae+Pediciidae, Psychodidae, Simuliidae, and Empididae.

In A1bis station, Simuliidae are the second most abundant taxon for aquatic invertebrates (1.0 – 9.9%). The biodiversity of aquatic insects in the non glacial stream was high and it included Ephemeroptera, Plecoptera, Coleoptera, and Trichoptera (relative abundance for each taxa between 0.1 and 0.9%).

Collembola (terrestrial insects) represented the second common and most abundant taxon in both streams (Table 8).

Drift samples

The 38 drift samples provided 5314 insects. Chironomidae was the most represented family (abundance ≥ 50%). In the glacier-fed, the aquatic biodiversity of A1 station included only Hydracarina and Nematoda (with Diptera), while in A1bis Diptera, Ephemeroptera, Plecoptera, Coleoptera, and Trichoptera (relative abundance for each taxa included between 0.1 and 0.9%) were found to be the most represented taxa (Table 8).

Emergence traps

A total of 45 samples from 6 emergence traps provided 392 insects, divided into 7 taxa from the glacier-fed stream and 8 taxa from the spring-fed stream. The majority of emerging insects belonged to the dipteran family of Chironomidae (≥ 50%) in A1 and to the family of Empididae (abundance range 10 – 49.9%) in A1bis station. Secondary groups for abundance were Plecoptera, Coleoptera and the families of Limoniidae, Tipulidae, Simulidae, and Empididae. Ephemeroptera were found only in A1bis. Other taxa were found at a relative lower abundance ≤ 1%.

Malaise traps

A total of 4376 insects was captured by means of malaise sampling technique. Empididae represented the most abundant family with a percentage close to 50 %, while, the second most represented taxon was Chironomidae (nearly 30%). Other frequent groups were: Limonidae+Pediciidae and Coleoptera. Other taxa were present with relative smaller abundances (≤ 1%, Table 8).

3.2.2. Terrestrial insects

In this study, a significant percentage of 4.1% of terrestrial insects were captured (Table 9).

The majority terrestrial insects belonged to the orders of Collembola (51.79%) and Hymenoptera (28.52%).

The terrestrial insects, instead of aquatic insects, develop their life cycle on the ground.

Table 8. Aquatic taxa found in two stations by Kick and Drift sampling, Emergence and Malaise Traps (Ad adults, juv juveniles). In the cells: number of specimens collected, colour of the cells according to the relative abundance of each taxon/station/sampling technique:

□ 0,1-0,9%, □ 1,0-9,9%, □ 10,0-49,9%, □ ≥ 50%.

	Kick Sampling		Drift Sampling		Emergence		Malaise
	A1	A1bis	A1	A1bis	A1	A1bis	A1
NEMATODA		2	2				
HYDRACARINA	2	1	1	1			
EPHEMEROPTERA							
Ephemroptera Ad.						2	
Ephemeroptera juv.				3			
Baetis		139		6			
Rhithrogena		2					
PLECOPTERA							
Plecoptera Ad.					10	8	30
Plecoptera juv.		33		5			
Protonemura		77					
Perlolidae juv.		18					
Dictyogenus		9					
Perlodes		4					
COLEOPTERA							
Coleoptera juv.		4	4	5			
Dytiscidae				1			
TRICHOPTERA							
Trichoptera juv		1		2			
Limnephilidae		166		2			
DIPTERA							
Nematocera							
Limoniidae+Pediciidae	23	24	22	5	17	27	92
Tipulidae		8			6	1	74
Psychodidae	1		1	1			60
Chironomidae	3795	16750	4253	598	181	29	1212
Ceratopogonidae		4					
Simuliidae	3	272	6	2	1	6	7
Thaumaleidae							36
Brachycera							
Empididae	6	72	5	2	50	48	1976

Table 9. Terrestrial taxa found in two stations by Kick and Drift sampling, Emergence and Malaise Traps (Ad adults, juv juveniles). In the cells: number of specimens collected, colour of the cells according to the relative abundance of each taxon/station/sampling technique:

□ 0,1-0,9%, □ 1,0-9,9%, □ 10,0-49,9%, □ ≥ 50%.

	Kick Sampling		Drift Sampling		Emergence		Malaise
	A1	A1bis	A1	A1bis	A1	A1bis	A1
COLLEMBOLA	36	571	204	97			1
ORTHROPTERA							1
PSOCOPTERA	1		1	5			2
HEMIPTERA	2	12	5	24			27
THYSANOPTERA	5	6	7	3			5
NEUROPTERA							20
COLEOPTERA					14	2	68
LEPIDOPTERA							20
DIPTERA							
Trichoceridae			2				13
Mycetophilidae							61
Sciaridae		4	8	3			217
Cecidomyiidae							1
Scatopsidae							1
Brachycera			5	8			
HYMENOPTERA	3	16	4	27			474

3.2.3. Biweekly sampling

Kick

The Tables 10 and 11 report the macroinvertebrate community composition in A1 and A1bis in the six sampling weeks in 2015.

A1

Table 10. Invertebrates found in A1 station. The codes refer univocally by each sampling: station_technique_date.

	A1_kick_7.7	A1_kick_7.23	A1_kick_8.6	A1_kick_8.26	A1_kick_9.10	A1_kick_9.26
Hydracarina	2					
Limoniidae+Pediciidae		1	1	8	9	4
Psychodidae				1		
Chironomidae	625	542	514	540	1158	408
Simuliidae			1	2		
Empididae					2	4

A1bis

Table 11. Invertebrates found in A1bis station. The codes refer univocally by each sampling: station_technique_date (juv juveniles).

	A1bis_kick_7.7	A1bis_kick_7.23	A1bis_kick_8.6	A1bis_kick_8.26	A1bis_kick_9.10	A1bis_kick_9.26
Nematoda		2				
Hydracarina		1				
Baetis	13	34	56	28	8	
Rhithrogena		2				
Plecoptera juv	21					
Protonemura	16	37	12	12		
Perlolidae juv	4	6		4	4	
Dictyogenus		5			4	
Perlodes				4		
Coleoptera juv					4	
Trichoptera juv	1					

Table 11. Invertebrates found in A1bis station. The codes refer univocally by each sampling: station_technique_date.

	A1bis_kick_7.7	A1bis_kick_7.23	A1bis_kick_8.6	A1bis_kick_8.26	A1bis_kick_9.10	A1bis_kick_9.26
Limnephilidae	33	49	40	40	4	
Limoniidae+Pediciidae			8	4	8	4
Tipulidae			8			
Chironomidae	4732	4272	2512	2032	1164	148
Ceratopogonidae			4			
Simuliidae	53	175	28	8	8	
Empididae	8	24	4	8	28	

Chironomidae was the most abundant family both in A1 and in A1bis stations. This family almost represented the totality of sampled insect in the glacial and in the non glacial stream with a percentage of 99% and 97% respectively. The table above shows a very low biodiversity in the A1 station while, in the A1bis station, besides Chironomidae, we found Trichoptera, Plecoptera, Ephemeroptera, Nematoda, and Hydracarina.

The ANOVA test, used to evaluate differences among sampling dates for each station, highlighted that in both A1 and A1bis station there are no significant differences among dates in insects composition ($p > 0.05$).

In the last sampling of the season (09/26/2015) there is a reduction in number of sampled insects (in A1 and A1 station) and a reduction in sampled taxa (only in A1bis) but, according with ANOVA, this change is not significant ($p > 0.05$).

The Mann - Whitney *U* test was used to analyze the spatial differences between the A1 and A1bis station.

Table 12 reports the arithmetic averages of seasonal kick sampling of A1 and A1bis station.

Table 12. Average of A1 and A1bis station used for *U* test (juv juveniles).

	A1 AVERAGE	A1bis AVERAGE
Nematoda		0.33
Hydracarina	0.33	0.17
Baetis		23.17
Rhithrogena		0.33
Plecoptera juv		3.50

Table 12. Average of A1 and A1bis station used for U test (juv juveniles).

	A1 AVERAGE	A1bis AVERAGE
Protonemura		12.83
Perlolidae juv		3.00
Dictyogenus		1.50
Perlodes		0.67
Coleoptera juv		0.67
Trichoptera juv		0.17
Limnephilidae		27.67
Limoniidae+Pediciidae	3.83	4.00
Tipulidae		1.33
Psychodidae	0.17	
Chironomidae	631.17	2476.67
Ceratopogonidae		0.67
Simuliidae	0.50	45.33
Empididae	1.00	12.00

The table shows a clear difference between the A1 and A1bis station which is confirmed by the significant U test (p value < 0.05).

Shannon (H), Evenness (J), and Dominance (D) indexes were calculated to understand the biodiversity of the single sample in each station.

Both in A1 and A1bis station (Tables 12 and 13) one taxon (Chironomidae family in Tables 10 and 11) dominates the community completely (Dominance close to 1).

Evenness index quantifies that the two stations are not numerically equal. Shannon index shows a community with a very low biodiversity in A1 station (H = 0.06) while, in the non glacial stream the value is higher (H = 0.27).

A1

Table 13. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1 station.

	A1_kick_7.7	A1_kick_7.23	A1_kick_8.6	A1_kick_8.26	A1_kick_9.10	A1_kick_9.26	AVERAGE	STD DV (±)
Taxa_S	2	2	3	4	3	3		
Individuals	627	543	516	551	1169	416		
Dominance_D	0.99	0.99	0.99	0.96	0.98	0.96	0.98	0.02
Shannon_H	0.02	0.01	0.03	0.11	0.06	0.11	0.06	0.04
Evenness_e^H/S	0.51	0.51	0.34	0.27	0.35	0.37	0.39	0.09

A1bis

Table 14. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1bis station.

	A1bis_kick_7.7	A1bis_kick_7.23	A1bis_kick_8.6	A1bis_kick_8.26	A1bis_kick_9.10	A1bis_kick_9.26	AVERAGE	STD DV (±)
Taxa_S	9	11	9	9	9	2		
Individuals	4881	4607	2672	2140	1232	152		
Dominance_D	0.94	0.86	0.88	0.90	0.89	0.95	0.91	0.03
Shannon_H	0.19	0.37	0.33	0.28	0.31	0.12	0.27	0.09
Evenness_e^H/S	0.13	0.13	0.15	0.14	0.15	0.56	0.21	0.17

Drift samples

The Tables 15 and 16 report the macroinvertebrate drift community composition at A1 and A1bis in the six sampling weeks in 2015.

A1

Table 15. Invertebrates found in A1 station. The codes refer univocally by each sampling: station_technique_date (juv juveniles)..

	A1_drift_7.23	A1_drift_8.6	A1_drift_8.26	A1_drift_9.10	A1_drift_9.26
Nematoda			1		
Coleoptera juv			1		
Limoniidae+Pediciidae			3		
Chironomidae	4	25	105	246	200
Simuliidae				1	
Empididae			1		

A1bis

Table 16. Invertebrates found in A1bis station. The codes refer univocally by each sampling: station_technique_date (juv juveniles).

	A1bis_drift_7.23	A1bis_drift_8.6	A1bis_drift_8.26	A1bis_drift_9.10	A1bis_drift_9.26
Ephemeroptera juv			1		
Baetis	3				
Coleoptera juv	2	1			
Limoniidae+Pediciidae		1			

Table 16. Invertebrates found in A1bis station. The codes refer univocally by each sampling: station_technique_date (juv juveniles)..

	A1bis_drift_7.23	**A1bis_drift_8.6**	**A1bis_drift_8.26**	**A1bis_drift_9.10**	**A1bis_drift_9.26**
Psychodidae					1
Chironomidae	19	25	19	35	47

During the seasonal collection, the totality of insects in A1 station belonged to the taxon of Diptera (exception for 1 Nematode and 1 juvenile of Coleoptera) while in the A1bis station the 97% were Diptera, 2% Ephemeroptera and 1% Coleoptera (Tables 14 and 15).

The ANOVA test, used to evaluate differences among sampling dates for each station, highlighted that in both A1 and A1bis station there are no differences in insects composition ($p > 0.05$).

Table 17 reports the arithmetic averages of seasonal drift sampling of A1 and A1bis station used to calculate the Mann – Whitney *U* test.

Table 17. Average of A1 and A1bis station used for *U* test (juv juveniles).

	A1 AVERAGE	**A1bis AVERAGE**
Nematoda	0.20	
Ephemeroptera juv		0.20
Baetis		0.60
Coleoptera juv	0.20	0.60
Limoniidae+Pediciidae	0.60	0.20
Psychodidae		0.20
Chironomidae	116.00	29.00
Simuliidae	0.20	
Empididae	0.20	

With a p value > 0.05, the Mann – Whitney test confirmed the null hypothesis: the two populations did not show any statistical difference.

Shannon (H), Evenness (J), and Dominance (D) indexes were calculated to understand the biodiversity of the single sample in each station.

As reported in Tables 18 and 19, both in A1 and A1bis station one taxon (Chironomidae) dominates the community completely (Dominance close to 1).

Evenness index, with a value near 1, quantifies that the two stations are not equally numerically allocated.

Shannon index shows a community with low biodiversity in A1 station (H = 0.06) while, in the non glacial stream the value is slightly higher (H = 0.25).

A1

Table 18. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1 station.

	A1_drift_7.23	A1_drift_8.6	A1_drift_8.26	A1_drift_9.10	A1_drift_9.26	AVERAGE	STD DV (±)
Taxa_S	1	1	5	2	1		
Individuals	4	25	111	247	200		
Dominance_D	1	1	0.90	0.99	1	0.98	0.05
Shannon_H	0	0	0.28	0.023	0	0.06	0.13
Evenness_e^H/S	1	1	0.26	0.51	1	0.76	0.37

A1bis

Table 19. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1bis station.

	A1bis_drift7.23	A1bis_drift_8.6	A1bis_drift_8.26	A1bis_drift_9.10	A1bis_drift_9.26	AVERAGE	STD DV (±)
Taxa_S	3	3	2	1	2		
Individuals	24	27	20	35	48		
Dominance_D	0.64	0.86	0.90	1	0.96	0.87	0.14
Shannon_H	0.65	0.32	0.20	0	0.10	0.25	0.25
Evenness_e^ H/S	0.64	0.46	0.61	1	0.55	0.65	0.21

Emergence

The tables 20 and 21 report the macroinvertebrate emergence community composition at A1 and A1bis in the six sampling weeks in 2015.

A1

Table 20. Adult insects found in A1 station. The codes refer univocally to each sampling: station_technique_date.

	Plecoptera	Limoniidae+Pediciidae	Tipulidae	Chironomidae	Simuliidae	Empididae
A1_Em_7.7-7.23			6			9
A1_emA_7.26-8.6	1	1		22		2
A1_emA_8.26-9.10	1			9		1
A1_emA_8.6-8.26	1	4		79	1	5
A1_emB_9.13-9.26		5		1		1
A1_emB_7.26-8.6				5		
A1_emB_8.26-9.10						2
A1_emB_8.6-8.26				2		
A1_emC_9.13-9.26		3		14		14
A1_emC_7.26-8.6	1	2		30		6
A1_emC_8.26-9.10	5			4		3
A1_emC_8.6-8.26	1	1		10		6

A1bis

Table 21. Adult insects found in A1 station. The codes refer univocally to each sampling: station_technique_date.

	Ephemeroptera	Plecoptera	Limoniidae+Pediciidae	Tipulidae	Chironomidae	Simuliidae	Empididae
A1bis_emA_9.13-9.26						1	4
A1bis_emA_7.26-8.6			6		1		17
A1bis_emA_8.26-9.10	1		2		2		2
A1bis_emA_8.6-8.26			7		4		8
A1bis_emB_9.13-9.26		2		1	3		16
A1bis_emB_7.26-8.6		1			4		
A1bis_emB_8.26-9.10						1	
A1bis_emB_8.6-8.26	1				2	2	1
A1bis_emC_9.13-9.26		1			2	1	
A1bis_emC_7.26-8.6					3		
A1bis_emC_8.26-9.10			1				
A1bis_emC_8.6-8.26		4	3		1	1	

The seasonal average emergence rate of the glacier-fed stream and the non glacial stream resulted in 79 ind/m^2 and 39 ind/m^2. The majority of organisms belonged to the family of Chironomidae, although the biodiversity distribution in both streams is similar (Tables 23 and 24).

The average of Shannon index resulted H = 0.58 in the A1 station and H = 0.72 in A1bis station. Even the average of Dominance (D) and Evenness (e^H/S) resulted quite similar in the glacial and non glacial stream (D= 0.67 and e^H/S = 0.76 in A1 and D = 0.59 and e^H/S = 0.88 in A1bis).

The seasonal pattern of emergence orders in A1 and in A1bis is shown in figure 23. Diptera and Plecoptera have a completely opposite trend in A1 than in A1bis. In both streams, Diptera had a negative trend in the date of 09/10/2015 whereas Plecoptera a positive one.

No Ephemeroptera were found in the A1 station.

The ANOVA test, used to evaluate differences among sampling dates for each station, indicated that in both A1 and A1bis station the null hypothesis is confirmed ($p > 0.05$).

There are not differences in the samples composition among all biweekly collections.

Table 22 shows the arithmetic averages of seasonal emergence sampling of A1 and A1bis station.

Table 22. Average of A1 and A1bis station used with *U* test.

	A1 AVERAGE	**A1bis AVERAGE**
Ephemeroptera		0.17
Plecoptera	0.83	0.67
Limoniidae+Pediciidae	1.33	1.58
Tipulidae	0.50	0.08
Chironomidae	14.67	1.83
Simuliidae	0.08	0.50
Empididae	4.08	4.00

With a p value > 0.05, the Mann – Whitney test confirmed the null hypothesis. The two populations did not show any statistical difference.

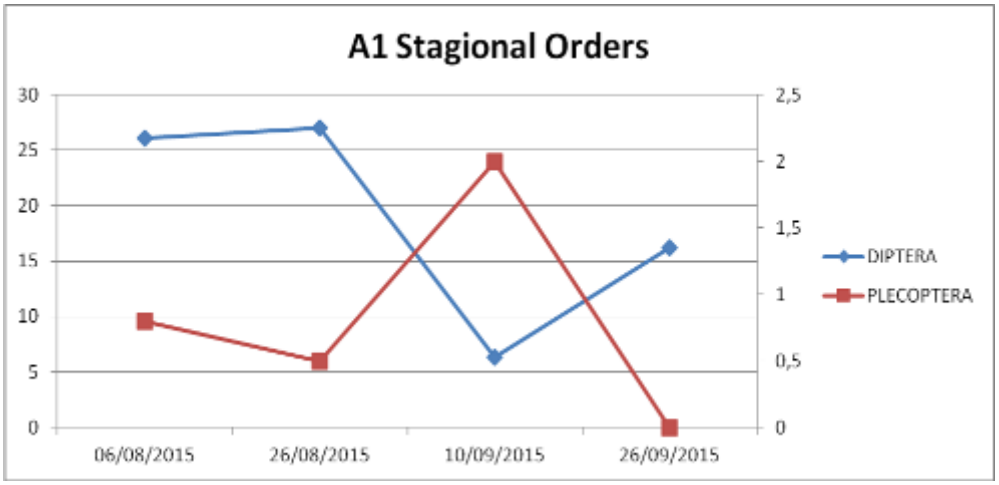

Figure 23. Seasonal pattern of emergence (X axis days, Yaxis number of organisms)

A1

Table 23. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1 station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1_Em_7.7-7.23	2	15	0.52	0.673	0.9801
A1_emA_7.26-8.6	4	26	0.7249	0.5893	0.4507
A1_emA_8.26-9.19	3	11	0.686	0.6002	0.6075
A1_emA_8.6-8.26	5	90	0.7758	0.5134	0.3342
A1_emB_9.13-9.26	3	7	0.551	0.7963	0.7391
A1_emB_7.26-8.6	1	5	1	0	1
A1_emB_8.26-9.10	1	2	1	0	1
A1_emB_8.6-8.26	1	2	1	0	1
A1_emC_9.13-9.26	3	31	0.4173	0.944	0.8568
A1_emC_7.26-8.6	4	39	0.6187	0.7361	0.5219
A1_emC_8.26-9.10	3	12	0.3472	1.078	0.9792
A1_emC_8.6-8.26	4	18	0.4259	1.014	0.6891
AVERAGE			0.67	0.58	0.76
STD DV (±)			0.24	0.39	0.24

A1bis

Table 24. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1bis station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1bis_emA_9.13-9.26	2	5	0.68	0.5004	0.8247
A1bis_emA_7.26-8.6	3	24	0.566	0.7233	0.687
A1bis_emA_8.26-9.10	4	7	0.2653	1.352	0.9661
A1bis_emA_8.6-8.26	3	19	0.3573	1.06	0.9622
A1bis_emB_9.13-9.26	4	22	0.5579	0.8618	0.5918
A1bis_emB_7.26-8.6	2	5	0.68	0.5004	0.8247
A1bis_emB_8.26-9.10	1	1	1	0	1
A1bis_emB_8.6-8.26	4	6	0.2778	1.33	0.9449
A1bis_emC_9.13-9.26	3	4	0.375	1.04	0.9428
A1bis_emC_7.26-8.6	1	3	1	0	1
A1bis_emC_8.26-9.10	1	1	1	0	1
A1bis_emC_8.6-8.26	4	9	0.3333	1.215	0.8425
AVERAGE			0.59	0.72	0.88
STD DV (±)			0.28	0.51	0.13

Malaise trap

Table 25 reports the macroinvertebrate malaise community composition at A1 and A1bis in the six sampling weeks in 2015.

A1

Table 25. Adult Insects found in A1 station. The codes refer univocally by each sampling: station_technique_date.

	A1_Mal_7.7-7.23	**A1_Mal_7.26-8.6**	**A1_Mal_8.6-8.26**	**A1_Mal_8.26-9.10**	**A1_Mal_9.13-9.26**
Plecoptera	2	5	7	13	
Limoniidae+Pediciidae	40	21	18	5	
Tipulidae	71			2	
Chironomidae	81	310	464	145	11
Simuliidae		2		1	1
Thaumaleidae	4	10	11	4	
Empididae	720	602	322	127	8

Five malaise samples were collected during the biweekly collection but for in the date of 09/26/2015, when the trap was found overthrown. The records of this date were not considered.
The only aquatic insect found in the malaise were Plecoptera and Diptera.
Diptera order represented the most abundant taxon collected in each collection with a peak of 657 specimens (Diptera is the sum of: Limoniidae+Pediciidae, Tipulidae, Chironomidae, Simuliidae, Thaumaleidae, and Empididae). The number of captured Plecoptera was never higher than 13 organisms.
The ANOVA test, used to evaluate differences among sampling dates for A1 station, was not significant ($p > 0.05$). There are no differences in insects' composition among seasons.
The average of Shannon index resulted ($H = 0.87$) is really high. The average of Dominance (D) and Evenness (e^H/S) resulted 0.50 and 0.48 respectively (Table 26).

A1

Table 26. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1 station.

	A1_Mal_7.7-7.23	A1_Mal_7.26-8.6	A1_Mal_8.6-8.26	A1_Mal_8.26-9.10	A1_Mal_9.13-9.26	AVERAGE	STD DV (±)
Taxa_S	6	6	5	7	3		
Individuals	918	950	822	297	20		
Dominance_D	0,6308	0,5087	0,4728	0,4236	0,465	0,50	0,08
Shannon_H	0,7763	0,8273	0,8719	1,03	0,8451	0,87	0,10
Evenness_e^H/S	0,3622	0,3812	0,4783	0,4001	0,7761	0,48	0,17

Malaise efficiency

The efficiency of trapping aquatic insects was calculated. These records can help understand the adults insects movement, which is one critical aspect of the colonization cycle. All data are reported in the table 27.

Table 27. Malaise efficiency during hourly and seasonal sampling.

	JULY (7.23-7.26)	SEPTEMBER(9.10-9.13)	SEASONAL	TOTAL
TOTAL	289	192	3979	4460
AQUATIC	251	174	3156	3581
% AQUATIC	86,9	90,7	79,3	80,3
TERRESTRIAL	38	18	823	879
% TERRESTRIAL	13,1	9,3	20,7	19,7

3.2.4. Hourly sampling

Drift (23- 26/07/2015)

The tables 28 and 29 report the macroinvertebrate drift community composition at A1 and A1bis in the first hourly sampling (07/23-07/26/2015).

A1

Table 28. Invertebrates found in A1 station. The codes refer univocally by each sampling: station_technique_date.

	Hydracarina	Coleoptera	Chironomidae	Simuliidae
A1_drift_7.23_6am		1	19	
A1_drift_7.23_9pm			33	
A1_drift_7.24_6am			9	1
A1_drift_7.24_9am	1		214	1
A1_drfit_7.24_12am			96	
A1_drift_7.24_6pm			24	
A1_drfit_7.24_9pm			20	
A1_drift_7.25_6am			50	1
A1_drift_7.25_9pm			82	
A1_drift_7.25_12am			203	
A1_drift_7.25_3pm			78	
A1_drift_7.25_6pm		2	30	
A1_drift_7.25_9pm			84	
A1_drift_7.26_6am			25	
A1_drift_7.26_9am			111	1
A1_drift_7.26_12am			32	
A1_drift_7.26_3pm			26	

A1bis

Table 29. Invertebrates found in A1bis station. The codes refer univocally by each sampling: station_technique_date.

	Hydracarina	Ephemeroptera juv	Baetis	Plecoptera juv	Coleoptera juv	Trichoptera juv	Limnephilidae	Limoniidae+Pediciidae	Chironomidae	Simuliidae	Empididae
A1bis_drift_7.23_6pm			1		1	2	1		7		
A1bis_drift_7.23_9pm								1	4		
A1bis_drift_7.24_6am									44	1	
A1bis_drift_7.24_9pm									10		
A1bis_drift_7.24_12am									3		
A1bis_drift_7.24_6pm				2					15		1
A1bis_drift_7.24_9pm							1		10		
A1bis_drift_7.25_6am									5		
A1bis_drift_7.25_9am									2		
A1bis_drift_7.25_12am	1	2							4		
A1bis_drift_7.25_3pm											
A1bis_drift_7.25_6pm								1	6	1	
A1bis_drift_7.25_9pm									2		
A1bis_drift_7.26_6am									1		
A1bis_drift_7.26_9am									15		
A1bis_drift_7.26_12am			1	1					2		
A1bis_drift_7.26_3pm									1		

During the first hourly collection 34 samples were collected. The samples of 07/24/2015 at 3pm were not collected due to bad atmospheric conditions.

In the glacial stream, the family of Chironomidae prevailed and only a few individuals belonged to the families of Simuliidae, Coleoptera, and Hydracarina.

In the non glacial stream the biodiversity is higher, with many sampled taxa.

This is in agreement with the diversity indices (Tables 31 and 32). The Shannon index, in A1 shows a very low biodiversity (H = 0.07) while the Dominance and the Evenness results respectively D = 0.96 and e^H/S = 0.82.

In A1bis the Shannon index show a higher biodiversity with a total H = 0.34 while Dominance = 0.81 and Evenness = 0.86

Chironomidae family results to be the major taxa represented.

The ANOVA test did not point out differences among sampling hour for each station, with a p = 0.956 in A1 and p = 0.800 in A1bis station.

In the table 30 the arithmetic averages of first hourly drift sampling (07/23-07/26/2015) of A1 and A1bis station used to calculate the Mann – Whitney test are shown.

Table 30. Average of A1 and A1bis station used for *U* test (Juv juvenile)

	A1 AVERAGE	**A1bis AVERAGE**
Hydracarina	0.06	0.06
Ephemeroptera juv		0.12
Baetis		0.12
Plecoptera juv		0.18
Coleoptera juv	0.18	0.06
Trichoptera juv		0.12
Limnephilidae		0.12
Limoniidae+Pediciidae		0.12
Chironomidae	66.82	7.71
Simuliidae	0.24	0.12
Empididae		0.06

The Mann – Whitney test resulted in p = 0.056. Although p is close to significance, the null hypothesis is confirmed.

A1

Table 31. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1 station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1_drfit_24.7_h12	1	96	1	0	1
A1_drfit_24.7_h21	1	20	1	0	1
A1_drift_21.7_9h	3	216	0.9816	0.05899	0.3536
A1_drift_23.7_h18	2	20	0.905	0.1985	0.6098
A1_drift_23.7_h21	1	33	1	0	1
A1_drift_24.7_h18	1	24	1	0	1
A1_drift_24.7_h6	2	10	0.82	0.3251	0.6921
A1_drift_25.7_h12	1	203	1	0	1
A1_drift_25.7_h15	1	78	1	0	1
A1_drift_25.7_h18	2	32	0.8828	0.2338	0.6317
A1_drift_25.7_h21	1	84	1	0	1
A1_drift_25.7_h6	2	51	0.9616	0.09651	0.5507
A1_drift_25.7_h9	1	82	1	0	1
A1_drift_26.7_6h	1	25	1	0	1

Table 31. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1 station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1_drift_26.7_h12	1	32	1	0	1
A1_drift_26.7_h15	2	28	0.8673	0.2573	0.6467
A1_drift_26.7_h9	2	112	0.9823	0.05102	0.5262
AVERAGE			0.96	0.07	0.82
STD DV (±)			0.06	0.11	0.23

A1bis

Table 32. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1bis station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1bis_drift_23.7_h18	5	12	0.3889	1.234	0.6872
A1bis_drift_23.7_h21	2	5	0.68	0.5004	0.8247
A1bis_drift_24.7_h6	2	45	0.9565	0.1066	0.5562
A1bis_drift_24.7_h9	1	10	1	0	1
A1bis_drift_24.7_h12	1	3	1	0	1
A1bis_drift_24.7_h18	3	18	0.7099	0.5566	0.5816
A1bis_drift_24.7_h21	2	11	0.8347	0.3046	0.6781
A1bis_drift_25.7_h6	1	5	1	0	1
A1bis_drift_25.7_h9	1	2	1	0	1
A1bis_drift_25.7_h12	3	7	0.4286	0.9557	0.8668
A1bis_drift_25.7_h18	3	8	0.5938	0.7356	0.6956
A1bis_drift_25.7_h21	1	2	1	0	1
A1bis_drift_26.7_h6	1	1	1	0	1
A1bis_drift_26.7_h9	1	15	1	0	1
A1bis_drift_26.7_h12	3	4	0.375	1.04	0.9428
A1bis_drift_26.7_h15	1	1	1	0	1
AVERAGE			0.81	0.34	0.86
STD DV (±)			0.24	0.44	0.17

Drift sampling (10- 13/09/2015)

The tables 33 and 34 report the macroinvertebrate drift community composition at A1 and A1bis in the second hourly sampling (10-13/09/2015).

A1

Table 33. Invertebrates found in A1 station. The codes refer univocally to each sampling: station_technique_date.

	Nematoda	Baetis sp. L	Plecoptera juv	Coleoptera juv	Dytiscidae	Limoniidae+Pediciidae Ad	Limoniidae+Pediciidae	Psychodidae	Chironomidae	Simuliidae	Empididae
A1_drift_9.10_6pm									111		
A1_drift_9.10_9pm						1	1		13		
A1_drift_9.11_12am							1		385	1	
A1_drift_9.11_3pm									125		
A1_drift_9.11_6pm									131		
A1_drift_9.11_9pm							2		158		2
A1_drift_9.11_9am	1			1			2		216		
A1_drift_9.12_12am							1		542		1
A1_drift_9.12_3pm							1		27		
A1_drift_9.12_6pm								1	32		
A1_drift_9.12_9pm							1		34		
A1_drift_9.12_6pm							1		113		
A1_drift_9.12_9pm							1		188		
A1_drift_9.13_12am							1		159		
A1_drift_9.13__6am							2		174		
A1_drift_9.13__9am							5		117		1

A1bis

Table 34. Invertebrates found in A1bis station. The codes refer univocally by each sampling: station_technique_date.

	Baetis sp.	Plecoptera juv	Dytiscidae	Limoniidae+Pediciidae	Chironomidae	Empididae
A1_drift_9.10_6pm					3	
A1_drift_9.10_9pm					1	
A1_drift_9.11_12am					15	
A1_drift_9.11_3pm					11	
A1_drift_9.11_6pm					11	
A1_drift_9.11_9pm					12	
A1_drift_9.11_9am	1	1			6	
A1_drift_9.12_12am					5	
A1_drift_9.12_3pm					2	
A1_drift_9.12_6pm		1		1	12	
A1_drift_9.12_9pm				1	7	
A1_drift_9.12_6pm					151	
A1_drift_9.12_9pm					7	

Table 34. Invertebrates found in A1bis station. The codes refer univocally by each sampling: station_technique_date.

	Baetis sp.	Plecoptera juv	Dytiscidae	Limoniidae+Pediciidae	Chironomidae	Empididae
A1_drift_9.13_12am			1		3	
A1_drift_9.13__6am					1	1
A1_drift_9.13__9am					6	

During the second hourly sampling 34 samples were collected. The samples of 11/09/2015 at 6 am were not collected due to bad atmospheric conditions.

In A1 and A1bis station the most abundant family was Chironomidae.

According with Shannon (H), Dominance (D), and Evenness (e^H/S), the biodiversity of both stream results to be very low with a strong dominance of only one taxa (Tables 36 and 37).

According with what expected the ANOVA test, used to evaluate differences among sampling dates for A1 station, highlighted that there are no differences in insects' composition during the this hourly collection.

Table 35 reported the arithmetic averages of first hourly drift sampling (09/10-09/13/2015) of A1 and A1bis station.

Table 35. Average of A1 and A1bis station used for *U* test (juv juveniles, Ad adults).

	A1 AVERAGE	**A1bis AVEAGE**
Nematoda	0.06	
Baetis		0.06
Plecoptera juv		0.12
Coleoptera juv	0.06	
Dytiscidae		0.06
Limoniidae+Pediciidae Ad	0.06	
Limoniidae+Pediciidae	1.19	0.12
Psychodidae	0.06	
Chironomidae	157.81	15.81
Simuliidae	0.06	
Empididae	0.25	0.06

With a p value > 0.05, the Mann – Whitney test, confirm the null hypothesis. The two populations did not show significant statistical difference.

A1

Table 36. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1 station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1_drift_9.10_6pm	1	111	1	0	1
A1_drift_9.10_9pm	3	15	0.76	0.4851	0.5414
A1_drift_9.11_12am	3	387	0.9897	0.03595	0.3455
A1_drift_9.11_3pm	1	125	1	0	1
A1_drift_9.11_6pm	1	131	1	0	1
A1_drift_9.11_9pm	2	160	0.9753	0.0672	0.5348
A1_drift_9.11_9am	4	220	0.9641	0.1098	0.279
A1_drift_9.12_12am	2	543	0.9963	0.01344	0.5068
A1_drift_9.12_3pm	3	29	0.8692	0.2988	0.4494
A1_drift_9.12_6pm	2	33	0.9412	0.1358	0.5727
A1_drift_9.12_9pm	2	35	0.9445	0.1297	0.5693
A1_drift_9.12_6am	2	114	0.9826	0.05028	0.5258
A1_drift_9.12_9am	2	189	0.9895	0.03301	0.5168
A1_drift_9.13_12am	3	161	0.9754	0.07547	0.3595
A1_drift_9.13_6am	2	176	0.9775	0.06218	0.5321
A1_drift_9.13_9am	2	122	0.9214	0.1711	0.5933
AVERAGE			0.96	0.10	0.58
STD DV (±)			0.06	0.13	0.22

.

A1bis

Table 37. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1bis station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1_drift_9.10_6pm	1	3	1	0	1
A1_drift_9.10_9pm	1	1	1	0	1
A1_drift_9.11_12am	1	15	1	0	1
A1_drift_9.11_3pm	1	11	1	0	1
A1_drift_9.11_6pm	1	11	1	0	1
A1_drift_9.11_9pm	1	12	1	0	1
A1_drift_9.11_9am	3	8	0.5938	0.7356	0.6956
A1_drift_9.12_12am	1	5	1	0	1
A1_drift_9.12_3pm	1	2	1	0	1
A1_drift_9.12_6pm	3	14	0.7449	0.5091	0.5546

Table 37. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1bis station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1_drift_9.12_9pm	2	8	0.7813	0.3768	0.7288
A1_drift_9.12_6am	1	151	1	0	1
A1_drift_9.12_9am	1	7	1	0	1
A1_drift_9.13_12am	2	4	0.625	0.5623	0.8774
A1_drift_9.13_6am	2	2	0.5	0.6931	1
A1_drift_9.13_9am	1	6	1	0	1
AVERAGE			0.89	0.18	0.93
STD DV (±)			0.18	0.29	0.14

Emergence (23-26/07/2015)

The tables 38 and 39 report the macroinvertebrate emergence community composition at A1 and A1bis in the first hourly sampling (23-26/07/2015).

A1

Table 38. Adult Insects found in A1 station. The codes refer univocally by each sampling: station_technique_date.

	Limoniidae+Pediciidae Ad	Chironomidae Ad	Empididae Ad
A1_EmC_23.7_h21			1
A1_EmC_24.7_h12		2	
A1_emC_25.7_h15	1	1	
A1_emC_25.7_h12		1	

A1bis

Table 39. Adult Insects found in A1 station. The codes refer univocally by each sampling: station_technique_date.

	Limoniidae+Pediciidae Ad	Chironomidae Ad
A1bis_emB_24.7_h6		1
A1bis_emC_24.7_h6		1
A1bis_emB_25.7_h12		1
A1bis_emB_25.7_h15		1
A1bis_emC_25.7_h15		1
A1bis_emA_26.7_h6	1	
A1bis_emA_26.7_h9		1
A1bis_emB_26.7_h9		1

During the first hourly collection 34 samples were collected. The samples of 07/24/2015 at 3pm were not collected due to bad atmospheric conditions.

In the glacier-fed stream a total of 6 insects were collected, all of them belonged to the Diptera order (4 individuals to Chironomidae family) as shown in the table 38. In the non glacial stream a total of 8 insects were collected, all of them belonged to the Diptera order (7 individuals to Chironomidae family) like the A1 station (Table 39).

The ANOVA test highlighted that there are no differences in the macroinvertebrate composition both in A1 than in A1bis station.

With a p value > 0.05, the Mann – Whitney test, confirm the null hypothesis. The two populations did not show any statistical difference (Table 40).

Table 40. Average of A1 and A1bis station used for *U* test.

	A1 AVERAGE	**A1bis AVERAGE**
Limoniidae+Pediciidae	0.17	0.10
Chironomidae	0.83	0.60
Empididae	0.17	

Emergence (10-13/09/2015)

During the second hourly collection 34 samples were collected. The samples of 11/09/2015 at 6 were not collected due to bad atmospheric conditions.

In the glacier-fed stream only 1 insect was collected (Chironomidae family) while, in the non-glacial stream no insects were collected.

Hourly intensity

The next figure shows the hour emergence pattern. This graphic was obtained using the first and the second hourly sampling in order to understand the emergence movement during the day.

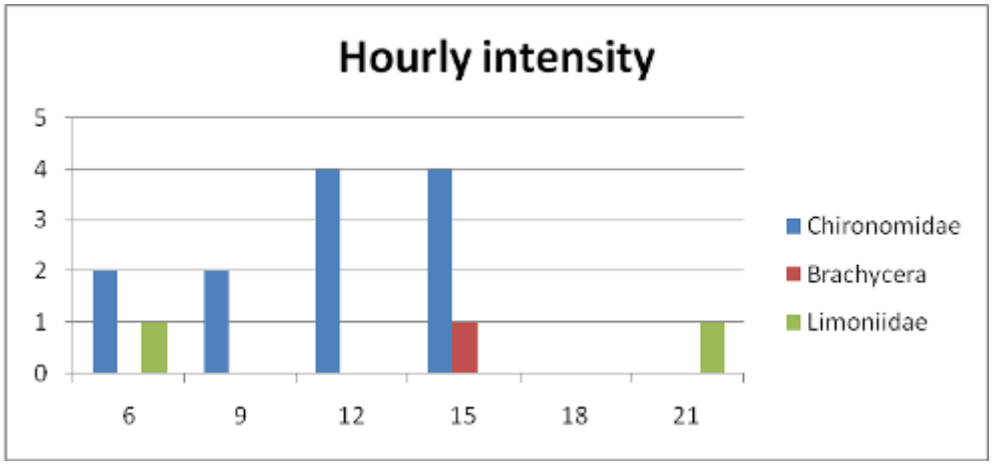

Figure 24. Hourly emergence intensity.

Emergence – Temperature patterns

Water and air temperature patterns were compared with the insects emergence. Figure 25 and 26 represent the relation among air, water temperature and emergence in A1 and A1bis respectively, during the hourly sampling of July.

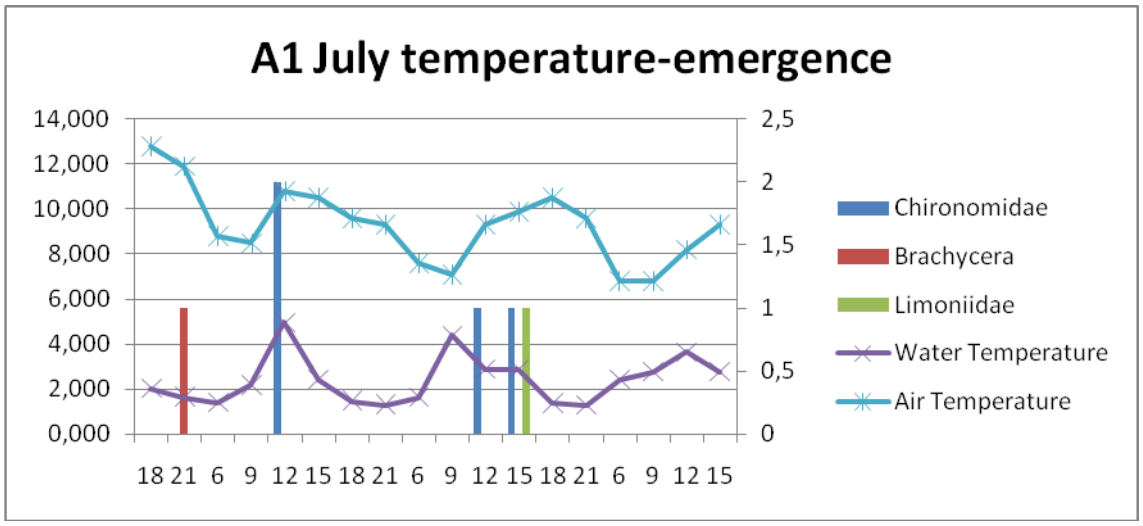

Figure 25. Emergence in relation with air and water temperature in A1 station.

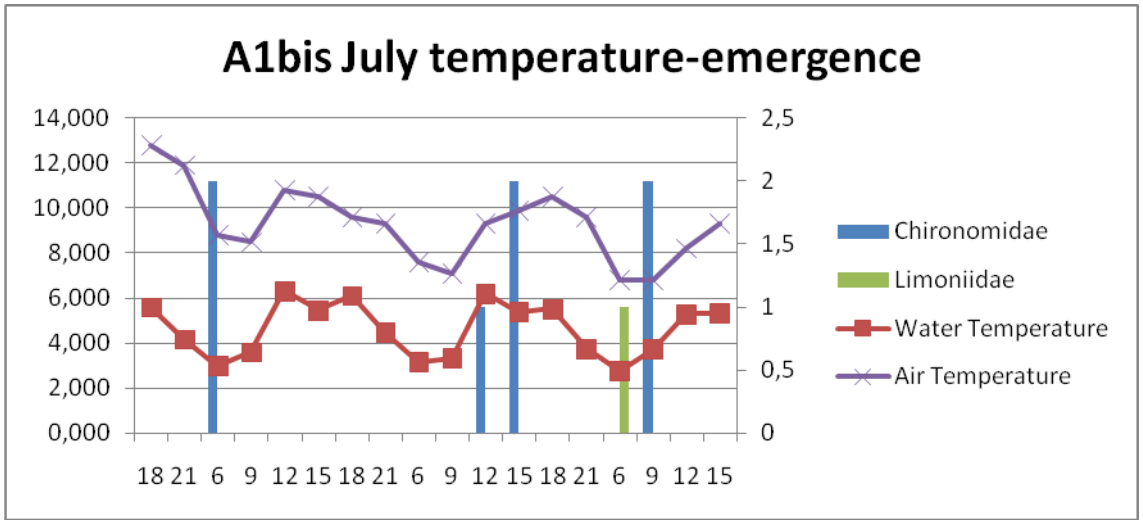

Figure 26. Emergence in relation with air and water temperature in A1bis station.

Emergence efficiency

Emergence trap efficiency was calculated only for the hourly sampling as percentage of observed captures vs. expected (if all emergency traps provided samples). During biweekly sampling all emergency traps provided samples (Table 41).

Table 41. Emergence efficiency during the first and the second hourly sampling.

	Theoretical Emergence sample	Emergence capture	Operation percentage %
Hourly 1	102	16	15,69
Hourly 2	102	1	0,98

Malaise trap (23-26/07/2015)

The table 42 reports the macroinvertebrate malaise community composition at A1 station in the first hourly sampling (23-26/07/2015).

A1

Table 42. Adult insects in A1 station. The codes refer univocally by each sampling: station_technique_date.

	Plecoptera	Limoniidae+Pediciidae	Tipulidae	Chironomidae	Empididae
A1_Mal_7.23_6pm					16
A1_Mal_7.23_9pm				4	17
A1_Mal_7.24_6am		4	1	15	7
A1_Mal_7.24_9am					3
A1_Mal_7.24_12am				2	19
A1_Mal_7.24_6pm				1	50
A1_Mal_7.24_9pm					1
A1_Mal_7.25_6am	1	1		4	5
A1_Mal_7.25_9am				3	2
A1_Mal_7.25_12am				4	10
A1_Mal_7.25_3pm	2			1	8
A1_Mal_7.25_6pm					11
A1_Mal_7.25_9pm					10
A1_Mal_7.26_6am		2		5	6
A1_Mal_7.26_9am				3	2
A1_Mal_7.26_12am				4	8
A1_Mal_7.26_3pm				2	13

During the first hourly collection 17 samples were obtained. The samples of 07/24/2015 at 3pm were not collected due to bad atmospheric conditions.

In the A1 site a total of 285 insects were collected, 188 of them were terrestrial insects while the composition of other 97 is shown in the figure 27.

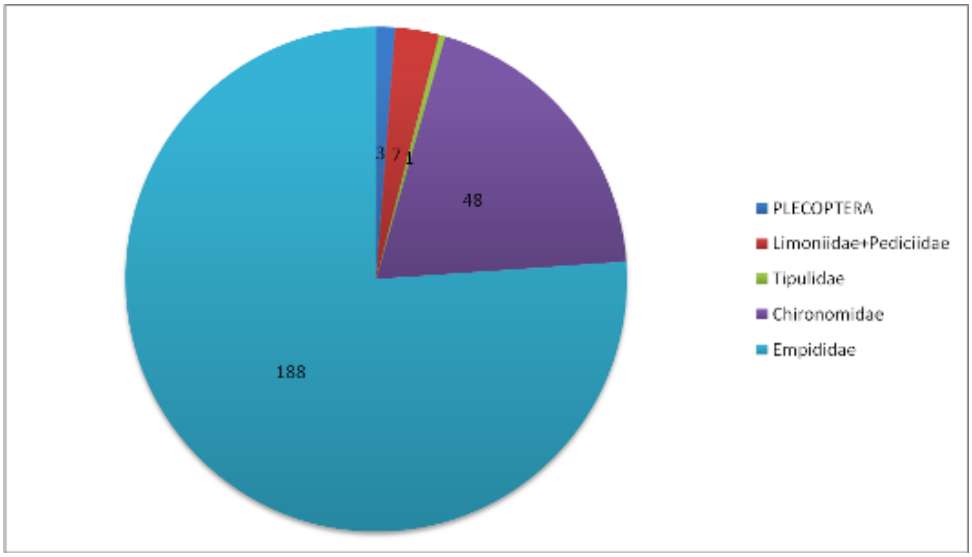

Figure 27. Malaise composition during the first hourly sampling.

ANOVA test highlighted no difference in the insect composition during the hourly sampling.

The diversity indices report a medium average biodiversity in the A1 station with a H = 0.48 and a good Dominance of one taxa D = 0.71 (Empididae family).

A1

Table 43. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1 station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
	5	247	0.6181	0.703	0.4039
A1_Mal_7.23_6pm	1	16	1	0	1
A1_Mal_7.23_9pm	2	21	0.6916	0.4869	0.8136
A1_Mal_7.24_6am	4	27	0.3992	1.081	0.7373
A1_Mal_7.24_9am	1	3	1	0	1
A1_Mal_7.24_12am	2	21	0.8277	0.3145	0.6848
A1_Mal_7.24_6pm	2	51	0.9616	0.09651	0.5507
A1_Mal_7.24_9pm	1	1	1	0	1
A1_Mal_7.25_6am	4	11	0.3554	1.162	0.7993
A1_Mal_7.25_9am	2	5	0.52	0.673	0.9801
A1_Mal_7.25_12am	2	14	0.5918	0.5983	0.9095
A1_Mal_7.25_3pm	3	11	0.5702	0.7595	0.7124
A1_Mal_7.25_6pm	1	11	1	0	1

Table 43. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1 station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1_Mal_7.25_9pm	1	10	1	0	1
A1_Mal_7.26_6am	3	13	0.3846	1.012	0.9173
A1_Mal_7.26_9am	2	5	0.52	0.673	0.9801
A1_Mal_7.26_12am	2	12	0.5556	0.6365	0.9449
A1_Mal_7.26_3pm	2	15	0.7689	0.3927	0.7405
AVERAGE			0.71	0.48	0.84
STD DV (±)			0.24	0.40	0.18

Malaise trap (10- 13/09/2015)

The table 44 reports the macroinvertebrate malaise community composition at A1 station during the second hourly sampling (09/10-09/13/2015).

A1

Table 44. Adult insects in A1 station. The codes refer univocally by each sampling: station_technique_date.

	Limoniidae+Pediciidae	Chironomidae	Simuliidae	Thaumaleidae	Empididae	Empididae
A1_Mal_9.10_9pm	1	5				
A1_Mal_9.11_9am		7				
A1_Mal_9.11_12am		5		1		
A1_Mal. 9.11_3pm		12		1		
A1_Mal_9.11_6pm		5			1	
A1_Mal_9.11_9pm		9				
A1_Mal_9.12_6am		7				
A1_Mal_9.12_9am		15	2	2		
A1_Mal_9.12_12am		18		1	2	
A1_Mal_9.12_3pm		3	1		2	
A1_Mal_9.12_6pm		1				
A1_Mal_9.12_9pm		13			2	
A1_Mal_9.13_6am		4				
A1_Mal_9.13_9am		29				
A1_Mal_9.13_12am		19		2	1	
A1_Mal_9.13_3pm		1			1	

During the second hourly collection 16 samples were collected. The sample of 09/10/2015 at 6 pm were not collected due to restoration of the collecting vessel, while the samples of 09/11/2015 at 6 am were not collected due to bad atmospheric conditions.

In the malaise trap a total of 192 insects were collected, 18 of them were terrestrial while 174 belonged to the aquatic Diptera order (Figure 28).

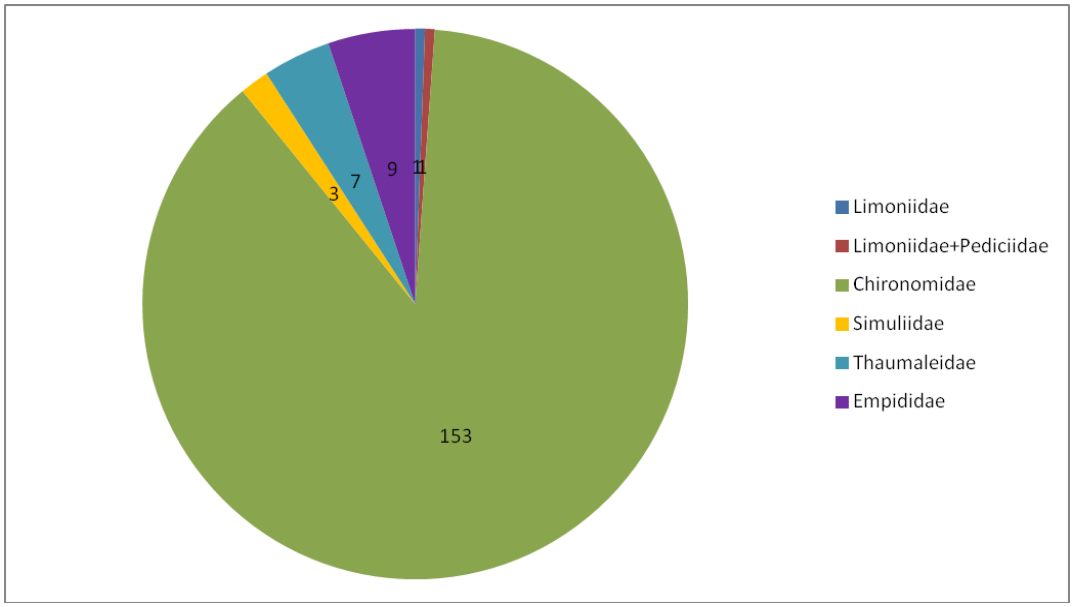

Figure 28. Malaise composition during the first hourly sampling.

A1

Table 45. Number of taxa, number of individuals, Dominance, Shannon, and Evenness indexes in A1 station.

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1_Mal_9.10_9pm	5	173	0.7868	0.4923	0.3272
A1_Mal_9.11_9am	2	6	0.7222	0.4506	0.7846
A1_Mal_9.11_12am	1	7	1	0	1
A1_Mal. 9.11_3pm	2	6	0.7222	0.4506	0.7846
A1_Mal_9.11_6pm	2	13	0.858	0.2712	0.6558
A1_Mal_9.11_9pm	2	6	0.7222	0.4506	0.7846
A1_Mal_9.12_6am	1	9	1	0	1
A1_Mal_9.12_9am	1	7	1	0	1
A1_Mal_9.12_12am	3	19	0.6454	0.6606	0.6453
A1_Mal_9.12_3pm	3	21	0.746	0.501	0.5501

	Taxa_S	Individuals	Dominance_D	Shannon_H	Evenness_e^H/S
A1_Mal_9.12_6pm	3	6	0.3889	1.011	0.9165
A1_Mal_9.12_9pm	1	1	1	0	1
A1_Mal_9.13_6am	2	15	0.7689	0.3927	0.7405
A1_Mal_9.13_9am	1	4	1	0	1
A1_Mal_9.13_12am	1	29	1	0	1
A1_Mal_9.13_3pm	3	22	0.7562	0.4851	0.5414
AVERAGE			0.80	0.34	0.81
STD DV (±)			0.19	0.30	0.21

No significant difference in the insect composition was recorded during the hourly sampling (ANOVA).

The diversity indices report a low average biodiversity in the A1 station with a H = 0.34 and a good Dominance of one taxa D = 0.71 (Chironomidae family).

Hourly intensity

The figure below reports the hour emergence pattern. For this graphic was used the first and the second hourly sampling in order to understand the emergence movement during the day.

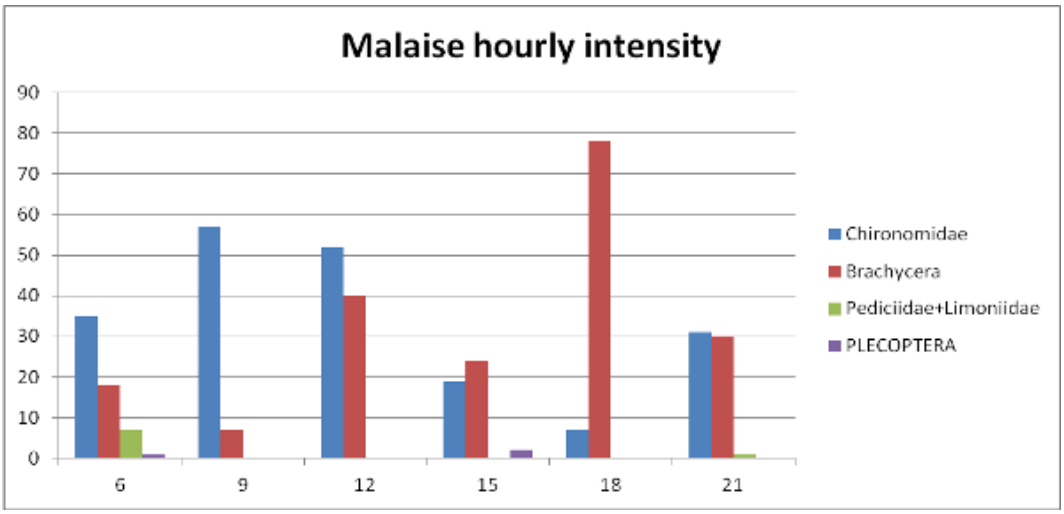

Figure 29. Malaise intensity of each taxon during the hourly collection (X axis hour, Y axis number of organism).

3.2.5 Seasonal pattern

In this paragraph, in order to better understand the emergence pattern during the seasonal collection, kick, drift, emergence and malaise records are shown in single subsequent graphics. Figures 30 to 39 show the trends of Diptera, Plecoptera, Chironomidae, and Brachycera taxa in the glacier-fed stream. Figures 40 to 47 show Diptera, Ephemeroptera, Plecoptera, Chironomidae and Brachycera trends in the non glacial stream.

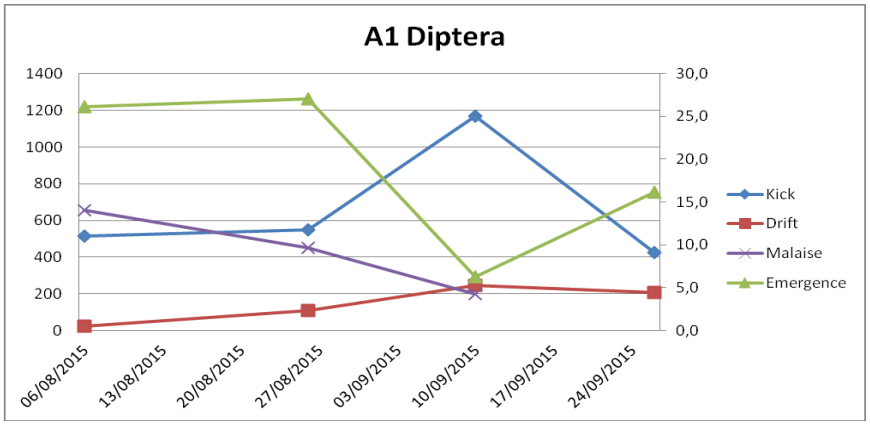

Figure 30. Patterns of Diptera order in A1 station (X axis sampling days, Y axis number of organisms).

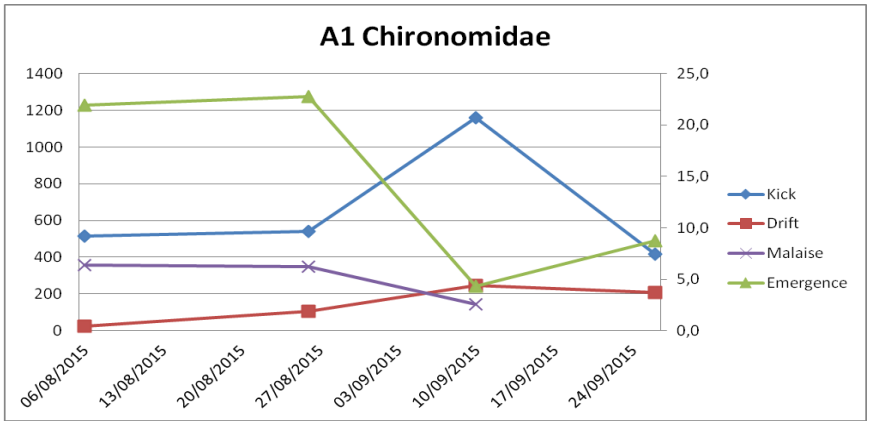

Figure 31. Patterns of Chironomidae family (X axis sampling days, Y axis number of organisms).

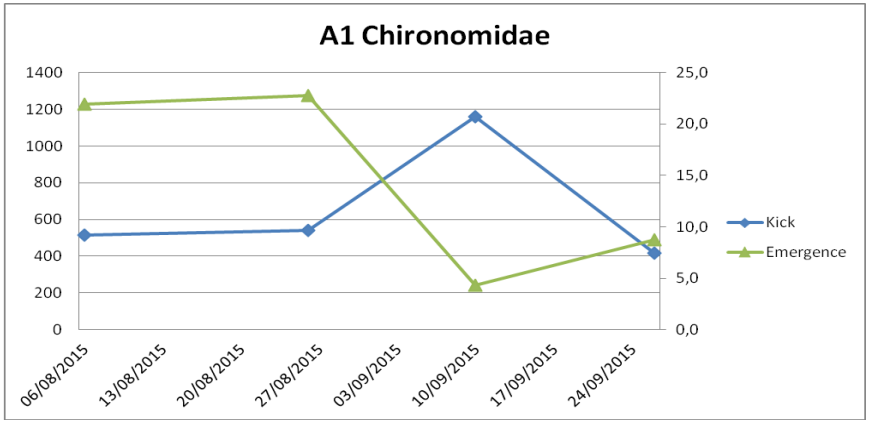

Figure 32. A focus on kick and emergence pattern (X axis sampling days, Y axis number of organisms).

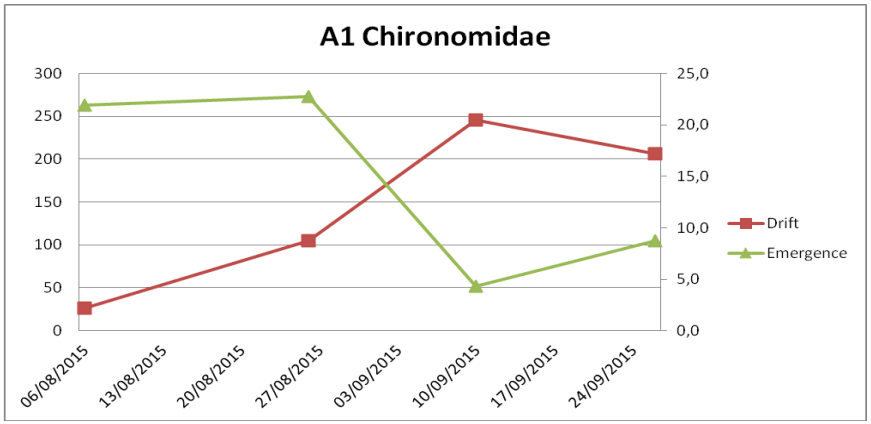

Figure 33. A focus on drift and emergence pattern (X axis sampling days, Y axis number of organisms).

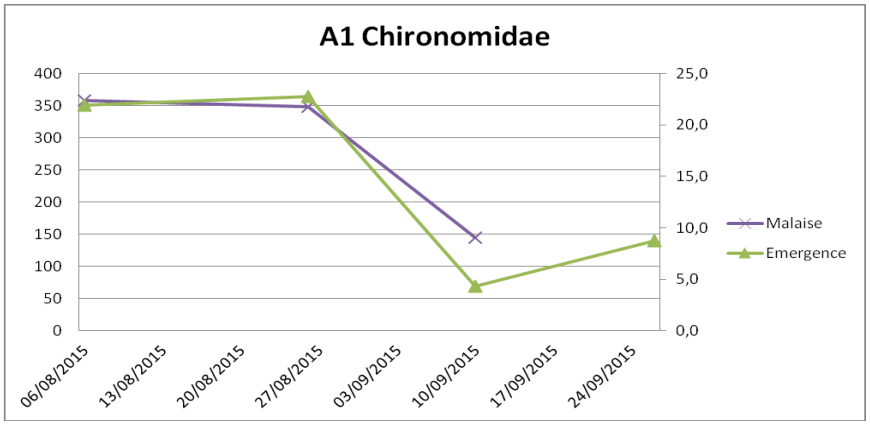

Figure 34. A focus on emergence and malaise (X axis sampling days, Y axis number of organisms).

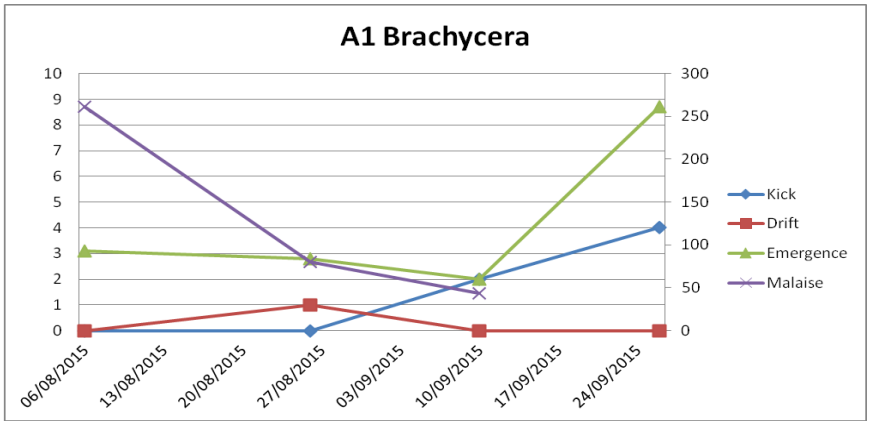

Figure 35. Patterns of Brachycera suborder(Empididae family) (X axis sampling days, Y axis number of organisms).

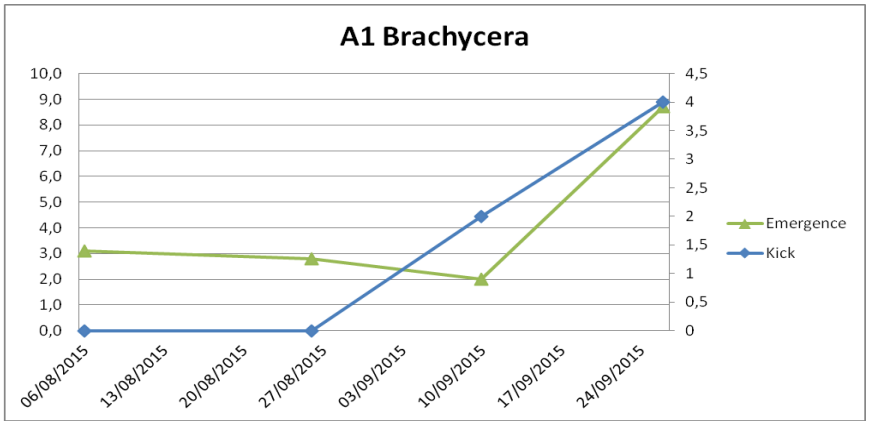

Figure 36. A focus on kick and emergence (X axis sampling days, Y axis number of organisms).

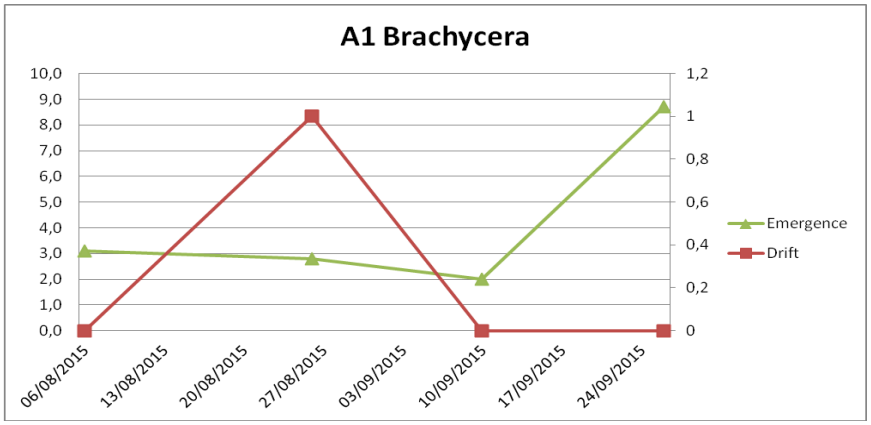

Figure 37. A focus on drift and emergence patterns (X axis sampling days, Y axis number of organisms).

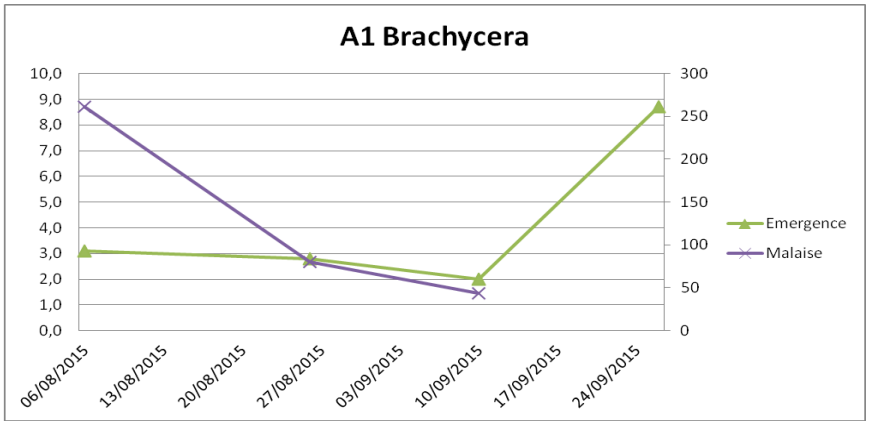

Figure 38. A focus on emergence and malaise patterns (X axis sampling days, Y axis number of organisms).

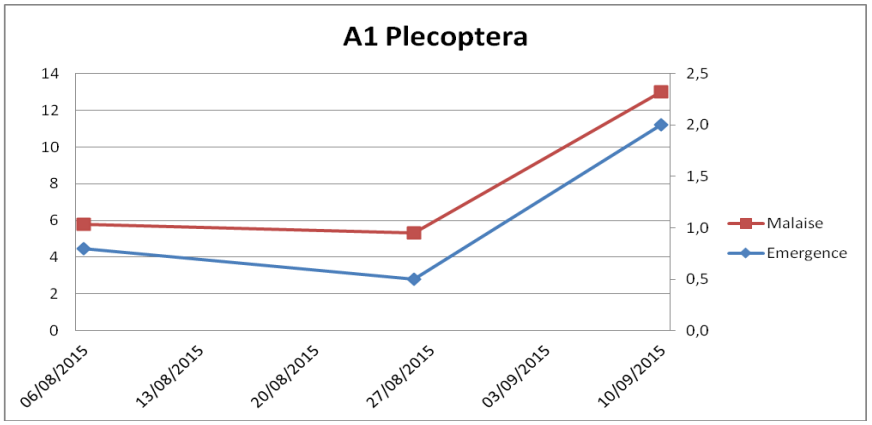

Figure 39. Malaise and Emergence patterns of Plecoptera order (X axis sampling days, Y axis number of organisms).

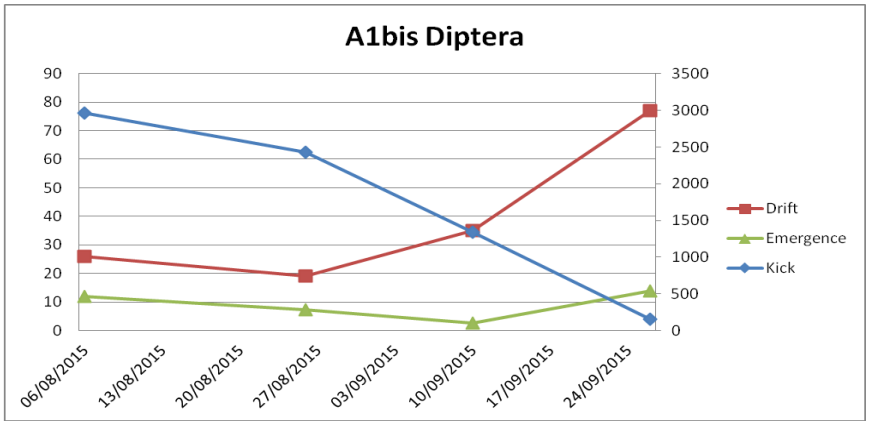

Figure 40. Patterns of Diptera order in A1bis station (X axis sampling days, Y axis number of organisms).

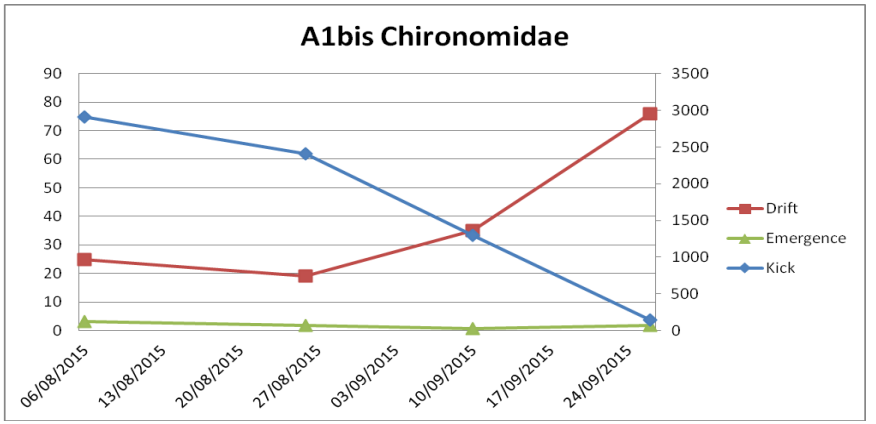

Figure 41. Chironomidae pattern in A1bis (X axis sampling days, Y axis number of organisms).

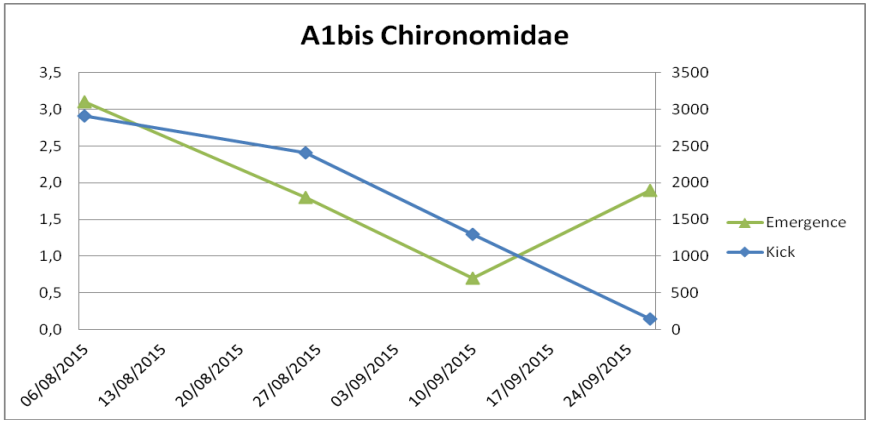

Figure 42. A focus on kick and emergence pattern (X axis sampling days, Y axis number of organisms).

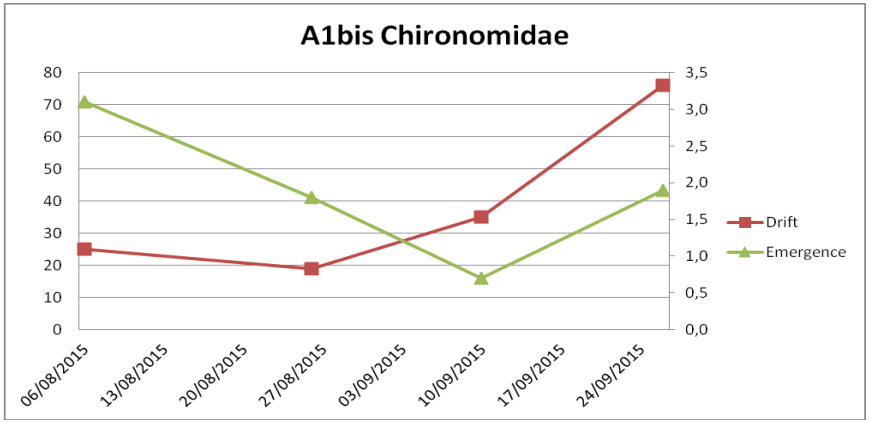

Figure 43. A focus on drift and emergence pattern (X axis sampling days, Y axis number of organisms).

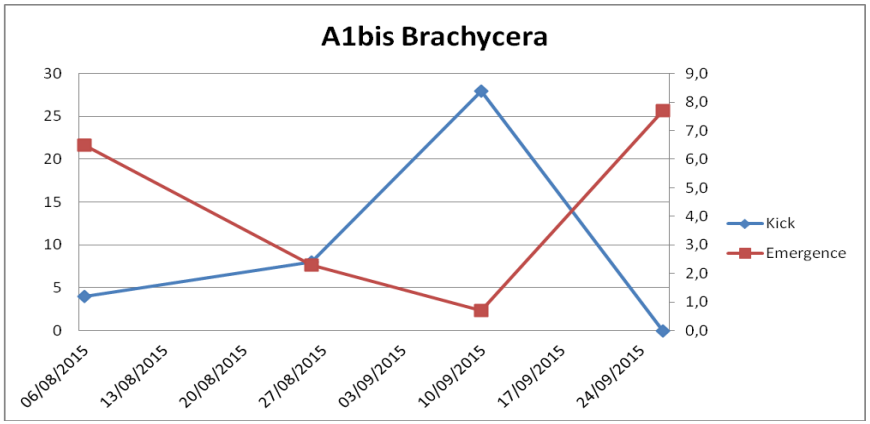

Figure 44. Trend of Brachycera suborder in A1bis station (X axis sampling days, Y axis number of organisms).

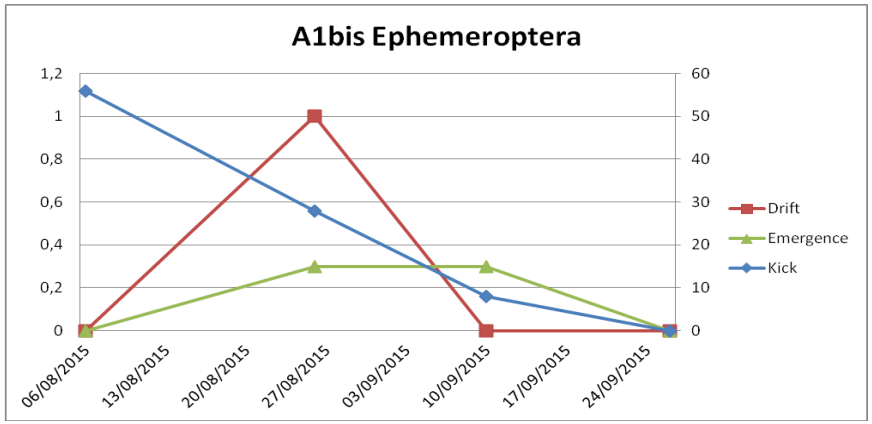

Figure 45. Trend of Ephemeroptera order in A1bis station (X axis sampling days, Y axis number of organisms).

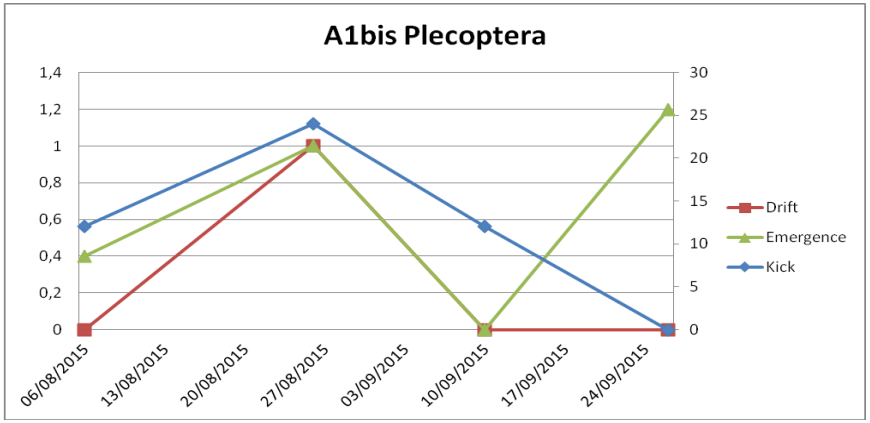

Figure 46. Trend of Plecoptera order in A1bis station (X axis sampling days, Y axis number of organisms).

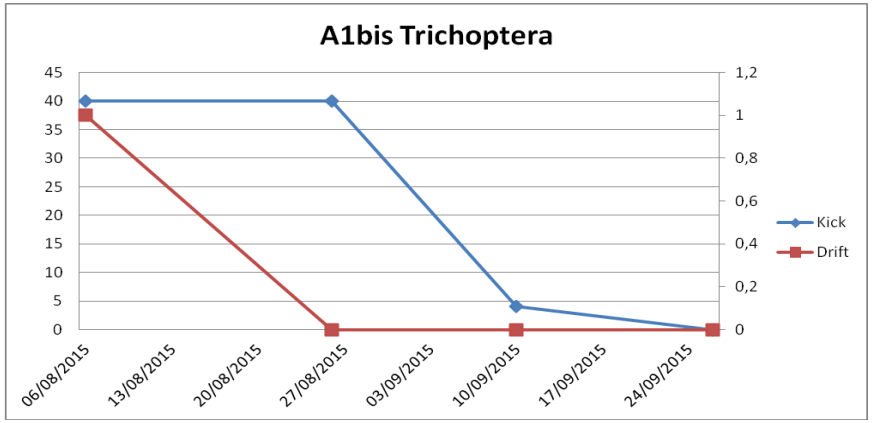

Figure 47. Trend of Trichoptera order in A1bis station (X axis sampling days, Y axis number of organisms).

4. Discussion

This study has revealed that Diptera is the most abundant taxon in terms of number of individuals in all samples and stations. Within Diptera, Chironomidae (suborder Nematocera) were the dominant taxon in the kick and drift samples in both glacier-fed and non-glacial streams, confirming what previously found by Lencioni and Rossaro (2005).

In contrast, Empididae (Brachycera suborder) were the dominant taxon in the emergence and malaise samples.

Kick

According to literature (Schültz 1999; Füreder et al. 2000; Gislason et al 2000; Füreder et al. 2001), we found clear differences in the benthos of the two streams: a higher biodiversity was recorded in the non-glacial stream than in the glacier-fed stream due to the severe environmental conditions of the glacier-fed stream (lower water temperature, higher discharge and current velocity, higher turbidity etc.).

Larvae of Chironomidae were essentially the unique inhabitants of the glacial station (excluded the presence of 23 Limoniidae+Pediicidae larvae) as reported for many glacier-fed streams (Lencioni and Rossaro, 2005).

Clear seasonal differences were not evident for A1 and A1bis stations even if some peaks of abundance were detected (in the glacial stream a peak of Chironomidae was observed on 09/10/2015 while in the non-glacial stream this occurred on 07/23/2015).

Drift

As observed for the benthic fauna collected with the pond net, the drift diversity was lower in the A1 station than the A1bis station, confirming what previously found by Ward (1994); however, no statistical differences were found between the two stations, probably due to the low number of specimens collected.

No statistical differences were found among sampling dates in each station as well.

Except for Nematoda, all orders founded in the A1bis kick collection were present in the A1bis drift collection.

According to literature (Lencioni et al. 2001; Fenoglio et al. 2004) Chironomidae were the prevalent component of the drift collection during the whole sampling campaign.

Both in A1 and in A1bis station, according to Maiolini et al. (2007), we hypothesise a strong connection between drifted organisms and water transparency: the number of drifted organisms decreased with water transparency. In addition, during the first hourly sampling (July) in A1 station, 3 of 5 peaks were between 9 am and 12 am during the peak of ice melt. Future data are needed to confirm this hypothesis. The other two peaks in A1 were at 9 pm and this is in agreement with Müller (1973): the drift increases during the night.

In the second hourly sampling (September), the drift samples collected in A1 presented a trend similar to the first hourly sampling (July). A similar trend was observed in the non glacial drift.

Emergence

Species richness and insect composition in the emergence traps reflect well the taxonomic composition of kick and drift sampling: all the insects found in the emergence traps were present also in the kick and in the drift samples. One interesting, unexpected finding was the collection of 10 Plecoptera in the emergence trap in A1 station because no Plecoptera were found in the kick or drift sampling in this station. We hypothesise they might come from upstream river sectors.

In contrast with Füreder et al. (2005), the number of specimens and taxon collected with the emergence traps was comparable between the two stations and not higher in A1bis as supposed according to the benthic community structure. This was probably influenced by the relatively low number of insects captured using these traps during the whole period, partly also due to bad wheatear conditions during sampling days, especially during the two “hourly sampling”.

Weather is a major direct and indirect factor limiting the abundance of many insect species (Gillott 2005). Flight activity is related to the wind condition: strong wind hinders the air movement but, at the same time it has an important role in dispersal. Rain influences indirectly the availability and the quantity of food, but, sometimes allows the egg-laying for some mosquitoes species. Beside weather condition, temperature is a limiting factor. During sampling temperature mean was 9.25 °C during the hourly sampling of July and 2.44 °C during the hourly sampling of September.

Temperature affects the activity of some insect species during emergence movement (Gillott 2005). According to Danks & Oliver (1972) in Chironomidae family an increase in water temperature induces emergence while a decrease inhibits it.

Although the study of Füreder et al. (2005) was based on a larger data set (sampling occurred over 1 year with 28 traps), we aimed at verifying their hypothesis: "*still moderate condition during morning hours before increase discharge around noon and maximum in the late afternoon, might enhance the ability to emerge at this time of the day even in periods of seasonal floods and harsh environmental conditions*". This was verified for Chironomidae during both hourly samplings (despite the low number of collected insects).

Malaise

No aquatic insects except Diptera and 30 Plecoptera were captured during the whole period with the malaise trap. This is in agreement with the taxa diversity observed with the other sampling methods.

Remarkably, the number of Empididae families were higher than the Chironomidae however these insects might come from neighbourhood habitats.

The Malaise traps had the highest terrestrial species richness and composition of any other sampling methods, as expected by their structure and position off the river flow.

During the hourly sampling no specific trend was highlighted with the malaise trap. Nevertheless, individuals of the Brachycera suborder were numerically prevalent than Chironomidae in July, while in September the number was closely equal. One explanation for this interesting finding is that only in July Brachycera suborder was represented by both terrestrial and aquatic taxa, therefore increasing the total abundance of this suborder.

An interesting pattern was observed with the evaluation of total hourly intensity (both July and September sampling) for the malaise trap (July): the Chironomidae have a sine wave during the day with the highest peak at 9 am and the lowest at 6 pm.

Seasonal pattern considering all data

Chironomidae in kick and drift samples represented almost the total component of Diptera order as also reported in literature (Ward 1994).

In this sense, the pattern of Chironomidae family in the glacier-fed stream exhibited an opposite trend when comparing kick and emergence patterns: an increase in the number of specimens collected with the pond net corresponded to a decrease in the number of emerged specimens.

Vice-versa, an decrease in the number of specimens collected with the kick sampling corresponded to an increase in the number of the emergence organisms.

This proportionally inverse relation reflects the life cycle of insects.

We observed a positive correlation when comparing drift and emergence patterns and between the malaise and emergence pattern for Chironomidae in A1bis station.

in the glacier-fed stream, we observed the same trend for Plecoptera in malaise traps as expected for these species, which are good flyers (Krest and Anderson 1973).

In the non-glacial stream, an autumnal peak of abundance (September) was observed for adult Plecoptera as known for stoneflies (Fureder et al., 2005).

5. Conclusions

The main goals of this study were to (1) implement knowledge on benthic and drift community in alpine streams with different origin and (2) better understand the patterns of insects' emergence in both glacial and in non-glacial streams by applying two different sampling strategies: the biweekly and hourly sampling. In particular, we focused on diurnal pattern from 6 am to 9 pm, rarely assessed in previous similar studies.

Our results improved the knowledge on the macrobenthic fauna in these remote areas, confirming the efficacy of kick and drift sampling techniques for the study of benthic fauna.

Emergence traps showed only few statistically significant trends. Rain, wind and fog compromised the sampling collection during the hourly sampling.

The application of emergence traps has also revealed a set of problems in the streams with strong discharge variation, suggesting caution in using them in alpine rivers.

During this study a higher number of aquatic adult insects was captured with malaise traps than with emergence traps. This suggests a better efficiency of malaise trap in these kind of studies, despite the non specificity for aquatic insects and the possibility that insects might come from neighbourhood habitats.

In order to improve this approach in high altitude stream we suggest extending the sampling plan over more days, implementing it every three hours. We also suggest to increase the number of emergence traps coupled with malaise traps.

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ACKNOWLEDGEMENTS

I would first like to thank Dr. Valeria Lencioni of the Museo delle scienze di Tento (MUSE) for her knowledge, kindness and for her manuscript reviews.

I am especially grateful to my thesis advisor Dr. Chiara Papetti, without her genuine enthusiasm, her passionate participation and input, this thesis could not have been successfully conducted.

I would also like to thank the experts who were involved in the validation survey for this research project: Alessandra Franceschini and Francesca Paoli for their field support and Francesco Bellamoli for his technical advices.

I also wish to thank Parco Adamello – Brenta for providing access to the study area, Meteotrentino for the historical data of temperature, precipitation and solar radiation, and Museo delle Scienze di Trento (MUSE) for the physico - chemical parameters of Sarca d'Amola of 2014.

Finally, I must express my authentic gratitude and friendship to: Matteo Planchestainer, for his infinite willingness and his "harsh" comments, Martina Saccani for her kindness and her English lessons, and Luca Bellini for his technical calls from Torino.

Last but not least, the deepest thanks to Teresa, my nudge.