MARGHERITA LUCADELLO

METAZOAN FAUNA OF CRYOCONITE HOLES IN HIGH ARCTIC GLACIERS OF SVALBARD, ECOLOGICAL FEATURES

UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS E TECNOLOGIA

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Mestrado em Biologia Marinha

Trabalho efetuado sob a orientação de:

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DECLARAÇÃO DE AUTORIA DE TRABALHO

METAZOAN FAUNA OF CRYOCONITE HOLES IN HIGH ARCTIC GLACIERS OF SVALBARD, ECOLOGICAL FEATURES

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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RESUMO

Os buracos de crioconita são pequenos habitats que se encontram na zona de ablação das superfícies glaciares de todo o mundo. Estes buracos surgem quando partículas inorgânicas e orgânicas (incluindo microrganismos), assentam no gelo e acabam por promover o aquecimento e consequente derretimento do gelo, porque absorvem mais radiação solar, devido ao seu albedo mais baixo em comparação com o gelo circundante.

Estes buracos são ecossistemas dinâmicos que podem ser encontrados em três diferentes estados – abertos, submersos e fechados – dependendo da temperatura, das descargas de sedimentos, dos fluxos da água e da radiação solar que os influencia. Normalmente, os buracos de crioconita de locais similares atingem profundidades de equilíbrio iguais.

Nos sedimentos dos buracos de crioconite encontra-se um pequeno ecosistema povoado por várias comunidades biologicas de bactérias, algas, fitoflagelados, fungos, vírus, nematóides, rotíferos, tardígrados e diatomáceas. Uma vez que o sedimento das crioconitas representa a principal fonte de nutrientes e microorganismos dos buracos, é expectável que a estrutura da comunidade de invertebrados varie de glaciar para glaciar, que seja semelhante à dos sistemas aquáticos próximos e que dependa dos anos que passaram desde a última deglaciação.

Tardígrados e rotíferos (eucariontes bilaterais) são os únicos invertebrados encontrados nas condições extremas dos buracos de crioconitas. Tardígrados são microrganismos que atingem 0,5 mm de comprimento, são geralmente fitófagos ou bacteriófagos, embora alguns possam ser carnívoros e comer outras espécies de tardígrados de menores dimensões. Geralmente encontram-se num estado de atividade vigorosa e geralmente rastejam quando não conseguem nadar. Os rotíferos variam entre os 0,1 a 0,5 mm de comprimento, são principalmente onívoros, alimentando-se de materiais orgânicos mortos ou em decomposição, algas unicelulares, bactérias, protozoários, fitoplâncton e outros produtores primários aquáticos. Assim como os tardígrados, algumas espécies apresentam uma dieta canibal. Os rotíferos movem-se, principalmente, por natação livre ou rastejando ao longo de um substrato, aderindo a cabeça e o pé ao substrato, em etapas alternadas. Ambos podem viver em quase todos os habitats da Terra, sobrevivendo facilmente em ambientes com condições extremas, experimentando um processo conhecido como criptobiose que lhes permite entraram num estado de latência. Tardígrados e rotíferos geralmente representam os roedores ou predadores na rede alimentar dos buracos de crioconita, tendo um importante papel no controlo top-down (de cima para baixo) em comunidades de bactérias e microalgas. Uma vez que as fontes de alimento das quais os tardígrados e rotíferos se alimentam são as mesmas, é provável que exista competição entre estes dois filos de invertebrados. Ademais, deve ser tido em consideração que as técnicas de alimentação destas duas espécies são diferentes. O objectivo deste estudo é compreender as interações entre estes metazoários presentes em buracos de crioconita e sua interação com o ambiente. Este projecto de investigação ocorreu em Svalbard, um aquipélago localizado na região Ártica. O estudo do Ártico e das regiões polares em geral é importante, tendo em conta que estes locais são bons indicadores de mudanças naturais e antrópicas no nosso planeta. O estudo dessas regiões é útil, uma vez que regiões polares e temperadas compartilham princípios ecológicos semelhantes embora em regiões polares as relações ecológicas são geralmente mais simples e menos diversificadas. O estudo de metazoários em buracos de crioconita pode revelar informações relevantes sobre a evolução da vida e a biodiversidade em ambientes extremos. Foram amostrados 150 buracos de crioconita de nove glaciares diferentes, em três áreas distintas para esta investigação em Svalbard, entre junho e agosto de 2017, sendo registados os seguintes parâmetros: variáveis geográficas (latitude, longitude, elevação, tipo glaciar), variáveis estruturais (estrutura do buraco de crioconita como área e profundidade), variáveis biológicas (presença e abundância de metazoários em crioconitas), variáveis sazonais (impacto do tempo de amostragem em parâmetros das amostras) e variáveis ambientais (presença de aves, características da área como a disponibilidade de sedimentos ou fluxos de água derretida e condições meteorológicas durante o período de amostragem). Foram efectuada análises uni e multivariadas para determinar o impacto das variáveis que determinam a presença e abundância dos metazoários nos buracos de crioconita de Svalbard. Os parâmetros ambientais, a variabilidade sazonal e as variáveis biológicas como a presença e abundância de outros metazoários são os mais importantes (com diferentes níveis de dependência) na presença e abundância de tardígrados e rotíferos. Ao invés disso, a estrutura dos buracos e o tipo de glaciar não parecem causar impacto nas populações de metazoários dos buracos de crioconita. A abordagem fragmentada do estudo de metazoários nos buracos de crioconita e dos buracos de crioconita conduziu a um quadro incompleto sobre sua interacção. Consequentemente, extrapolar o seu comportamento em diferentes escalas espaciais e temporais ou como estes metazoários responderão às mudanças das condições ambientais com os dados actuais torna-se um exercício especulativo. Para melhor entender estes metazoários num ecossistema tão particular como o dos buracos de crioconita será necessária uma aproximação integrada e abrangente, que tópicos relacionados com a alimentação, reprodução, e estratégias de persistência.

Palavras-chave: glaciares de Svalbard, furos de crioconita, tardígrados, rotíferos, ecologia

ABSTRACT

Cryoconite holes are small habitats found on the ablation zone of glacier surfaces worldwide. They are created when dust (a mixture of inorganic and organic particles, including microorganism), mainly windblown on the glacier, absorbs more solar radiation then the surrounding ice due to a lower albedo. The heating makes the ice melting underneath and the sediments sinking into the hole of melted water. This study aims to understand the interactions between the metazoans presents in cryoconite holes and their interaction with the environment. From June 2017 to August 2017, 150 cryoconite holes from nine different glaciers from three different areas were sampled for this research. Multivariate and univariate statistic was run to understand the impact of the variables driving the presence and abundance of the metazoans in the Svalbard's cryoconite holes: geographic variables (latitude, longitude, elevation, glacier type), structural variables (structure of the cryoconite hole as area and depth), biological variables (presence and abundance of metazoan in cryoconites), seasonal variables (impact of the sampling time on parameters of the samples) and environmental variables (bird's presence, area's features as sediment input or flows of melted water, and weather conditions of the sampling period). The variables were tested for significant differences between the nine glaciers and the three areas, and the outcomes discussed. Dependency levels and interconnected impacts of these biotic and abiotic variables were hypothesized. From the analyses of the results, environmental variables, seasonal variability and biological variables are proposed as most critical controls with different levels of dependency. Instead, cryoconite holes structural variables and the geographical variables are proposed to do not have an impact as a driver of the presence and abundance of the metazoan populations of cryoconite holes.

Keywords: Svalbard glaciers, cryoconite holes, tardigrades, rotifers, ecology

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ACKNOWLEDGMENTS

Deciding to work for this master thesis project allowed me to learn what I would have never learnt, to see places I would have never seen, to meet people I would have never met and live adventures that I would have never lived. This project brought me from Portugal to Svalbard and from there to Czech Republic. I have grown personally and professionally living in a research station for two months in between icebergs and polar bears, rifles, glaciers, and a lot of tardigrades, rotifers and cryoconite holes. All this would have not been possible without the help of my family and of my supervisors. First of all, thanks to mamma Patrizia, sorella Virginia (e Stefano), papà Giuseppe e la giovane Clara. Besides, I want to thank Miloslav Devetter, and Bob the dog for the great possibility and the space that they left me in the lab, and I want to profoundly thanks Marie Šabacká and Rita Casthilo for the precious help that I received from them. Last, but not least, a special thanks goes to who helped me to start this project, to who helped me keep going with it during the sampling months and who helped me ending it. Packo!

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a.s.l.: above sea level

CPE: Centre for Polar Ecology; http://polar.prf.jcu.cz/index.php?id=home / CPE

Sd: standard deviation of the mean

E: Ebbabreen

GS: Gåsbreen

HA: Hansbreen

HC: Hørbyebreen

HD: Hornsund målestasjon (99754)

HO: Hornbreen

NA: not available

NAN: Nansenbreen

NC: Nördenskildbreen

PY: Pyramiden observation station (99880)

R: Rotifers

SEF: Sefströmbreen

T: Tardigrades

TO: Torellbreen

UAlg: Universidade do Algarve; https://www.ualg.pt/pt

CHAPTER 1

1. Introduction

Cryoconite holes have been recognized as an important habitat as early as 1936 (Steinböck *et al.* 1957) but only in recent years that they have been recognised as important hydrological and biological systems in cold environments. However, being the Arctic itself and the Arctic glaciers an extreme environment difficult to reach and expensive to study, the available information on the metazoan fauna of cryoconite holes in high Arctic glaciers of Svalbard are still limited. Worldwide there are a high similarity between different cryoconite holes habitats in which the same main primary producers and ecosystem dynamics are presented (Anesio *et al.* 2017). However, the main features driving the metazoan presence and the composition of cryoconite holes' environment are not yet established. The influence of the cryoconite hole location, of the type of glacier, of the cryoconite hole structure, of the micro community composition and of the sampling time is also still unclear.

The study of the Arctic and of polar regions in general, it is important since these places are exceptional indicators of natural and anthropogenic changes in our planet. Indeed, polar regions are more affected by environmental changes than the rest of the world (Holland *et al.* 2003). The study of these regions is useful since polar and temperate regions share similar ecological principles (Gaston *et al.* 2000). Furthermore, since most of temperate regions were glaciated during the last glacial maximum, these regions are now evolving, and the evolution of periglacial environment is a great model of how we can study long term nature development (Schirrmeister *et al.* 2008). Moreover, ecological study of extreme polar environment, such as cryoconite holes, can be also used as a model for the study of life in Martian Life in the Solar System by astrobiologists (Schulze-Makuch *et al.* 2005). In fact, researchers have pointed out the utility of cryoconite hole components as proxies for larger systems, for example as refuges for life on other planets (MacClune *et al.* 2003), Snowball Earth theory (Vincent *et al.* 2000; Porazinska *et al.* 2004) or as predictors of regional climatic changes.

The study of metazoans in cryoconite holes is giving us information about life evolution and the biodiversity in extreme environments, being cryoconite holes a geographically isolated and extremely localised ecosystem.

The microbial communities that populate cryoconite holes show distinct interactions with their physical environment (Anesio *et al.* 2017). Recently, some of these interactions have already been object of study. In this thesis project, more sampling locations and more sampling variables are considered compared to the previous studies (Vonnahme *et al.* 2015, Zawierucha *et al.* 2016). The variables were analysed with univariate and multivariate statistic and a wider overview about the interactions among the metazoans in cryoconite holes and their environment was obtained.

1.1 Glaciers

Extended frozen areas of the planet Earth are designated as cryosphere. These areas are characterized by remarkably low temperatures turning liquid water into ice. The cryosphere is composed by snow, sea ice, ice shelves, ice sheets, glaciers, ice caps, permafrost continuous, permafrost discontinuous or permafrost isolated (Figure 1.1.).

Figure 1.1. Cryosphere map (Ahlenius *et al*. 2018).

Glaciers are persistent bodies of dense ice moving under its own weight. They constitute presently 10% of the land area on Earth in form of ice caps and ice sheets, which correspond to 15 million km² and represent the greatest reservoir of fresh water (NSIDC, 2018). Glaciers are formed by snow transformation process caused by changes in pressure and temperature on the snow layers. Their ice is characterised by constant downslope or outward movements due to the force of gravity.

The masses of ice are constantly affected by stresses induced by their own weight that are making the ice to deform and flow, abrading rocks as a result. Stress events are the explanations why some of the englacial system are formed. Englacial system are meltwater system that are present within the glaciers. They can include crevasses, glacier mill, vertical holes of different diameters that usually transport meltwater towards the bottom of the glacier. Moreover, the movement of the glacial mass could cause moraine, debris and rocks on the glacier surface or margin, usually moving collectively with the glacier (Benn *et al.* 2014).

Glaciers have a complex drainage system divided in subglacial drainage system, a system of meltwater channels underneath the glacier, and in supraglacial system, a meltwater system on the glacier surface. The movement of the water mass along the glaciers is affected by turbulences that are also affecting the distribution of organic matter and nutrients both at supraglacial and subglacial level (Poraziska *et al.* 2004). These are influencing the formation of melting areas and of new habitats as well. The factors that are disturbing the drainage system are not linked with the intra-granular permeability of the ice, that is instead insignificant, nonetheless the factors are associated with the macroscale structure of the glacial drainage network (Paterson *et al.* 2008) (Figure 1.2.).

The process leading to the glacier formation starts with overlying snow subjected to pressure that causes recrystallization of the snow underheat and increasing grainsize of the snowflakes. Therefore, snow loses air bubbles and its density slowly increases until it reaches that of ice. By continuing this process, the ice state is reached. The formed ice can present different characteristics in density, air content, temperature and colour, depending on the age and the position on the ice in the glacier column. (NSIDC, 2018)

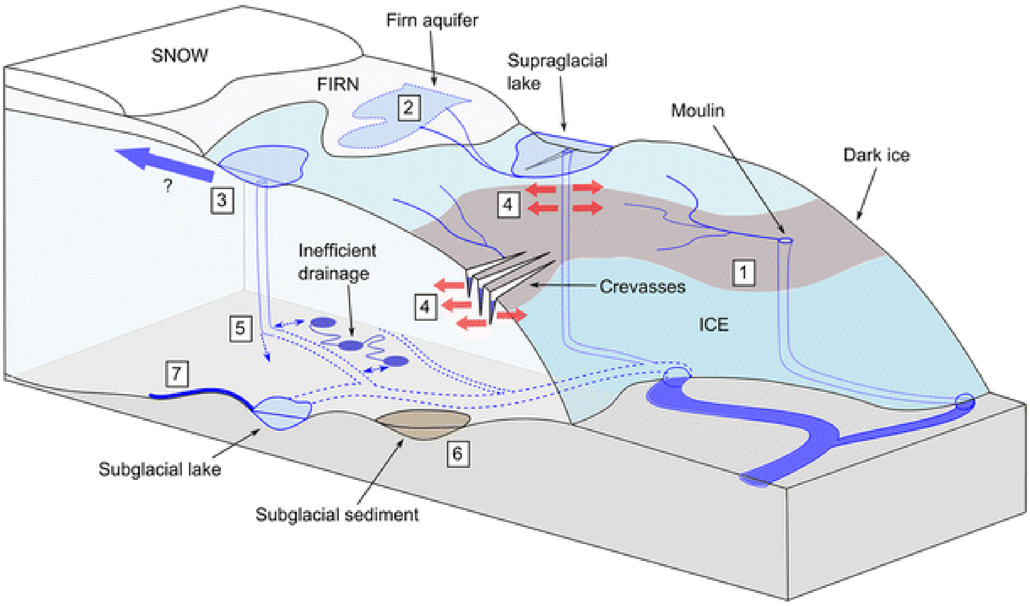

Figure 1.2. Schematic illustration of glacier drainage system with land-terminating ice sheet, highlighting the main meltwater pathways and stores in the hydrological system. (1) Darkening of the ice sheet, (2) surface firn densification processes, (3) surface to bed connections at higher elevations, (4) cryo-hydrologic warming, (5) rates of channelization at the ice bed interface, (6) subglacial sediments and till deformation, and (7) basal melt rates. All these processes are also relevant to tidewater glacier systems (Nienow *et al.* 2017).

The basal temperature of a glacier is an important pivotal factor and glaciers can be cold-based and/or warm-based. The cold-based glaciers have an underlying substrate that is frozen. The warm-based glaciers are over or at a freezing temperature in the interface and warmer at the base. Glaciers which are partly cold-based and partly warm-based are known as polythermal. Polythermal glaciers have an active subglacial drainage system and they present an area of the glacier that is frozen on the bed rock and another area that is made by water flowing underneath (Irvine-Fynn *et al.* 2011).

Although glaciers surface seems quite uniform in appearance, they are divided in different zones due to the difference in behaviour of the glacier ice depending on their compactness, density, internal temperature or temperature at the glacier base or on the characteristics of the rocky substratum. The accumulation zone is characterized by snow falling during winter time not melting during the hot season. This represents the glacier intake. Ablation zone instead represents zones of ice loss troughs melting and icebergs formation. It is possible to recognize the accumulation zone in summer because it is a white surface covered with snow and firn, while the ablation zone shows ice is called "black ice" since is usually looking soiled. The extension of the two zones is not fixed: in fact, they are bounded by the equilibrium line, which coincides, roughly, with the limit of perennial snows (Figure 1.3.) (Nienow *et al.* 2017).

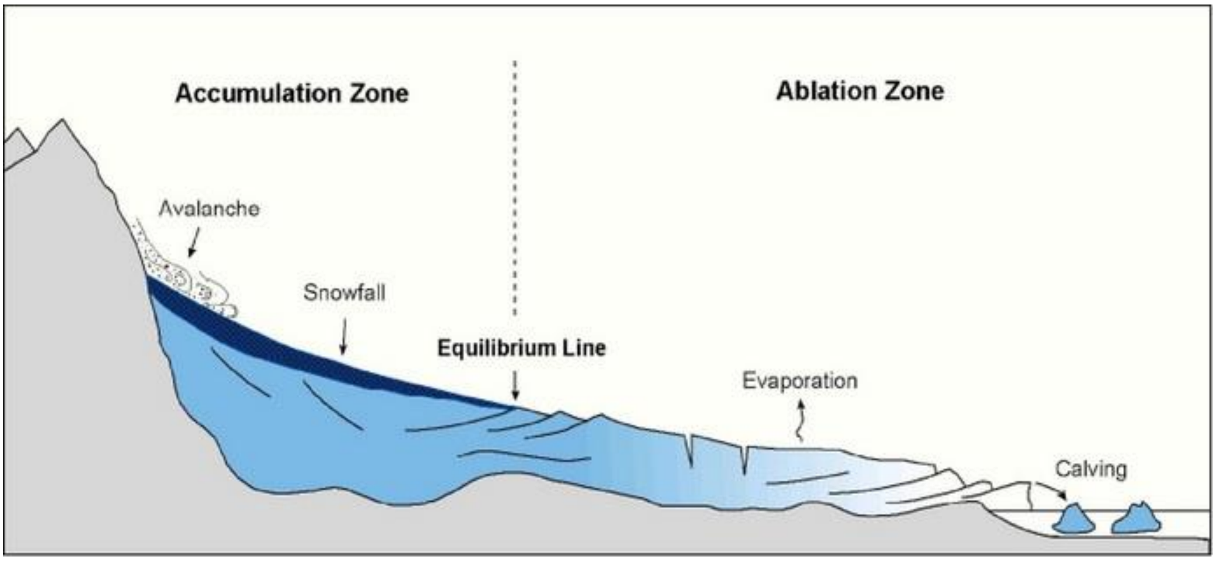

Figure 1.3. Glaciers areas. Graphic representation of accumulation zone (characterised by avalanche, snowfall and a general ice intake) and ablation zone (characterised by evaporation, calving and a general ice output) (Nienow *et al.* 2017).

There are different types of glaciers. Located at high elevation in small areas there are mountain glaciers. Cirque glaciers, valley glaciers, tide water glaciers, piedmont glaciers, ice cup or continental glaciers are all sub groups deriving from mountain glaciers. Cirque glaciers are small glaciers that can be found in depressions on the sides of the mountains. Valley glaciers are cirque glaciers that while growing follow the topography of the area in which are located. Tide water glaciers extended to the sea level carving a valley down into the coast. Piedmont glaciers are valley glaciers that extended down a valley and covered a slope beyond the mountain range. Instead, the largest type of mountain glaciers is defined as ice cups or continental glaciers which can include other smaller glaciers (NSIDC, 2018).

1.2. Svalbard

Svalbard is a Norwegian archipelago located in the Arctic Ocean positioned between 74° and 81° N latitude and in between 10° and 35° E longitude (Figure 1.4.). The main and largest island is Spitsbergen, other islands include Nordaustlandet and Edgeøy. Despite the archipelago position, Svalbard's mean annual temperature is kept moderate by the presence of the West Spitsbergen current into the Barents Sea, an extension of the Gulf Stream. Svalbard is characterized by five different seasons: light winter, spring, summer, autumn, dark winter. The snow usually starts melting in spring during May. Once the snow melts, in summer, small flowers bloom, several migrating birds appear for their nesting season and the glaciers start calving. Svalbard's summer is characterized by the presence of the midnight sun, during these months generally is moving from zero to eight degrees Celsius with insignificant day/night temperature excursions. Large inter-annual differences in mean precipitation and temperatures do occur. The warmest month of the year is July, with a mean temperature of 6.2°C (Hagen *et al.* 1993).

About 60% of Svalbard is glacier-covered, these areas are continuously retreating since the end of the little ice age around 1910 (ending in Svalbard during 20th century). Most glaciers in the dry interior of Spitsbergen are cold-based and move only one-two meters per year. In more humid regions along the coasts, glacier velocities frequently are higher than 10-30 m per year, causing large crevasses to form. Nearly 30% of glaciers in Svalbard are surging-type glaciers (but possibly up to 60%), which is characterised by switching between a surging phase lasting months or years, when the glacier is rapidly advancing due to increase in velocity; and a quiescent phase, when the glacier remains inactive for 50-100 years (Dowdeswell *et al.* 1999, Hambrey *et al.* 2005).

Figure 1.4. Landscape types of Svalbard archipelago (Norwegian Polar Institute).

Cryoconite holes are present only in high alpine and high latitude areas, as well as glaciers, where the low melting rates are allowing them to form not washing off the sediments from the surface

(MacDonell *et al.* 2008) (Figure 1.5.). They are one of the most productive cryo-habitat of the snow-free area of the glaciers. The first cryoconite holes have been documented in 1870 in Greenland by Adolf Erik Nordenskiöld, a Swedish scientist describing it as water filled depressions on the surface of the ice sheet annoying him during his crossing of Greenland. Cryoconite holes are most common in the supraglacial ecosystem in the ablation zone, areas often affected by weathering crusts and ablation cusps, ideal for storing sediment (Fountain *et al.* 2004, Hodson *et al.* 2008).

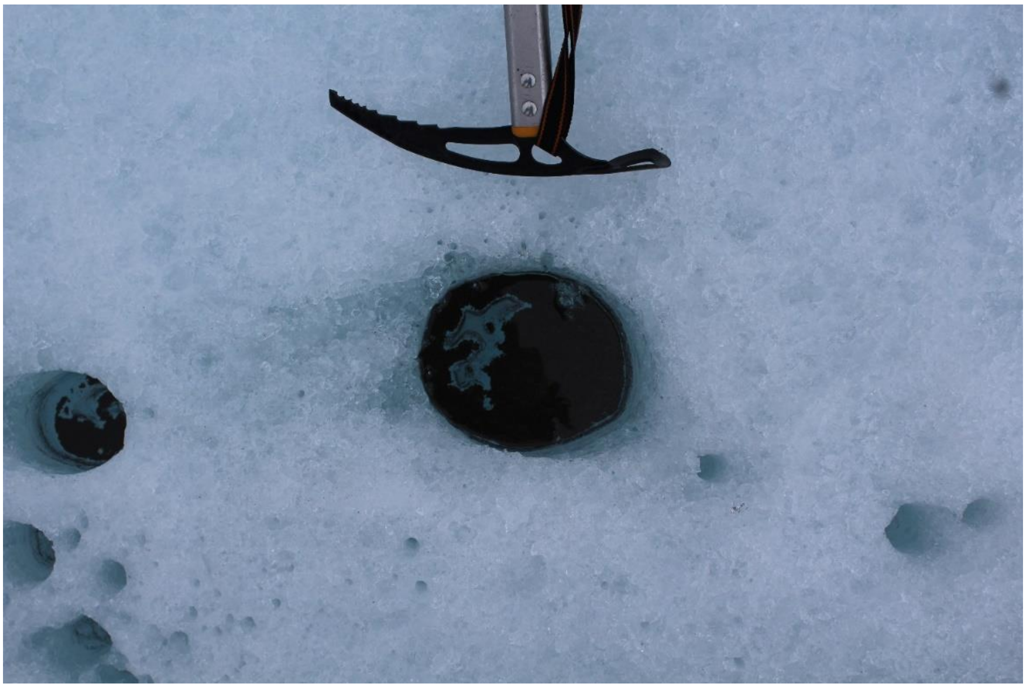

Figure. 1.5. Cryoconite hole (© Margherita Lucadello).

1.3. Cryoconite holes

Cryoconite holes powder is sinking into the ice due to the lower albedo of the sediment compared to the surrounded ice and due to the increased absorption of shortwave radiation of the sediment area (Adhikary *et al.* 2000, Takeuchi *et al.* 2001, MacDonell *et al.* 2008) (Figure 1.6.). This sediments layer must be at least two millimetres thick (MacDonell *et al.* 2008). The formation process is influenced by the timing of the sediment loading on the surface and by the melting point period. When the sediment load occurs exactly before the melting point period, the whole formation reaches his maximum level (Adhikary *et al.* 2000, Hodson *et al.* 2008).

Cryoconite holes are dynamic ecosystems that can become enclosed in new depressions of the ice or washed into streams depending on the environmental conditions (Takeuchi *et al.* 2001; MacDonnel *et al.* 2008; Shelley *et al.* 2008). A cryoconite hole contains melted water unable to leave the hole because of the permeability of the hole ice walls. They can be found in several conditions, in an open state (melted water pot exposed to the atmosphere), in a submerged state (melted water covered by a layer of air covered by a thin layer of ice) or in a close state (absence of air layer, all cryoconite hole's components such as melted water, sediments are frozen), depending on the temperature and on the weather conditions (Porazinska *et al.* 2004).

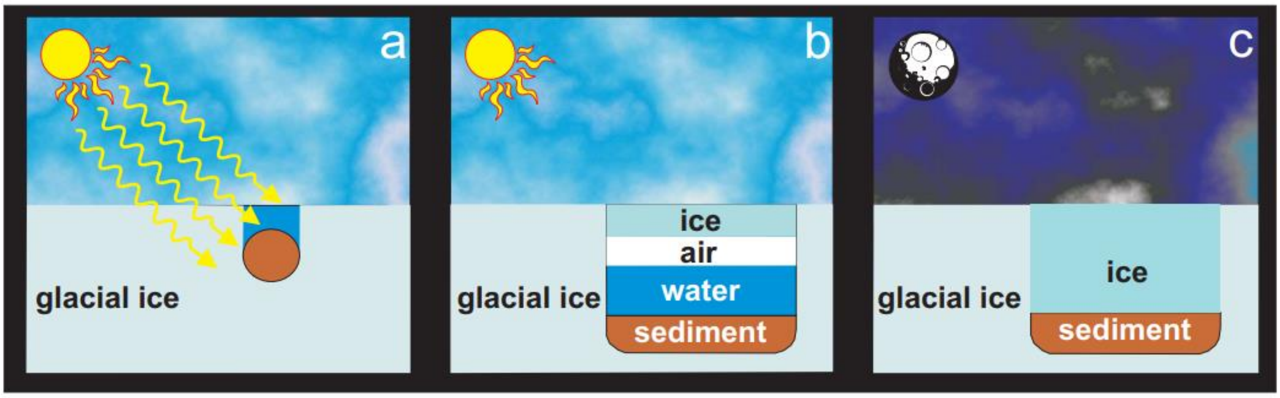

Figure 1.6. Cryoconite hole formation and behaviour in different seasons. In summer, sediment accumulates on glacial surfaces, and exposure to solar irradiation produces (a) melt pools within the ice, which may subsequently freeze on the surface with the end of the summer (b), (c) and completely freeze during the winter (Priscu *et al.* 2004).

Moreover, addition of new sediments causes widening of the hole because of the lateral thermal conduction from the debris to the hole wall, causing the widening of the cryoconite holes by melt and a redistribution of the sediments. As the hole widen, the layer of sediments becomes thinner. This can also reduce the self-shading effect and thus maximise the exposure of cryoconite to solar radiation

(Cook *et al.* 2010). Therefore, solar radiations, temperature, flows of water and sediments input are the environmental conditions to which the cryoconite holes' state is depending.

The shape of the hole is driven mainly by the distribution of the sediments on the glacier surface and by the shortwave radiation patter that is received by them (Porazinska *et al.* 2004, Hodson *et al,* 2008). According to the energy fluxes and the sediments distribution, cryoconite holes can have a diameter going from few cm to several meters (Hodson *et al.* 2008). Usually, holes from a similar site are reaching an equal equilibrium depth (Podgorny *et al.* 1996). This can be affected by changes in albedo parameters, variations in the ice grain dimensions, variations in the density of air bubbles, variations in the size of intergranular spaces and in the proportions of air and water (Hodson *et al.* 2008). Moreover, the morphology of the wall is also related to the path of the sun during the melting period (Wharton *et al.* 1985; Christner *et al.* 2003). Indeed, it seems that the shape of the internal area (wall) of the cryoconite holes is different weather if the hole is located at high latitude or at low latitude. The more circular sun's path of polar region seems to affect the shape of the cryoconite' wall shaped more circularly as well. Instead, at lower latitude, the cryoconite wall seems to form a D-shaped wall, always in dependence of the sun's path (Mc Intyre, 1984).

1.4. Invertebrates in cryoconite holes

Where there is liquid water there is life and glaciers are not an exception. Cryoconite holes shows enough liquid water to be able to sustain a variability of microbial life (Anesio *et al.* 2017). Cryoconite holes' powder represent the main source of nutrients and microorganisms for cryoconite holes. The origin of the nutrients input to cryoconite holes are affected by changes to the external sediment income. Sediments can be blown by the wind, brought by the water flush or by the birds (Porazinska *et al.* 2004). The presence of flying birds and bird's remnants (excrements, carcasses) are a proxy for cryoconite hole's nutrient inputs. Bird's guano is highly nutritive, and its presence or absence is affecting the cryoconite holes' micro-communities (Vonnahme *et al.* 2015). Thanks to the presence of water and nutrients, cryoconite holes ecosystems are inhabited by several biological communities of bacteria, algae, phytoflagellates, fungi, viruses, nematodes, rotifers and tardigrades (Anesio *et al.* 2017; Zawierucha *et al.* 2015). Mainly, cryoconite holes' communities are composed by bacteria, cyanobacteria, phytoflagellates, algae, and micro invertebrates (Shain *et al.* 2001, Takeuchi *et al.* 2001, Sawstrom *et al.* 2002, Porazinska *et al.* 2004, Priscu *et al.* 2004; Kastovska *et al.* 2005).

Because the micro communities are depending on the local source, their community composition is expected to differ from glacier to glacier and/or like aquatic systems nearby. Cryoconite holes might also act as inoculum for proglacial systems. The community structure in meltwater channels and cryoconite holes is similar (Vonnahme *et al.* 2015) and the micro-communities are also shared with the neighbouring deglaciated areas. The micro-communities present on the deglaciated areas are depending on the age of the deglaciation. Indeed, in recently deglaciated area the soil will not show vegetated areas, when, in hundreds of years old deglaciated areas the vegetated areas are present. In these different conditions the micro-communities inhabiting these areas are different, they usually show a higher presence of invertebrates on the older soil substrate (Kaufmann *et al.* 2001, Stibal *et al.* 2008).

The most abundant invertebrates in glacial ecosystems worldwide are tardigrades and rotifers, as well in cryoconite holes (De Smet *et al.* 1994; Grongaard *et al.* 1999). In order to survive in cryoconite holes, establishing a stable population, they required preadaptation to withstand freeze thaw cycles, high UV radiation, low nutrient environments. Typical preadaptation are: cryptobiosis (tolerance to the cold by slowing metabolisms to ~0.01% of normal activity and losing up to 99% of the total moisture), anhydrobiosis (desiccation tolerance) and resting stages (tun state) (Sømme *et al.* 1996). Moreover, to maximize their dispersal, tardigrades and rotifers use parthenogenic reproduction (de Smet *et al.* 1994) and wide distribution abilities such as the possibility of dispersal by wind and overflying birds (de Smet *et al.* 1994; Grongaard *et al.* 1999).

The first investigations of the tardigrades of Svalbard took place in the early 20th century and nearly 30 papers on the subject have been published to date (Zawierucha *et al.* 2013). Typically, a single species of bdelloid rotifers and a single species of tardigrades is dominating the entire system. For instance, it was found that the dominating tardigrade species on a Svalbard glacier was *Isohypsibius granulifer* (90%) (Grongaard et al, 1999) and that the rotifer *Macrotrachella insolita* represents the 99% of the rotifer fraction of the same glacier (Hyrnebeen) (Smet et al, 1994). But generally, several species have been found in the same area (Zawierucha *et al.* 2013).

1.4.1. Tardigrades

Tardigrades are commonly known as water bears because of their appearance, but the term tardigrade literally means "slow stepper" describing the bear's gait rather than the speed with which they move. Indeed, water bears are usually in a state of vigorous activity, they usually move crawling as they are unable to swim (Shcherbakov *et al.* 2010) (Figure 1.7.). Tardigrades are bilaterally symmetrical, segmented and they present a pair of legs in each of the three body segment, followed by a caudal segment with a fourth pair of leg.

Figure 1.7. Microscope picture of a tardigrade (© Robert Pickett).

The first three pairs of legs attached to their cylindrical body have motile functions, meanwhile, the last segment is primarily for grasping the substrate. Water bears are considerably small, up to 0.5 mm. The mouth of the tardigrade presents a sucking tube with a circular external portion, and a pair of arming stylets that are used to pierce their nutrient source (Kinchin, *et al.* 1994, Schmidt-Rhaesa *et al.* 2001). Most tardigrades are phytophagous (plant eaters) or bacteriophagous (bacteria eaters) but some are carnivorous and can eat other smaller animals such as nematodes, rotifers and tardigrades (Seki *et al.* 1998, Jönsson *et al.* 2008).

Tardigrades have been found in several and diverse types of habitats, from the highest mountains to the deepest sea, from the tropical rain forest to the Antarctic and Arctic area (Seki *et al.* 1998, Jönsson *et al.* 2008). They can sustain prolonged period of dehydration but need liquid water to stay active and are thus mostly found in areas where liquid water is present for at least a short period (Nelson *et al.* 2000). Tardigrades can stand temperatures as low as -200 °C and as high as 151 °C, they can survive to freezing and thawing processes, changes in salinity, lack of oxygen, lack of water, high levels of X-ray radiation, some noxious chemicals, boiling alcohol, vacuum and high pressure (Seki *et al.* 1998; Hengherr *et al.* 2009, Persson *et al.* 2011). To survive environmental stress, tardigrades experience a process known as cryptobiosis, which can be further divided into: anhydrobiosis (surviving prolonged periods in dehydrated states), cryobiosis (surviving sub-zero temperatures), osmobiosis (increased solute concentration, such as salt water), anoxybiosis (lack of oxygen). In this state several metabolic activities move into a reversible stand still. Tardigrades can stay in a cryptobiotic state for years, from which they are able to recover successfully within hours, once conditions become favourable again (Sømme *et al.* 1996). Nevertheless, tardigrade cannot be considered extremophiles (organism that prospers in physically or geochemically extreme conditions) because they are surviving these conditions and are not anatomically or behaviourally adapted to live in them, indeed, the possibility for them to die is increasing more and more with the time of exposure to the extreme environment (Sømme, *et al.* 1996).

1.4.2. Rotifers

Rotifers means “wheel bearer” referring to the cilia that are present around the mouth apparatus and that move similarly to a wheel (Figure 1.8.). The phylum rotifer contains approximately 1850 species of bilateral eukaryotes. They are microscopic aquatic animals, usually inhabiting freshwater environment and moist soil, such as lake bottoms, flowing water, rivers and streams (Örstan, 1998).

Figure 1.8. Microscope picture of a rotifer (© Chloe Givens).

They are small size (up to 0.1–1 mm) long soft body multicellular invertebrate with an externally segmented body, telescopic, semi flexible, extendible and with a transparent cuticle covering it. Rotifers are mainly free swimming, or they are moving with an inch-worming movement along a substrate taking alternate steps while attaching at the substrate with the head and tail. Rotifers are characterised by the presence of a complete digestive tract. Their diet is primarily omnivorous, and they can eat up to 10 µm in size particles. Rotifers can feed on dead or decomposing organic materials, unicellular algae, bacteria, protozoans, phytoplankton and other water primary producers. Nevertheless, it has been noticed that some species present a cannibalistic diet and are feeding on other rotifers.

Rotifers can also enter cryptobiosis, which enables them to survive high desiccation (Lapinski *et al.* 2003), low humidity and low temperatures as well as tardigrades. Moreover, they also use parthenogenic reproduction (de Smet *et al.* 1994) and wide distribution abilities as the possibility of dispersal by wind and overflying birds (de Smet *et al.* 1994; Grongaard *et al.* 1999) to maximize their dispersal.

1.4.3. Tardigrades/rotifers relationship

Tardigrades and rotifers are generally representing the grazers or predators in the cryoconite hole food web, showing their important role also as top down controller on bacteria and microalgae communities. The cryoconite food chain is likely multi trophic (Cameron *et al.* 2012). Generally, tardigrades and rotifers influence the food web feeding on ciliates or nanoflagellates, or other rotifers and tardigrades. Nonetheless, tardigrades that have been found in cryoconite holes so far were species grazing both on microalgae and bacteria or on other tardigrades or predatory rotifers, as, for example, the species of the genera *Encentrum* (Fountain *et al.* 2004). The species of rotifers that have been found in cryoconite holes are planktonic or predatory: *Keratella* sp. and *Dicranophorus permullis permullis* but also benthic species grazing on microalgae, bacteria and detritus (de Smet *et al.* 1994). The presence of omnivorous rotifers could make them become secondary consumers since these rotifers could feed on grazers as ciliates and nanoflagellates.

Since the sources of food that tardigrades and rotifers are feeding on are the same, they are likely competing for it. However, the feeding techniques of these two species are different. While rotifers show a filter feeder behaviour, often limited to small particular cells (Ricci *et al.* 2000), tardigrades can pierce their prey with stylets and feed on the released cell contents (Nelson *et al.* 2002). In this way they can possibly consume bigger cells and large colonies as *Nostoc* sp.. Hence, competition between these species might be weakened and the impact of each taxonomic group is probably different (Vonnahme *et al.* 2015).

What is driving the tardigrade and rotifers presence and abundance in cryoconite holes is still unsure. The influence of tardigrades presence on rotifers presence, and vice versa, is still unknown. Some studies showed rotifers displaying a constant greater presence compared to other invertebrates (Porazinska *et al.* 2004). In other cases, positive correlations between tardigrades and rotifer have been found (Porazinska *et al.* in 2004; Cameron *et al.* in 2012).

2. Objectives

This master thesis project aims to contribute to the study of the drivers of the metazoan presence and abundance in cryoconite holes. Biotic and abiotic interactions between the different metazoan components and the cryoconite ecosystem were recorded on each cryoconite sampled:

Location variables: geographic area (latitude, longitude, elevation), glacier type and characteristics.

Cryoconite holes structural variables: structure of the cryoconite hole (depth, length, width).

Biological variables: the presence or absence of metazoan in cryoconites, the abundance of each phyla in each cryoconite.

Environmental variables: other environmental parameters (area's features, bird's impact, sediment input, flows of melted water, sampling days weather conditions).

Seasonal variables: differences between the samples along the season.

The work centred around the question: "is the metazoans' presence related to the cryoconite hole location, the structure of the cryoconite hole, the presence or absence of other metazoans, the environmental variables and the sampling time?". Previous study considered some of these variables separately, but these have never been considered together and tested on both the phyla of invertebrates (tardigrades and rotifers) before.

3. References

Adhikary, S., Nakawo, M., Seko, K., & Shakya, B. (2000). Dust influence on the melting process of glacier ice: experimental results from Lirung Glacier, Nepal Himalayas. *IAHS PUBLICATION*, 43-52.

Anesio, A. M., Lutz, S., Chrismas, N. A., & Benning, L. G. (2017). The microbiome of glaciers and ice sheets. *NPJ biofilms and microbiomes*, *3*(1), 10.

Benn, D., & Evans, D. J. (2014). *Glaciers and glaciation*. Routledge.

Cameron, K. A., Hodson, A. J., & Osborn, A. M. (2012). Carbon and nitrogen biogeochemical cycling potentials of supraglacial cryoconite communities. *Polar biology*, *35*(9), 1375-1393.

Christner, B. C., Kvitko, B. H., & Reeve, J. N. (2003). Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. *Extremophiles*, *7*(3), 177-183.

Cook, J., Hodson, A., Telling, J., Anesio, A., Irvine-Fynn, T., & Bellas, C. (2010). The mass–area relationship within cryoconite holes and its implications for primary production. *Annals of Glaciology*, *51*(56), 106-110.

De Smet, W. H., & Van Rompu, E. A. (1994). Rotifera and Tardigrada from some cryoconite holes on a Spitsbergen (Svalbard) glacier. *Belgian Journal of Zoology*, *124*, 27-27.

Dowdeswell, J. A., Unwin, B., Nuttall, A. M., & Wingham, D. J. (1999). Velocity structure, flow instability and mass flux on a large Arctic ice cap from satellite radar interferometry. *Earth and Planetary Science Letters*, *167*(3-4), 131-140.

Fountain, A. G., Tranter, M., Nylen, T. H., Lewis, K. J., & Mueller, D. R. (2004). Evolution of cryoconite holes and their contribution to meltwater runoff from glaciers in the McMurdo Dry Valleys, Antarctica. *Journal of Glaciology*, *50*(168), 35-45.

Gaston, K. J. (2000). Global patterns in biodiversity. *Nature*, *405*(6783), 220.

Grongaard, A., Pugh, P. J., & McInnes, S. J. (1999). Tardigrades, and other cryoconite biota, on the Greenland ice sheet. *Zoologischer Anzeiger*, *238*(3-4), 211-214.

Hagen, J. O., Eiken, T., Kohler, J., & Melvold, K. (2005). Geometry changes on Svalbard glaciers: mass-balance or dynamic response?. *Annals of Glaciology*, *42*, 255-261.

Hagen, J. O., Liestøl, O., Roland, E., & Jørgensen, T. (1993). Glacier atlas of Svalbard and Jan Mayen.

Hambrey, M. J., Murray, T., Glasser, N. F., Hubbard, A., Hubbard, B., Stuart, G., ... & Kohler, J. (2005). Structure and changing dynamics of a polythermal valley glacier on a centennial timescale: Midre Lovénbreen, Svalbard. *Journal of Geophysical Research: Earth Surface*, *110*(F1).

Hengherr, S., Worland, M. R., Reuner, A., Brümmer, F., & Schill, R. O. (2009). Freeze tolerance, supercooling points and ice formation: comparative studies on the subzero temperature survival of limno-terrestrial tardigrades. *Journal of Experimental Biology*, *212*(6), 802-807.

Hodson, A., Anesio, A. M., Tranter, M., Fountain, A., Osborn, M., Priscu, J., ... & Sattler, B. (2008). Glacial ecosystems. *Ecological monographs*, *78*(1), 41-67.

Holland, M. M., & Bitz, C. M. (2003). Polar amplification of climate change in coupled models. *Climate Dynamics*, *21*(3-4), 221-232.

Hugo Ahlenius, UNEP/GRID-Arendal, http://www.grida.no/graphicslib/detail/the-cryosphere-world-map_e290. Retrieved July 2018

Irvine-Fynn, T. D., Hodson, A. J., Moorman, B. J., Vatne, G., & Hubbard, A. L. (2011). Polythermal glacier hydrology: A review. *Reviews of Geophysics*, *49*(4).

Jönsson, K. I., Rabbow, E., Schill, R. O., Harms-Ringdahl, M., & Rettberg, P. (2008). Tardigrades survive exposure to space in low Earth orbit. *Current biology*, *18*(17), R729-R731.

Kaštovská, K., Elster, J., Stibal, M., & Šantrůčková, H. (2005). Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (High Arctic). *Microbial ecology*, *50*(3), 396

Kaufmann, R. (2001). Invertebrate succession on an alpine glacier foreland. *Ecology*, *82*(8), 2261-2278.

Kinchin, I. M. (1994). *biology of tardigrades*. Portland.

Lapinski, J., & Tunnacliffe, A. (2003). Anhydrobiosis without trehalose in bdelloid rotifers. *FEBS letters*, *553*(3), 387-390.

MacClune, K. L., Fountain, A. G., Kargel, J. S., & MacAyeal, D. R. (2003). Glaciers of the McMurdo dry valleys: Terrestrial analog for Martian polar sublimation. *Journal of Geophysical Research: Planets*, *108*(E4).

MacDonell, S., & Fitzsimons, S. (2008). The formation and hydrological significance of cryoconite holes. *Progress in Physical Geography*, *32*(6), 595-610.

Nelson, D. R. (2002). *Defects and geometry in condensed matter physics*. Cambridge University Press.

Nelson, D. R., & Marley, N. J. (2000). The biology and ecology of lotic Tardigrada. *Freshwater Biology, 44*(1), 93-108.

Nienow. P. W., Sole. A. J., Slater. D. A., & Cowton. T. R. (n.d.) (2017). Recent Advances in Our Understanding of the Role of Meltwater in the Greenland Ice Sheet System. https://doi.org/10.1007/s40641-017-0083-9. Retrieved July 2018

Norwegian Polar Institute Website; http://www.npolar.no/en/. Retrieved July 2018

NSIDC. National Snow and Ice Data Center. (n.d.), https://nsidc.org/. Retrieved July 2018.

Örstan, A. (1998). Microhabitats and dispersal routes of bdelloid rotifers. *Scientiae Naturae, 1*, 27-36.

Paterson, W. S. B. (2016). *The physics of glaciers*. Elsevier.

Persson, D., Halberg, K. A., Jørgensen, A., Ricci, C., Møbjerg, N., & Kristensen, R. M. (2011). Extreme stress tolerance in tardigrades: surviving space conditions in low earth orbit. *Journal of Zoological Systematics and Evolutionary Research*, *49*, 90-97.

Podgorny, I. A., & Grenfell, T. C. (1996). Absorption of solar energy in a cryoconite hole. *Geophysical Research Letters*, *23*(18), 2465-2468.

Priscu, J. C., & Christner, B. C. (2004). Earth's icy biosphere. In *Microbial diversity and bioprospecting* (pp. 130-145). American Society of Microbiology.

Ricci, C., & Balsamo, M. (2000). The biology and ecology of lotic rotifers and gastrotrichs. *Freshwater Biology, 44*(1), 15-28.

Säwström, C., Mumford, P., Marshall, W., Hodson, A., & Laybourn-Parry, J. (2002). The microbial communities and primary productivity of cryoconite holes in an Arctic glacier (Svalbard 79 N). *Polar Biology*, *25*(8), 591-596.

Schirrmeister, L., Grosse, G., Kunitsky, V., Magens, D., Meyer, H., Dereviagin, A., ... & Grigoriev, M. (2008). Periglacial landscape evolution and environmental changes of Arctic lowland areas for the last 60 000 years (western Laptev Sea coast, Cape Mamontov Klyk). *Polar Research*, *27*(2), 249-272.

Schmidt-Rhaesa, A. (2001). Tardigrades—are they really miniaturized dwarfs?. *Zoologischer Anzeiger-A Journal of Comparative Zoology, 240*(3-4), 549-555.

Schulze-Makuch, D., & Grinspoon, D. H. (2005). Biologically enhanced energy and carbon cycling on Titan?. *Astrobiology, 5*(4), 560-567.

Seki, K., & Toyoshima, M. (1998). Preserving tardigrades under pressure. *Nature, 395*(6705), 853.

Shain, D. H., Mason, T. A., Farrell, A. H., & Michalewicz, L. A. (2001). Distribution and behavior of ice worms (Mesenchytraeus solifugus) in south-central Alaska. *Canadian Journal of Zoology, 79*(10), 1813-1821.

Shcherbakov, D., Schill, R. O., Brümmer, F., & Blum, M. (2010). Movement behaviour and video tracking of Milnesium tardigradum Doyère, 1840 (Eutardigrada, Apochela). *Contributions to Zoology, 79*(1).

Sømme, L. (1996). Anhydrobiosis and cold tolerance in tardigrades. *European Journal of Entomology, 93*, 349-358.

Steinböck, O. (1957). *Über die Fauna der Kryokonitlöcher alpiner Gletscher*. na.

Stibal, M., Tranter, M., Benning, L. G., & Řehák, J. (2008). Microbial primary production on an Arctic glacier is insignificant in comparison with allochthonous organic carbon input. *Environmental microbiology, 10*(8), 2172-2178.

Takeuchi, N. (2000). Characteristics of cryoconite holes on a Himalayan glacier, Yala Glacier Central Nepal. *Bull. Glaciol. Res., 17*, 51-59.

Takeuchi, N., Kohshima, S., & Seko, K. (2001). Structure, formation, and darkening process of albedo-reducing material (cryoconite) on a Himalayan glacier: a granular algal mat growing on the glacier. *Arctic, Antarctic, and Alpine Research, 33*(2), 115-122.

Vincent, W. F., & Howard-Williams, C. (2000). Life on snowball Earth. *Science, 287*(5462), 2421b-2421b.

Vonnahme, T. R., Devetter, M., Žárský, J. D., Šabacká, M., & Elster, J. (2015). Controls on microalgal community structures in cryoconite holes upon high Arctic glaciers, Svalbard. *Biogeosciences Discussions, 12*(14).

Wharton Jr, R. A., McKay, C. P., Simmons Jr, G. M., & Parker, B. C. (1985). Cryoconite holes on glaciers. *BioScience*, 499-503.

Zawierucha, K., Coulson, S. J., Michalczyk, Ł. U., & Kaczmarek, Ł. U. (2013). Current knowledge of the Tardigrada of Svalbard with the first records of water bears from Nordaustlandet (High Arctic). *Polar Research*, *32*(1), 20886.

Zawierucha, K., Kolicka, M., Takeuchi, N., & Kaczmarek, Ł. (2015). What animals can live in cryoconite holes? A faunal review. *Journal of Zoology*, *295*(3), 159-169.

Zawierucha, K., Vonnahme, T. R., Devetter, M., Kolicka, M., Ostrowska, M., Chmielewski, S., & Kosicki, J. Z. (2016). Area, depth and elevation of cryoconite holes in the Arctic do not influence Tardigrada densities. *Polish Polar Research*, *37*(2), 325-334.

CHAPTER 2

METAZOAN FAUNA OF CRYOCONITE HOLES IN HIGH ARCTIC GLACIERS OF SVALBARD, ECOLOGICAL FEATURES

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ABSTRACT

Cryoconite holes are small habitats found on the ablation zone of glacier surfaces worldwide. They are created when dust (a mixture of inorganic and organic particles, including microorganism), mainly windblown on the glacier, absorbs more solar radiation then the surrounding ice due to a lower albedo. The heating makes the ice melting underneath and the sediments sinking into the hole of melted water. This study aims to understand the interactions between the metazoans presents in cryoconite holes and their interaction with the environment. From June 2017 to August 2017, 150 cryoconite holes from nine different glaciers from three different areas were sampled for this research. Multivariate and univariate statistic was run to understand the impact of the variables driving the presence and abundance of the metazoans in the Svalbard's cryoconite holes: geographic variables (latitude, longitude, elevation, glacier type), structural variables (structure of the cryoconite hole as area and depth), biological variables (presence and abundance of metazoan in cryoconites), seasonal variables (impact of the sampling time on parameters of the samples) and environmental variables (bird’s presence, area’s features as sediment input or flows of melted water, and weather conditions of the sampling period). The variables were tested for significant differences between the nine glaciers and the three areas, and the outcomes discussed. Dependency levels and interconnected impacts of these biotic and abiotic variables were hypothesized. From the analyses of the results, environmental variables, seasonal variability and biological variables are proposed as most critical controls with different levels of dependency. Instead, cryoconite holes structural variables and the geographical variables are proposed to do not have an impact as a driver of the presence and abundance of the metazoan populations of cryoconite holes.

Keywords: Svalbard glaciers, cryoconite holes, tardigrades, rotifers, ecology

Introduction

Svalbard is an archipelago located between 74° and 81° latitude N and 10° and 35° longitude E. The study of the Arctic, and of polar regions in general, is important since these places are great indicators of natural and anthropogenic changes in our planet. The study of these regions is useful since polar and temperate regions share similar ecological principles, nevertheless, in polar regions ecological relationships are generally simpler and less diverse (Holland *et al.* 2003). The study of metazoans in cryoconite holes is giving us information about life evolution and the biodiversity in extreme environment, being cryoconite holes a geographically isolated and extremely located ecosystem.

Glaciers are persistent bodies of dense ice moving under their own weight. They constitute presently 10% of the land area on Earth. Located at high elevation in small areas there are one of the different types of glaciers, the mountain glaciers. Cirque glaciers, valley glaciers, tide water glaciers, piedmont glaciers, ice cup or continental glaciers are all sub groups deriving from mountain glaciers (NSIDC, 2018). Glaciers are formed by snow transformation process caused by changes in pressure and temperature on the snow layers. Their ice is characterised by constant downslope or outward movements due to the force of gravity. The masses of ice are constantly affected by stresses induced by their own weight deforming and making the ice flow, and abrading rocks as a result. Stress events are the explanations why some of the englacial system are formed. Englacial system are meltwater system that are present within the glaciers. They can include crevasses, glacier mill, vertical holes of different diameters that usually transport meltwater towards the bottom of the glacier (Benn *et al.* 2014). Glaciers have a complex drainage system divided in subglacial drainage system, a system of meltwater channels underneath the glacier, and in supraglacial system, a meltwater system on the glacier surface. These are influencing the formation of melting areas and of new habitats as well (Paterson *et al.* 2008, Benn *et al.* 2014). Based on their differences in behaviour, glaciers area can be separated in accumulation zone and ablation zone. Accumulation zones are characterized by snow falling during winter time, representing the glacier intake. Ablation zone represent zones of ice loss, troughs melting, and icebergs loss (NSIDC, 2018). This zone harbour cryoconite holes, one of the most active cryo-habitat in this zone. Cryoconite holes are formed due to the presence of powdery dust called cryoconite on the glacier surface that is commonly blown by the wind to the glacier from fore field areas but that could also depend by fluvial, aeolian and mass movement and/or melt out processes (Adhikary *et al.* 2000, Takeuchi *et al.* 2001, Takeuchi *et al.* 2002, MacDonell *et al.* 2008).

Due to the lower albedo of the sediment compared to the surrounded ice and due to the increased absorption of shortwave radiation of the sediment area these are sinking into the ice (Adhikary *et al.* 2000, MacDonell *et al.* 2008). This process is influenced by the timing of the sediment loading and the melting point period. Cryoconite holes are dynamic ecosystems that with the passing of the seasons could become enclosed in new depressions of the ice, or that could be washed into streams (Takeuchi *et al.* 2000; MacDonnel *et al.* 2008). Cryoconite hole can be found in an open hole's state, submerged holes state and closed holes state depending on the temperature, the amount of sediment input, the flows of water and the solar radiation (Porazinska *et al.* 2004). The shape of the hole it's driven by different reasons, from the sediment's distribution on the glacier surface, to the shortwave radiation patter that is receipt by them (Hodson *et al.* 2008). Usually, cryoconite holes from a similar site are reaching an equal equilibrium depth (Podgorny *et al.* 1996). Moreover, their shape appears to be related to the path of the sun during the melting period (Wharton *et al.* 1985; Christner *et al.* 2003).

Cryoconite's powder represent the main source of nutrients and microorganisms for cryoconite holes (Vonnahme *et al.* 2015). Their microbial life is composed by bacteria, algae, phytoflagellates, fungi, viruses, nematodes, rotifers, tardigrades and diatoms (Zawierucha *et al.* 2015). The invertebrate community structure is expected to differ from glacier to glacier, to be like aquatic systems nearby and to depend on the years that the deglaciated areas passed from the last deglaciation (Kaufmann *et al.* 2001, Porazinska *et al.* 2004). Cryoconites might well act as inoculum for proglacial systems.

Tardigrades and rotifers (bilateral eukaryotes) were the only invertebrates found in the extreme conditions of cryoconite holes. Tardigrades are microorganisms up to 0.5 mm in length (Schmidt-Rhaesa *et al.* 2001) phytophagous (plant eaters) or bacteriophages (bacteria eaters) while some are carnivorous and can eat other smaller species of tardigrades (Seki *et al.* 1998, Jönsson *et al.* 2008). They are usually in a state of vigorous activity and they usually crawl as they are unable to swim. Rotifers are up to 0.1–1 mm in length (Kinchin *et al.* 1994, Shcherbakov *et al.* 2010), they are primarily omnivorous, feeding on dead or decomposing organic materials, unicellular algae, bacteria, protozoans, phytoplankton and other water primary producers. As well as tardigrades, some species present a cannibalistic diet. Rotifers are mainly free swimming, or they are moving with an inch-worming movement along a substrate taking alternate steps attaching at the substrate with the head and tail. Both can live almost on every habitat on Earth (Seki *et al.* 1998, Jönsson *et al.* 2008). They can easily survive in hard conditions environments experiencing a process known as cryptobiosis and entering in a tun state (Sømme *et al.* 1996; Lapinski *et al.* 2003). Tardigrades and rotifers are generally representing the grazers or predators on the cryoconite hole food web, showing their important role also as top down controller on bacteria and microalgae communities (Cameron *et al.* 2012). Since the sources of food on which tardigrades and rotifers are feeding on are the same, is luckily to be present a competition between these two phyla of invertebrates. Besides, it must be taken in consideration that the feeding techniques of these two species are different.

This study aims to contribute to the research regarding the metazoan presence in cryoconite holes. Both the biotic and abiotic interactions between the different metazoan components and the cryoconite ecosystem were studied on each cryoconite sampled. Several variables have been considered: A) Location variables: geographic area (latitude, longitude, elevation), glacier type. B) Cryoconite holes structural variables: structure of the cryoconite hole (depth, area). C) Biological variables: the presence or absence of metazoan in cryoconites, the abundance of each phyla in each cryoconite.

D) Environmental variables: Other environmental parameter (bird's impact, area's features as sediment input and flows of melted water, sampling days weather conditions). E) Seasonal variables: differences between the samples along the Svalbard summer sampling season. The work centred around the question: Is the metazoans' presence related to the cryoconite hole location, the structure of the cryoconite hole, the presence or absence of other metazoans, the environmental variables and the sampling time? To achieve the research purpose from the variables' data multivariate and univariate statistic were applied to explore the most important controls, interspecific interactions and their significance for the metazoan presence in cryoconite holes high arctic ecosystem.

Material and methods

Sampling areas

Nine glaciers from three different geographical areas of Svalbard were sampled: Nordenskiöldbreen (NC), Hørbybreen (HC), Ebbabreen (E) in Billefjorden area, Sefströmbreen (SEF), Nansenbreen (NAN) in Isfjorden area, Hornbreen (HO), Hansbreen (HA), Gåsbreen (GS), Torellbreen (TO) in Hornsund area. Variables as locality's longitude, latitude and altitude, glacier type, glacier dimensions, glacier features were noted about each glacier. It was not possible to assess every variable at each sampling site (see Table 2.1. and Figure 2.1. for sampling details and localities). In all the areas bird's presence was spotted, as Pomarine Skua, Fulmars and Glaucous gulls, Black legged kittiwates, Purple sandpiper, Ivor Gull, Pomarine Jaeger, Rock ptarmigan, Arctic tern, Thick-billed Murre. Glacier forefront connected to NC peninsula, Rettretøya, is an Arctic tern nesting site.

Sampling stations

The research was conducted thought the Czech arctic infrastructure Josef Svoboda station in Svalbard. The station consists of several facilities: a research vessel motorboat (see Figure 2.2. in the Annex), a field station in Petuniabukta (see Figure 2.3. in the Annex) and base station in Longyearbyen (see Figure 2.4. in the Annex, Table 2.2.). In the case of Billefjorden area, thanks to the presence of Nostoc station, more samplings sessions and time variability analyses (comparisons between samples from the same site along the season) overview relatively this area were possible.

Table 2.1. Sampling areas details.

Location	Glacier ID	Sampling days	Samples number	Coordinates, altitude	Glacier type	Dimensions	Notes
Billefjorden area	NC	07/07/2017, 15/07/2017, 20/08/2017	30	78.6387 N, 16.9904 E, 151.05 m a.s.l.	Tidewater glacier	Length: 25 km,Width: 11 km, Area: 242 km^2	Flow velocity: 60-150 m/year
	HC	03/07/2017, 19/07/2017, 14/08/2017	30	78.7533 N, 16.3448 E, 173.64 m a.s.l.	Valley glacier	Area: 13.9 km^2	Flow velocity: 3 - 12.1 m/year.
	E	12/07/2017, 20/07/2017, 22/08/2017	30	78.7409 N, 16.8695 E, 452.90 m a.s.l.	Valley glacier	Area: 20.4 km^2	Flow velocity: 10.8 m/year
Isfjorden area	NAN	04/08/2017	10	78.3572 N, 14.0088 E, 187.00 m a.s.l.	Tidewater glacier	Length: 14 km, Area: 9.0 km^2	Significant surge of about 2 km in 1946, since gradually retreated
	SEF	02/08/2017	10	78.7269 N, 14.1784 E, 229.00 m a.s.l.	Valley glacier	NA	Surged 6.5 km across an arm of the sea (1882/1886)
Hornsund area	HA	26/07/2017	10	77.0748 N, 15.6505 E, 303.00 m a.s.l.	Tidewater glacier	Length: 15 km, Area: 53.1 km^2	NA
	HO	27/07/2017	10	77.0662 N, 16.6893 E, 205.00 m a.s.l.	Tidewater glacier	Length: 15 km,Width: 8 km	NA
	GS	28/07/2017	10	76.9128 N, 15.9461 E, 160.00 m a.s.l.	Valley glacier	Length: 5 km, Area: 5 km^2	NA
	TO	29/07/2017	10	77.1792 N, 14.8627 E, 50.00 m a.s.l.	Tidewater glacier	Length: 21 km, Area: 136.2 km^2.	NA

Sampling

Samples were collected in between 24/06/2017 and 26/08/2017. The ablation ice surfaces on all the nine glaciers were densely covered with cryoconite holes. Each sampling day 10 cryoconite holes were sampled. From the total 150 cryoconites sampled, 26 sampled were lost during the procedures, and 124 were analysed (76 from Billefjorden area, 29 from Hornsund area, 19 from Isfjorden area).

Data related to the temperature, wind condition and precipitation were taken from the Norwegian Meteorological institute (stations Pyramiden (Pyramiden målestasjon - 99880) and Hornsund (Hornsund målestasjon - 99754) (NSIDC). The data were used to look at the sampling days weather conditions and check if any difference in the weather variables (temperature, wind condition and precipitation) changed close to the sampling day, possibly impacting the sample.

Abundance estimates

The samples for abundance analyses were sampled using a polyethylene ring (diameter of 4.5 cm - area 31.8 cm^2) (see Figure 2.8. in the Annex) by sucking the sediment from that precise area of the cryoconite holes bottom's using a plastic 500 ml polyethylene siphon actioned by mouth into a 500 ml polyethylene bottle (both washed with the meltwater of the sampled cryoconite to avoid cross-contaminations) (see Figure 2.9. in the Annex).

From every sample 1/3 of the sediments have been checked by looking with an inverted microscopy Olympus^{TM} for tardigrades and rotifers numbers of individuals per square centimetre (n°/cm^2) of cryoconite sediment layer. Tardigrades and rotifers have been recognized based on the different morphology, colours (tardigrades are pinkish, instead rotifers result light blue) and locomotion techniques. The number of rotifer and tardigrades have been counted for 1/3 of the total amount of the sediments and then multiplied for three to estimate the total amount per sample area.

Variables measurements

Cryoconite holes structural measurements comprised: depth, width and length. The sediment coverage areas were calculated supposing each cryoconite had an oval shape or a round shape if noted.

Sampling location data have been taken by looking for presence or absence of birds (flying birds, dead animals, bird guano, nesting bird's area in the proximities) and the presence or absence of englacial systems on the glacier. Bird's presence and deglaciated areas, represent the main sources of nutrients (birds and deglaciated areas) and sediment input (deglaciated areas) for cryoconite holes. Meanwhile, the presence of englacial systems is a proxy for the activity of the drainage system of the glaciers.

Statistical analyses

The results obtained relatively the structural features of cryoconite holes and the abundance of invertebrate were statistically analysed through Excel and RStudio (package stats for statistical test and package graphics for plots). The statistical analyses that have been done have been univariate and multivariate to obtain univariate and multivariate analyses to consider the effects of all variables on the responses of interest. In all the tests a confidence level of 95% was used ($\alpha = 0.05$).

Sampling days variables: The weather variables (temperature, wind condition and precipitation) during the sampling months (June 2017 and August 2017) were tested for normality with a Shapiro-Wilk test. Afterwards, the statistical difference between the data have been tested. The aim of the test was to check if between the weather variables of the sampling days, did happen any statistically different fact that could have affected the samples features (H_0: The group comes from a normally distributed population).

Cryoconite holes' structural features variables: Cryoconite holes structural features (depth, width, length) were tested to underline significant differences between the structure of cryoconite holes between different sites. Therefore, the variables were tested using the non-parametric Mann–Whitney test to test the presence of significant differences between the variables of each area. The assumption that the true location shift between the two groups of data tested was not equal to zero, and thus that these are non-identical groups, was used as H_0 (Hodges *et al.* 1956).

Moreover, regarding Billefjorden area, a further test was possible since the cryoconite of NC, HC, E were sampled more than one time along the season. Significant differences and changes of the cryoconite structure along the sampling season were found by testing the samples from the same site, from different sampling moments, with a Mann–Whitney U test (1st sampling at the beginning of the season, 3rd sampling at the end of the season).

The variables (cryoconite's depth, length, width, tardigrades abundance, rotifers abundance) were first tested for normality with a Shapiro–Wilk test. Afterwards, the variables were tested with a non-parametric Spearman's correlation coefficient test to examine the strength of a monotonic relationship between paired variables.

Cryoconite holes invertebrates: Cryoconite holes differences between abundance of tardigrades and abundance of rotifers of the all 124 samples / of the different areas / of the different glaciers were described and analysed. The percentage of cryoconite holes showing invertebrates presence, and more in detail the percentage of them showing tardigrades or rotifers presence have been calculated, as well as the percentage of cryoconite holes showing a higher abundance of tardigrades compared to rotifers (T > R), and the percentage of cryoconite holes showing a higher percentage of rotifers compared to tardigrades (T < R). These values were calculated for all the 124 samples, for each area and each glacier. The percentages were checked for significant values. Afterwards, the data regarding the percentage of abundance of tardigrades and the percentage of abundance of rotifers was checked for significant differences between the abundance of the invertebrate's population in different areas or in different glaciers.

Moreover, a Mann–Whitney U test was used to test the differences between tardigrades and rotifers population of each group of glaciers (valley glaciers: HC, E, GS, SEF / tide water glaciers: NC, HA, HO, TR, NAN), to check if a significant difference was present between the abundance of the invertebrates population of these two different glacier type.

The abundance of tardigrades and the abundance of rotifers in the 124 samples were statistically checked for significant differences with a non-parametric test Mann–Whitney U test. The same test has been repeated to check significant differences between different areas.

Tardigrades/rotifers relationship: the correlation between the abundance of tardigrades and the abundance of rotifers populations in the 124 samples was checked with a Shapiro–Wilk test and the strength of the correlation (ϱ) was checked. The same groups of data have been plot using RStudio to show the tendency of their relationship.

Relatively Billefjorden area a further test was possible since the cryoconite of NC, HC, E were sampled more than one time along the season. The differences between the tardigrades abundance and the rotifers abundance were tested under a seasonal variability overview, by testing the samples from the same site, from different sampling moments, with a Mann–Whitney U test (1st sampling at the beginning of the season, 3rd sampling at the end of the season) to underline the presence of significant differences. Moreover, the significant results of NC were statistically tested with a non-parametric test Mann–Whitney U test, to test the difference between the number of tardigrades and the number of rotifers. These data were represented in a box plot and the respective quartiles were shown.

Results

Sampling days variables

Slightly higher mean temperatures were recorded from Pyramiden observation station (99880) than from Hornsund målestasjon (99754) station area. Slightly lower average wind speed was recorded from Pyramiden observation station (99880) (PY) than from Hornsund målestasjon (99754) (HD) station area. The weather variables (temperature, wind condition and precipitation) relatively the sampling months (Table 2.2.) showed to came from a normally distributed group of data (PY p-value: 0.3338, HD p-value: 0.4532) and did not show any significant difference of the variables along the sampling months with no exception of the sampling days. The precipitation values were not significant since were ≈ to zero millimetres.

Table 2.2. Weather variables (temperature, wind) of the sampling days.

Observation station	**Sampling day**	**Max T (°C)**	**Min T (°C)**	**Mean T (°C)**	**Max Wind (m/s)**	**Mean Wind (m/s)**
Pyramiden observation station (99880) data	03/07/2017	5.9	3.0	4.7	7.6	5.3
	07/07/2017	6.9	4.9	6.0	5.2	2.4
	15/07/2017	8.9	5.9	7.8	5.5	1.7
	19/07/2017	10.1	7.5	7.7	4.6	2.2
	14/08/2017	8.2	6.2	7.1	9.1	5.4
	20/08/2017	6.5	2.9	4.7	5.1	2.0
	22/08/2017	8.0	4.7	5.3	8.1	3.5
Hornsund målestasjon (99754) data	26/07/2017	7.3	3.5	5.4	3.8	1.7
	27/07/2017	6.3	4.1	5.2	4.4	1.7
	28/07/2017	7.3	2.0	5.3	7.8	2.6
	29/07/2017	7.1	3.4	5.1	4.9	1.8

Cryoconite holes' structural features variables

Cryoconite holes structural features (depth, width, length) showed the cryoconite holes of Billefjorden to be significantly deeper than those from Hornsund area (p-value: 0.007077) and Istfjorden area (p-value: 0.0001867) (Table 2.3.), otherwise, not other significant difference was shown from area to area.

Table 2.3. Cryoconite holes structural features in different areas (Billefjorden, Hornsund, Istfjorden).

Location	Variable	Min value (cm)	Max value (cm)	Mean value (cm)	Sd
Billefjorden	Depth	2.00	85.00	14.64	12.75
	Length	9.00	100.00	29.36	17.80
	Width	9.00	83.00	27.11	16.67
Hornsund	Depth	1.00	18.00	7.47	3.90
	Length	16.00	266.00	71.84	63.42
	Width	11.00	120.00	33.42	28.10
Isfjorden	Depth	0.50	25.00	9.67	6.41
	Length	14.00	220.00	45.66	40.18
	Width	10.00	120.00	30.83	23.11

Regarding Billefjorden area, statistical tests showed a significant increase of the mean length (p-value: 0.0103) and mean width (p-value: 0.002843) of cryoconite holes of NC along the sampling time (from the 1st sampling to the 3rd sampling), meanwhile the mean depth values remained stable in time (Table 2.3.).

Table 2.4. Cryoconite holes structural features (depth, length, width) along the season (1st sampling, 3rd sampling) in Nordenskiöldbreen, Hørbybreen and Ebbabreen glacier.

Location	Variables	1st sampling	3rd sampling
NC	Mean depth (cm)	22.65	18.85
	Mean length (cm)	25.15	52.10
	Mean width (cm)	21.05	42.90
HC	Mean depth (cm)	9.30	19.6
	Mean length (cm)	26.40	33.60
	Mean width (cm)	16.35	29.80
E	Mean depth (cm)	8.28	13.30
	Mean length (cm)	28.89	18.00
	Mean width (cm)	27.00	23.70

1st sapling mean value, 3rd sampling mean value

The cryoconite holes structural features (cryoconite's depth, length and width) and other invertebrates variables (tardigrades abundance, rotifers abundance) paired to each other showed a weak strength of correlation with ϱ values comprised between 0.00 - 0.19

Cryoconite holes invertebrates

In the 124 cryoconite holes samples analysed, invertebrates were found in 86 % (107/124) of them, while in the 22% (17/124) cryoconites, no invertebrates were found. In all the cryoconites sampled, a higher presence of rotifers compared to tardigrades were found. In details, tardigrades were found in the 69% (85/124) of the cryoconite sampled and rotifers were found with a greater percentage, in the 74% (92/124) of the cryoconites.

Moreover, on the singular cryoconite hole, rotifers were again found in higher abundance compared to tardigrades abundance. In detail, the cryoconite holes showing a higher percentage of tardigrades compared to the percentage of rotifers have been 35% (37/107), meanwhile the cryoconite holes showing a higher percentage of rotifers compared to the percentage of tardigrades were 50% (54/107) In the rest of the cases the abundance was equal.

In the 124 cryoconite holes samples analysed the mean abundance (n°/cm^2) of tardigrades was 13, with a minimum value equal to zero and a maximum value equal to 180, meanwhile the mean abundance (n°/cm^2) of rotifers was 16, with a minimum value equal to zero and a maximum value equal to 147.

The data about the invertebrate's populations of different areas (Table 2.5.) and glaciers (Table 2.6.) showed that in Billefjorden area a significantly greater percentage of cryoconite holes habited by invertebrates was present. No significant differences between the sampled glaciers was found.

Table 2.5. Description of invertebrate's presence in different areas (Billefjorden, Horsund and Istfjorden).

Area	**Invertebrates presence (%)**	**Tardigrade presence (%)**	**Rotifers presence (%)**	**T > R (%)**	**T < R (%)**
Billefjorden	92	73	87	29	51
Hornsund	76	91	91	27	64
Isfjorden	79	93	73	60	27

Invertebrates presence (%), cryoconites with invertebrates' presence, tardigrade presence (%), cryoconites with invertebrates' presence; rotifers presence (%), cryoconites with invertebrates' presence; T > R (%), cryoconite with higher abundance of tardigrades; T < R (%), cryoconite with higher percentage of rotifers.

Table 2.6. Description of invertebrate's presence in different glaciers (NC, HC, E, HA, HO, GS, TO, SEF, NAN).

Glacier	**Invertebrates presence (%)**	**Tardigrade presence (%)**	**Rotifers presence (%)**	**T > R (%)**	**T < R (%)**
NC	96	69	92	19	65
HC	97	83	79	41	35
E	79	60	100	20	60
HA	100	100	100	38	62
HO	63	80	10	60	100
GS	75	83	100	33	67
TO	60	100	33	67	0
SEF	67	83	67	50	17
NAN	90	100	78	67	33

Invertebrates presence (%), cryoconites with invertebrates' presence, tardigrade presence (%), cryoconites with invertebrates' presence; rotifers presence (%), cryoconites with invertebrates' presence; T > R (%), cryoconite with higher abundance of tardigrades; T < R (%), cryoconite with higher percentage of rotifers.

The sampled glaciers belonged to two different types of glaciers (valley glaciers: HC, E, GS, SEF and tide water glaciers: NC, HA, HO, TR, NAN) but no significant difference between the abundance of tardigrades and abundance of rotifers of the different type of glaciers was found.

Generally, the difference between the number of tardigrades and the number of rotifers from the 124 samples have not been found statistically different (p-value: 0.3265). Moreover, the abundance of tardigrades and the abundance of rotifers showed no significant difference between the populations of tardigrades present in cryoconite of all the three areas (Table 2.7.).

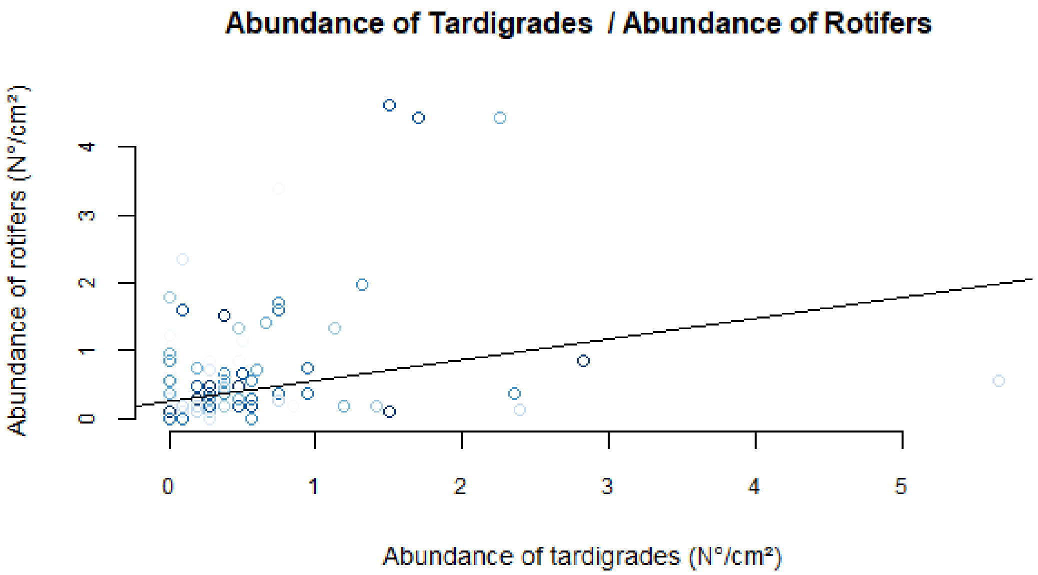
Table 2.7. Tardigrade and rotifers abundance in different areas (Billefjorden, Horsund and Istfjorden).

Location	Variables	Min value (n°/cm^2)	Max value (n°/cm^2)	Mean value (n°/cm^2)
Billefjorden	Tardigrades	0	72	9
	Rotifers	0	141	18
Hornsund	Tardigrades	0	180	23
	Rotifers	0	147	17
Isfjorden	Tardigrades	0	48	11
	Rotifers	0	54	7

Tardigrades/rotifers relationship

The strength of the correlation (ϱ: 0.5340951) existing between tardigrades and rotifers showed to be a moderate value, between 0.40 - 0.59. Moreover, the two abundances have been plot using RStudio, showing a positive correlation underlined by the positive trend line (Graph 2.1.).

Figure 2.10. Abundance of tardigrades / Abundance of rotifers (RStudio).



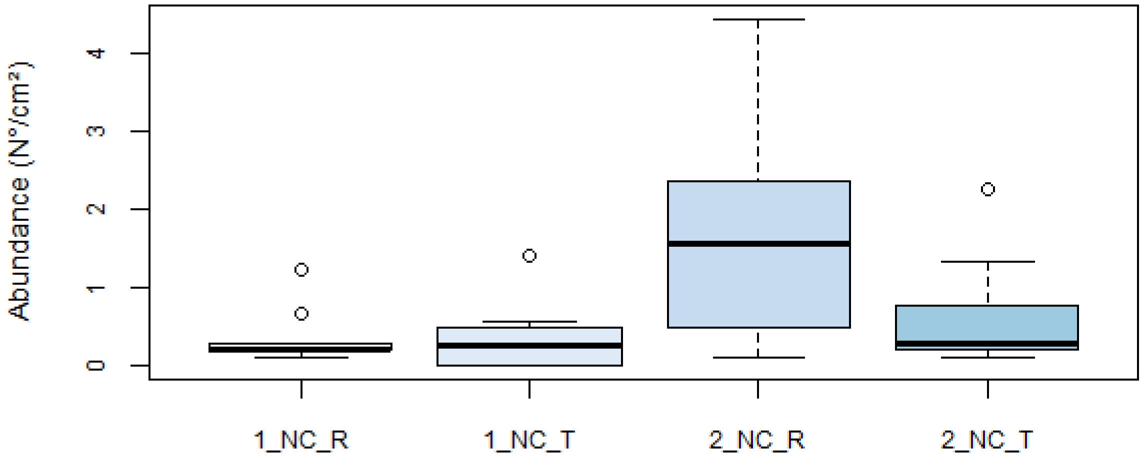
x, abundance of tardigrades, y, abundance of rotifers

Differences between tardigrades and rotifers showed a significant increase of the number of rotifers along the season (p-value: 0.003782) (Table 2.8.) (Graph. 2.2. and quartile at Table 2.9.). The difference in the 2nd quartile in time is meaningful of a big change in rotifers' abundance.

Table 2.8. Invertebrates abundance variables along the season (1st sampling, 3rd sampling) in Nordenskiöldbreen.

Location	Variables	Mean 1st sampling	Mean 3rd sampling
NC	Tardigrades (n°/cm^2)	11	19
	Rotifers (n°/cm^2)	11	56

Figure. 2.11. Nordenskiöldbreen., tardigrades and rotifers abundance variability along the season (Boxplot, R Studio).



1_NC_R, rotifers abundance in NC at the beginning of the season; 1_NC_T, tardigrade abundance in NC at the beginning of the season; 2_NC_R, rotifers abundance in NC at the end of the season; 2_NC_T: tardigrade abundance in NC at the end of the season (RStudio)

Discussion

Metazoan presence / Cryoconite holes' area

Is the metazoans presence and composition related to the cryoconite hole location? In Billefjorden area the presence of invertebrates in cryoconite holes was higher (92% of cryoconite holes contained invertebrates) than in the other sampled areas (Hornsund 76%, Isfjorden 79%). However, the relative abundance of tardigrades and rotifers showed no significant difference between the populations of tardigrades and rotifers present in cryoconite holes of all the three areas (Table 2.6). It was proposed that the higher presence of invertebrates in Billefjorden area could be not directly dependent to the geographical position in which the glaciers are located. Instead, it could be related with the characteristics of the areas. Two parameters were considerate as possible explanation: sediment input impact and birds' impact (Vonnahme *et al.*2015).

Sediment input impact: the area neighbouring the glaciers provide the innocuous for cryoconite sediment. Differences in environmental conditions, as age of the soil, soil formation and vegetation development (Stibal *et al.* 2008) can change the faunal succession on these areas adn the presence and number of microorganisms present in it. Furthermore, sun and light conditions as the moisture of snow cover regime can influence the successional process and the micro-communities (Darci, 2017). Svalbard glaciers typically have a substantial allochthonous input of sediment and nutrients from local sources which is due to their small size compared to larger ice sheets. It was proposed that factors such as environmental conditions, proximity of the closest deglaciated area and age of the deglaciated area, could have affected the higher number of invertebrates in Billefjorden area, increasing the amount and richness in invertebrates of cryoconite sediments reaching the glacier surface. Usually, after the input of new sediments, the hole becomes wider because of melting processes. Indeed, new sediments input, increase the melting rate, regulating the thickness of the layer of sediment at the bottom of the cryoconite hole (Cook *et al.* 2010). Since the equilibrium depth is controlled by the spreading of melt over the glacier (Podgorny *et al.* 1996), the input of new sediments could have affected the depth of the cryoconite holes. It was proposed that, due to the environmental conditions of the area, a higher amount of sediments was blown on the sampled areas in Billefjorden, explaining also why the cryoconite holes in that area were significantly deeper compared to those from Hornsund area (p-value: 0.007077) and Istfjorden area (p-value: 0.0001867).

Birds impact: the impact of birds could be a proxy of nutrient input and a possible explanation of the higher abundance of invertebrates in cryoconite holes from Billefjorden area. Presence of birds, typically increase the nutrient levels and thus the biomass of the glaciers (Stibal *et al.* 2008). In Billefjorden area sea birds are nesting, arriving in April-May and leaving by August-September, with the exclusion of same species that have been spotted all years around (Rachlewicz *et al.* 2007). In Billefjorden area *Ivory gulls*, *Fulmars, Glaucous gulls, Black legged kittiwakes* and *Arctic terns* (in Rettretøya) were observed near the glaciers. It was proposed that the higher presence of invertebrates in Billefjorden area could be not related with the geographical position of the area but could be possibly related with the features typical of the sampling areas as sediment input (see previous paragraph) and the nutrient input trough birds' impact. However, sediment transportation on the glacier are not only aeolian but also a fluvial process, as well as a mass movement and/or a melt out processes (Takeuchi *et al.* 2002).

Metazoan presence / Cryoconite holes' glacier type

Is metazoans presence and composition related to the type of glacier in which the cryoconite holes are located? The nine types of glaciers studied belonged to two types of mountain glaciers: tide water glaciers (NC, NAN, HA, HO, TO) and valley glaciers (GS, HC, E, SEF). These did not show any significant difference (p-value: 0.3265) relatively the metazoan fauna composition and the type of glacier in which the cryoconite have been sampled. Likewise, the invertebrate's populations of the nine different glaciers compared to each other did not show any significant difference. It was proposed that what could explain the presence and abundance of metazoan in cryoconite holes are the inner propriety of the glacier, as their supraglacial water flow activity, their thermal regime, size, slope, orientation, geological bedrock and the geology of the fore fields and nearby areas.

Metazoan presence / Cryoconite holes' structural features

Is metazoans' presence and composition related to the structure of the cryoconite hole they are inhabiting? The cryoconite holes of Billefjorden were significantly deeper than those from Hornsund area (p-value: 0.007077) and Istfjorden area (p-value: 0.0001867), and no other significant difference was shown from area to area. Moreover, higher abundance of invertebrates was found in Billefjorden area (92% of cryoconite holes contained invertebrates) than in the other sampled areas (Hornsund 76%, Isfjorden 79%). The correlation between the depth of the cryoconite and the sediment input of open cryoconite holes on the glacier is possibly present since more sediments increase the thickness of the layer of the sediments at the bottom of the cryoconite holes, increasing the melting processes, redistributing the new sediment layers, and changing the equilibrium depth, that is controlled by the spreading of the melting over the glacier (Podgorny *et al.* 1996; Cook *et al.* 2010). Thus, the presence and amount of metazoan is probably not directly related with the structure of cryoconite holes, but it can be driven by some of the drivers that are also controlling the structure of cryoconite holes such as amount of sediments, temperature and solar radiation and timing of the sediment loading.

Metazoan presence / Sampling time

Is metazoans presence and composition related to the sampling time? Seasonal changes are having an impact on cryoconite holes, especially on high Artic glacier, where with the changing of the seasons, the sun is changing its position in the sky, vanishing over the horizon for long months. When the sun first sunrise, the temperature in Svalbard starts to increase, and the solar radiation start to affect the cryoconite holes, opening them, helping the formation process of new once, causing more flows of melted water, more washing of the cryoconites, more sediments and nutrients input (Christner *et al.* 2004). These processes are having an impact also on the invertebrate's populations, that are increasing rotifers abundance along the summer season. For instance, in the 124 cryoconite holes sampled rotifers were found with a greater percentage in cryoconite holes (74%; 92/124). However, along the season, NC showed a significant increase of the number of rotifers (p-value: 0.003782) from 1st sampling to the 3rd sampling. Additionally, in NC cryoconite holes showed a significant increase of the mean length (p-value: 0.0103) and mean width (p-value: 0.002843) of cryoconite holes along the sampling time (from the 1st sampling to the 3rd sampling). It was proposed that all these founding could relate to the seasonal environmental changes. The ablation zone during the summer season is more affected by changes of the solar radiations on the melting flows (Fountain *et al.* 2004, Hodson *et al.* 2008). Moreover, cryoconite holes are affected by daily fluctuations, partly with multiple peaks in the middle of the day at the beginning of the season, and higher stability at the end of the sampling season, showing the impact of the summer temperature changing on the cryoconite hole watery ecosystem. (Vonnahme *et al.* of 2015). The proceeding of the season, the increase of the temperature, the consequent increased on melting flows, is affecting the cryoconite holes ecosystem and the invertebrate's population, by increasing the flushing of the cryoconite holes. Tracer experiment showed that the surface water during summer season is exchanged within five minutes, while the bottom water remained in the hole up to seven days (Vonnahme *et al.* 2015). Summer flows are thus likely increasing the possibility of losing some of the animals. Indeed, since rotifers are able to attach to the substrate, they may be able to resist to be flushed away from the cryoconite holes more than tardigrades, that are mainly crawling in the sediments, resulting in an increase of available resources for rotifers and an increase in rotifers population. The increase in dimension of the cryoconite holes during the season could have also relate to the increased in melted water flows, causing the widening of the cryoconite holes by melt, and a redistribution of the sediments (Cook *et al.* 2010). The fact that these data are significant just in NC glacier could be due to the NC's lowest altitude causing the highest disturbances by glacial melt (Vonnahme *et al.* 2015) and the high slope of the sampling area that could have increased the melting flow activities.

Metazoan relationships

Is metazoans presence and composition related to the presence or absence of other metazoans? The presence of tardigrades is positively influencing the presence of rotifers, and vice versa, with a moderate strength of correlation (ϱ: 0.5340951). In cryoconite holes most tardigrades are phytophagous (plant eaters) or bacteriophages (bacteria eaters), while some are carnivorous to the extent of eating other smaller species of tardigrades. Instead rotifers diet its primarily omnivorous and they can feed on dead or decomposing organic materials, unicellular algae, bacteria, protozoans, phytoplankton and other water primary producers. However, it has been noticed that some species present a cannibalistic diet and are feeding on other rotifers. The positive correlation between tardigrades and rotifers could be due to low competition, different life behaviour and feeding strategies (filter feeding, grasping feeding, carnivorous diet) that allows them to coexist with each other. Similarly, in a previous study of Vonnahme and colleges (2015) a positive correlation between tardigrades and rotifers was found, as well as it was reported in other occasion from the literature (Cameron *et al.* 2012).

Method testing

In the aftermath of the present master thesis, several comments relatively the methods were expressed. It was proposed to concentrate the next research project on a smaller number of areas and glaciers. Field research in the Arctic is very challenging, and a reduced number of sampling sites would allow an easier sampling and a deeper approach. For instance, it would be better to choose two different areas, instead of three, preferably far from each other (as Billefjorden and Hornsund areas). It was proposed to choose two types of glacier per area, located as far as possible (as Nördenskioldbreen and Hørbybreen in Billefjorden, and Hornsund and Gåsbreen in Honrsund area). It was proposed to sample the highest number of cryoconite holes in order to get the wider number of variables possible. Cryoconite holes should be sampled along a vertical line at different elevation values, in order to check if and how the micro-communities are changing along the melting flows paths. It was suggested to sample separated cryoconite holes and not the ones connected through channels of melted water. It could be better to keep sampling the same cryoconites along the season, taking smaller samples to do not destroy the entire ecosystem and to keep enough sediments for the next sampling. It was suggested taking precise locations of the closest deglaciated area with a GPS, and to check the wind direction, temperature and weather during and before the sampling days. It would be important to take precise data regarding the bird passages and nesting sites. Precise analyses of the temperature of the water could also help to understand the changing happening in the cryoconite holes environment. It was suggested taking samples for genetic analyses, both from the cryoconite holes and from the deglaciated area. From these samples the metazoans could be extracted using the method explained by Devetter and colleges (2010) to obtain the highest concentration of metazoan possible. Afterwards, the DNA could be extract by using a DNeasy Power Soil Kit (Qiagen) to then analyse it and list the species present in the cryoconite holes and in the fore field area nearby to better understand the metazoan behaviour, relationships and provenience.

Conclusions

The ecology of the metazoans living in cryoconite holes of the high glaciers of Svalbard, their relationship with the environment, and the factors driving their presence and abundance inside the cryoconite holes were investigated. Dependency levels and interconnected impacts of the biotic and abiotic variables driving the presence and abundance of the metazoans in high arctic glacier of Svalbard were hypothesized. The five main variables investigated as drivers of metazoan presence and abundance were: geographic area, cryoconite holes structure, glacier type, seasonal variability and presence and abundance of other metazoans. Several interconnections variables were found, as well as: area's characteristics, glaciers' characteristics, melted water flows, sediments input and bird's impact. It was proposed that the geographic area (latitude, longitude, elevation) and the glacier type (valley glacier and tide water glacier) did not have a direct impact on the presence and abundance of the metazoans (tardigrades and rotifers). Instead, it was proposed that the seasonal changes are affecting the metazoan population through a direct impact on the amount of melted water flows. It was proposed that with the changing of the seasons and the solar radiations, the melted water flow is increasing, effecting the sediment amount input (affecting themselves the water flows), effecting the cryoconite structure (depth, length and width) and thus the presence of other metazoan (tardigrades and rotifers). Moreover, the seasonal changes were proposed to affect indirectly the presence and abundance of other metazoans. Besides, the presence of other metazoan showed a direct and positive correlation with the presence and abundance of the metazoans (positive correlation between tardigrades and rotifers). Instead, the cryoconite structure was proposed to do not show any direct or indirect effect on the presence and abundance of the metazoans but to share with it several drivers as well as: seasonal variation, glacier characteristics, melted water flows, sediment input, area's characteristics.

Summarising, from the analyses of the results, environmental parameters, seasonal variability and the presence and abundance of other metazoans are proposed as most critical controls with different levels of dependency. Instead, cryoconite holes structure and the glacier type are proposed to do not have an impact on the metazoan populations of cryoconite holes.

The fragmented approach to the study of metazoans in/and cryoconite holes until now led to an incomplete picture about their ecology. Consequently, it is problematic to extrapolate their general behaviour at different spatial and temporal scales, or how they would respond to changing environmental conditions. To better understand metazoans in cryoconite hole ecosystem more researches related to this topic are needed.

References

Adhikary, S., Nakawo, M., Seko, K., & Shakya, B. (2000). Dust influence on the melting process of glacier ice: experimental results from Lirung Glacier, Nepal Himalayas. *IAHS PUBLICATION*, 43-52.

Benn, D., & Evans, D. J. (2014). *Glaciers and glaciation*. Routledge.

Cameron, K. A., Hodson, A. J., & Osborn, A. M. (2012). Carbon and nitrogen biogeochemical cycling potentials of supraglacial cryoconite communities. *Polar biology, 35*(9), 1375-1393.

Christner, B. C., Kvitko, B. H., & Reeve, J. N. (2003). Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. *Extremophiles*, *7*(3), 177-183.

Cook, J., Hodson, A., Telling, J., Anesio, A., Irvine-Fynn, T., & Bellas, C. (2010). The mass–area relationship within cryoconite holes and its implications for primary production. *Annals of Glaciology*, *51*(56), 106-110.

Darcy, J. L. (2017). Biogeographic and Biogeochemical Drivers of Microbial Community Assembly (Doctoral dissertation, University of Colorado at Boulder).

Devetter, M. (2010). A method for efficient extraction of rotifers (Rotifera) from soils. *Pedobiologia*, *53*(2), 115-118.

Fountain, A. G., Tranter, M., Nylen, T. H., Lewis, K. J., & Mueller, D. R. (2004). Evolution of cryoconite holes and their contribution to meltwater runoff from glaciers in the McMurdo Dry Valleys, Antarctica. *Journal of Glaciology*, *50*(168), 35-45.

Hodges, J. L., & Lehmann, E. L. (1956). The efficiency of some nonparametric competitors of the $ t $-test. *The Annals of Mathematical Statistics*, *27*(2), 324-335.

Hodson, A., Anesio, A. M., Tranter, M., Fountain, A., Osborn, M., Priscu, J., ... & Sattler, B. (2008). Glacial ecosystems. *Ecological monographs*, *78*(1), 41-67.

Holland, M. M., & Bitz, C. M. (2003). Polar amplification of climate change in coupled models. *Climate Dynamics*, *21*(3-4), 221-232.

Jönsson, K. I., Rabbow, E., Schill, R. O., Harms-Ringdahl, M., & Rettberg, P. (2008). Tardigrades survive exposure to space in low Earth orbit. *Current biology*, *18*(17), R729-R731.

Kaufmann, R. (2001). Invertebrate succession on an alpine glacier foreland. *Ecology*, *82*(8), 2261-2278.

Kinchin, I. M. (1994). *biology of tardigrades*. Portland.

Lapinski, J., & Tunnacliffe, A. (2003). Anhydrobiosis without trehalose in bdelloid rotifers. *FEBS letters*, *553*(3), 387-390.

MacDonell, S., & Fitzsimons, S. (2008). The formation and hydrological significance of cryoconite holes. *Progress in Physical Geography*, *32*(6), 595-610.

MacDonell, S., & Fitzsimons, S. (2008). The formation and hydrological significance of cryoconite holes.*Progress in Physical Geography*, *32*(6), 595-610.

Norwegian Polar Institute Website; http://www.npolar.no/en/. Retrieved July 2018

NSIDC. National Snow and Ice Data Center. (n.d.), https://nsidc.org/. Retrieved July 2018.

Podgorny, I. A., & Grenfell, T. C. (1996). Absorption of solar energy in a cryoconite hole. *Geophysical Research Letters*, *23*(18), 2465-2468.

Porazinska, D. L., Fountain, A. G., Nylen, T. H., Tranter, M., Virginia, R. A., & Wall, D. H. (2004). The biodiversity and biogeochemistry of cryoconite holes from McMurdo Dry Valley glaciers, Antarctica. *Arctic, Antarctic, and Alpine Research*, *36*(1), 84-91.

Rachlewicz, G., Szczuciński, W., & Ewertowski, M. (2007). Post-"Little Ice Age" retreat rates of glaciers around Billefjorden in central Spitsbergen, Svalbard. *Polish Polar Research*, *28*(3), 159-186.

Schmidt-Rhaesa, A. (2001). Tardigrades—are they really miniaturized dwarfs?. *Zoologischer Anzeiger-A Journal of Comparative Zoology*, *240*(3-4), 549-555.

Seki, K., & Toyoshima, M. (1998). Preserving tardigrades under pressure. *Nature*, *395*(6705), 853.

Shcherbakov, D., Schill, R. O., Brümmer, F., & Blum, M. (2010). Movement behaviour and video tracking of Milnesium tardigradum Doyère, 1840 (Eutardigrada, Apochela). *Contributions to Zoology*, *79*(1).

Sømme, L. (1996). Anhydrobiosis and cold tolerance in tardigrades. *European Journal of Entomology*, *93*, 349-358.

Stibal, M., Tranter, M., Benning, L. G., & Řehák, J. (2008). Microbial primary production on an Arctic glacier is insignificant in comparison with allochthonous organic carbon input. *Environmental microbiology*, *10*(8), 2172-2178.

Takeuchi, N. (2000). Characteristics of cryoconite holes on a Himalayan glacier, Yala Glacier Central Nepal. *Bull. Glaciol. Res.*, *17*, 51-59.

Takeuchi, N. (2002). Optical characteristics of cryoconite (surface dust) on glaciers: the relationship between light absorbency and the property of organic matter contained in the cryoconite. *Annals of Glaciology*, *34*, 409-414.

Vonnahme, T. R., Devetter, M., Žárský, J. D., Šabacká, M., & Elster, J. (2015). Controls on microalgal community structures in cryoconite holes upon high Arctic glaciers, Svalbard. *Biogeosciences Discussions*, *12*(14).

Wharton Jr, R. A., McKay, C. P., Simmons Jr, G. M., & Parker, B. C. (1985). Cryoconite holes on glaciers. *BioScience*, 499-503.

Zawierucha, K., Kolicka, M., Takeuchi, N., & Kaczmarek, Ł. (2015). What animals can live in cryoconite holes? A faunal review. *Journal of Zoology*, *295*(3), 159-169.

ANNEX

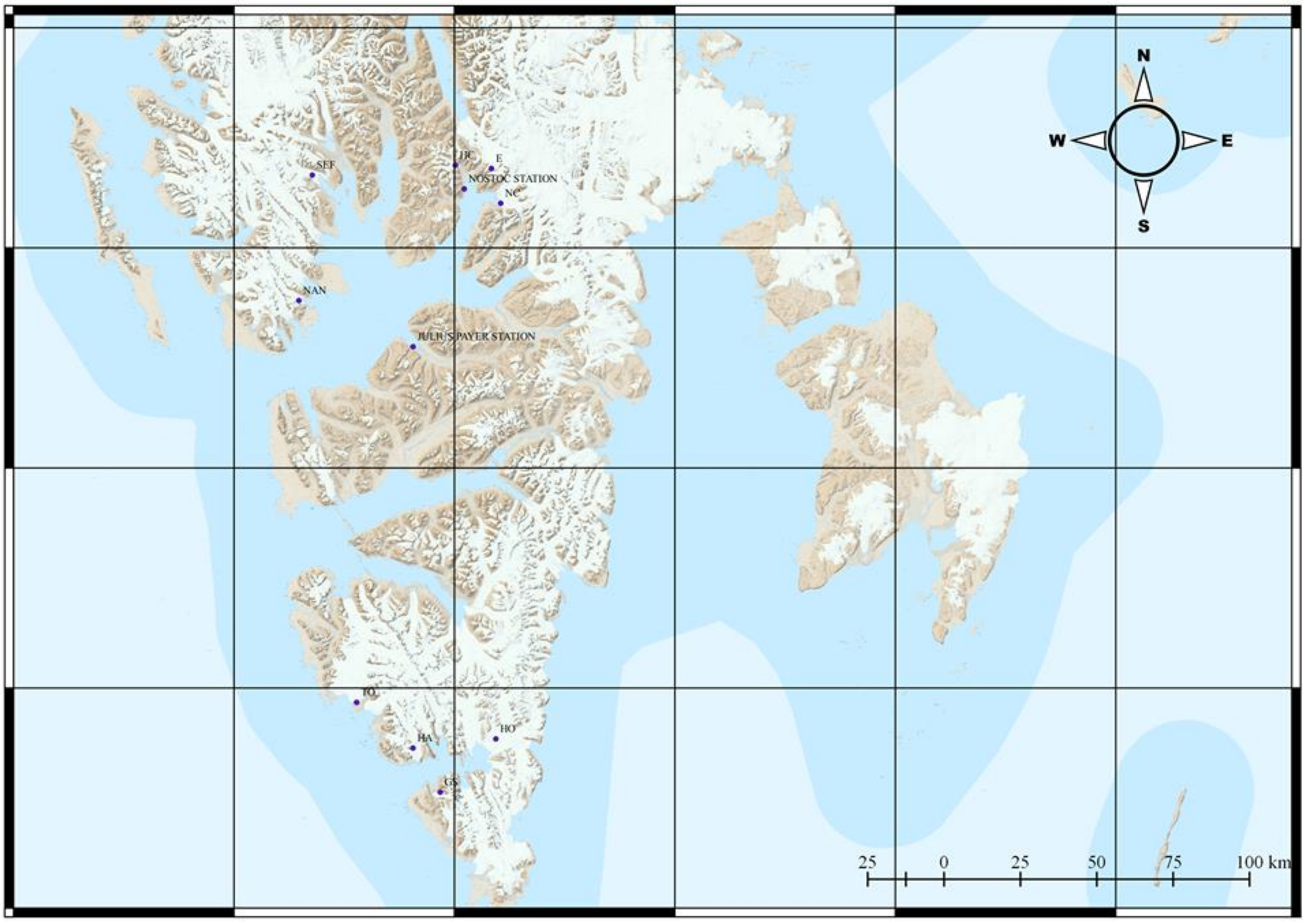


Figure 2.1. Representation of the nine sampling sites on a topographic map of Svalbard, Nostoc and Julius Payer stations (QGIS).

Figure 2.2. Clione (© Margherita Lucadello).

Figure 2.3. Nostoc Station (© Margherita Lucadello).

Figure 2.4. Julius Payer Station (© Margherita Lucadello).

Figure 2.5. Representation of the three sampling sites (Nordenskiöldbreen, Hørbybreen and Ebbabreen in red circles) of Billefjorden area on a topographic map of Svalbard and of Nostoc station (topographic map of Svalbard from Norwegian Polar Institute).

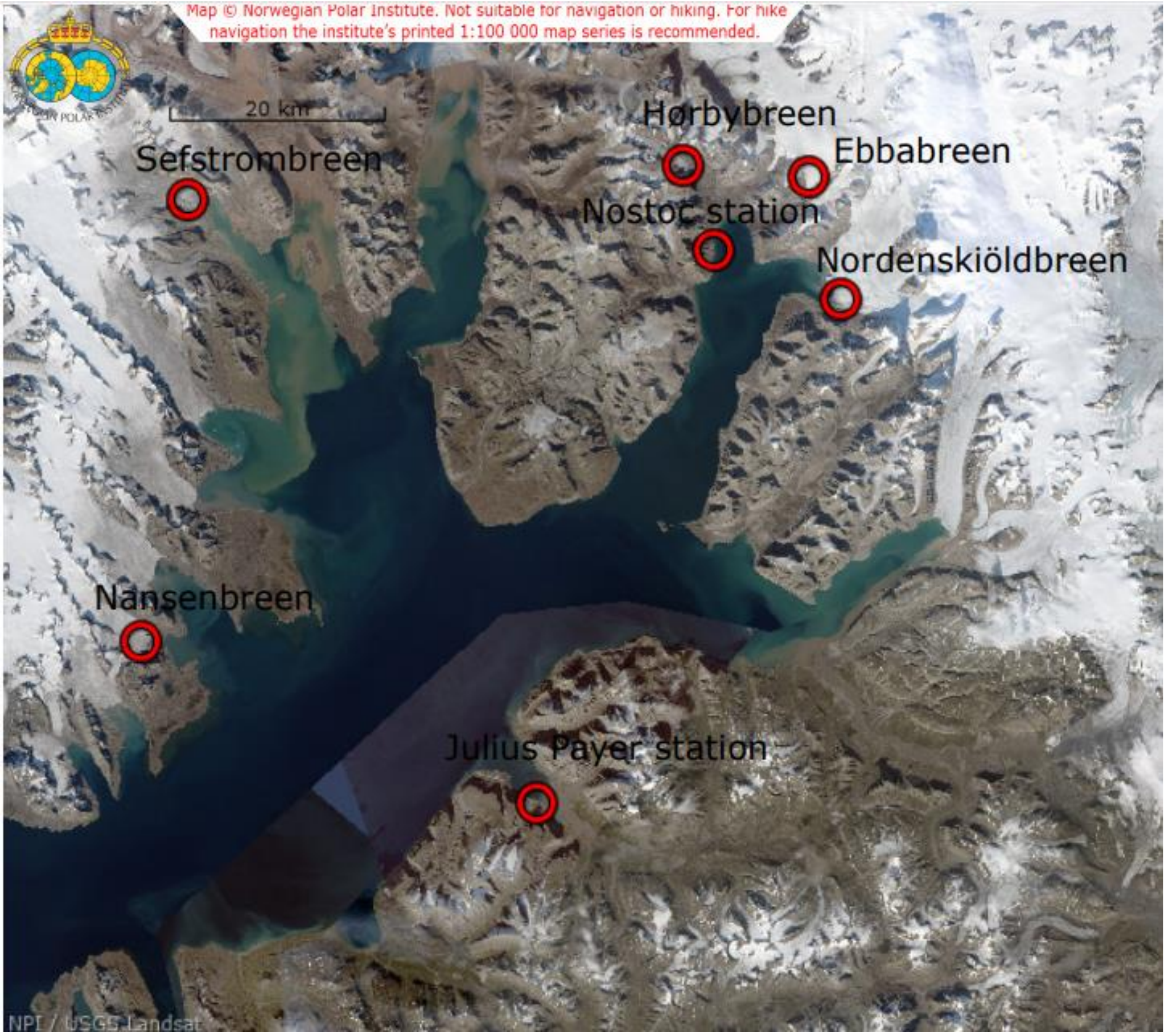


Figure 2.6. Representation of the two sampling sites. In red circles on the left side of the map are visible Sefströmbreen and Nansenbreen of Isfjorden area. In red circles on the right side of the map are visible also the sampling sites of Billefjorden area and the stations Nostoc and Julius Payer in order to show the relative positions of the glaciers of the two areas (topographic map of Svalbard from Norwegian Polar Institute).

Figure 2.7. Representation of the four sampling sites (in red circles Hornbreen, Hansbreen, Gåsbreen, and Torellbreen) of Hornsund area on a topographic map of Svalbard (topographic map of Svalbard from Norwegian Polar Institute).

Figure 2.8. Cryoconite holes and sampling ring (© Margherita Lucadello).

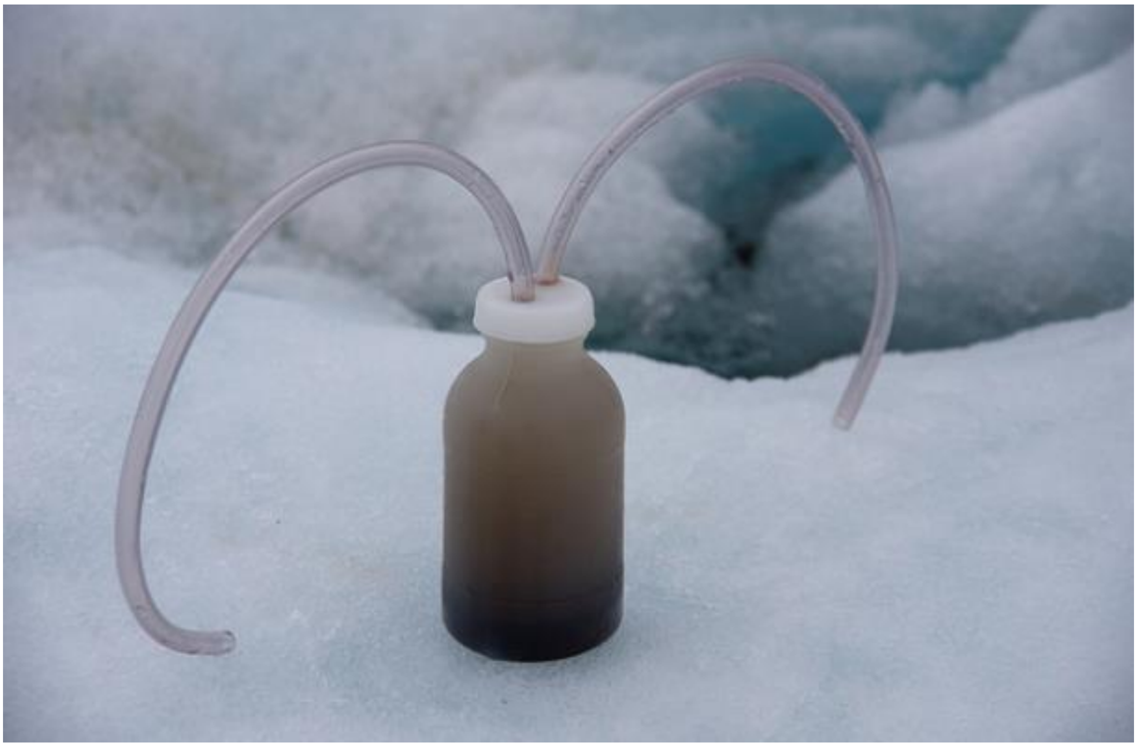


Figure 2.9. Polyethylene bottle syphon (© Jakub Ondruch).

GLOSSARY

Ablation zones: the low-altitude area of a glacier or ice sheet below firn with a net loss in ice mass due to melting, sublimation, evaporation, ice calving, aeolian processes like blowing snow, avalanche, and any other ablation.

Accumulation zones: the area above the firn line, where snowfall accumulates and exceeds the losses from ablation (melting, evaporation, and sublimation).

Anhydrobiosis: the life away from water. Anhydrobiosis is the most studied form of cryptobiosis and occurs in situations of extreme desiccation.

Anoxybiosis: life away of oxygen. In situations lacking oxygen, many cryptobionts take in water and become turgid and immobile but can still survive for prolonged periods of time just as with other crypto biological processes.

Box plot: in descriptive statistic, a box plot is a method for graphically depicting groups of numerical data through their quartiles.

Cirque glaciers: cirque glaciers are small glaciers that can be found in hollows or bowl-shaped depressions on the sides of the mountains.

Cold-based glaciers: the cold-based glaciers have an underlying substrate that is frozen.

Cryoconite: cryoconite is powdery windblown dust made of a combination of small rock particles, soot and microbes which is deposited and builds up on snow, glaciers, or ice caps.

Cryoconite holes: cylindrical holes made by sediments and melted water, small cold adapted ecosystems situated on the ablation zone of glaciers.

Cryosphere: those portions of Earth's surface where water is in solid form, including sea ice, lake ice, river ice, snow cover, glaciers, ice caps, ice sheets, and frozen ground.

Cryptobiosis: metabolic state of life entered by an organism in response to adverse environmental conditions such as desiccation, freezing, and oxygen deficiency.

Descriptive statistic: summary statistic that quantitatively describes or summarizes features of a collection of information.

Firn: type of snow that has been left over from past seasons and has been recrystallized into a substance denser.

Food web: series of organisms related by predator-prey and consumer-resource interactions. The entirety of interrelated food chains in an ecological community.

Glaciers surge: short-lived events where a glacier can advance substantially, moving at velocities up to 100 times faster than normal.

Glacier: persistent body of dense ice that is constantly moving under its own weight; it forms where the accumulation of snow exceeds its ablation (melting and sublimation) over many years, often centuries.

Grazers: feeding method in which an herbivore feeds on plants, or algae as for instance in the case of multicellular organisms.

Ice calving: the breaking of ice chunks from the edge of a glacier.

Ice caps: mass of ice that covers less than 50,000 km^2 of land area.

Ice shelves: thick floating platform of ice that forms where a glacier or ice sheet flows down to a coastline and onto the ocean surface.

Invertebrates: animals that neither possess nor develop a vertebral column (commonly known as a backbone or spine), derived from the notochord.

Melting: physical process that results in the transition phase of a substance from a solid state to a liquid state.

Metazoan: synonymous with Animalia.

Permafrost: ground, including rock or soil, at or below the freezing point of water zero degrees Celsius for two or more years.

Piedmont glacier: a valley glacier that have extended down a valley and then have covered a slope beyond the mountain range.

Polythermal: glaciers which are partly cold-based and partly warm-based are known as polythermal.

Rotifers: phylum of microscopic and near-microscopic pseudocoelomate animals.

Sloppy feeding: production of dissolved organic matter by aquatic animals during imperfect absorption of food particles.

Snowline: the boundary between a snow-covered and snow-free surface.

Subglacial level: Pertaining or belonging to the underside of a glacier.

Tardigrades: a phylum of water-dwelling, eight-legged, segmented micro-animals.

Tide water glacier: glaciers that have extended down to the sea level and that carve a valley into the coastline.

Valley glaciers: cirque glaciers that once grown bigger, follow the topography of the area in which they are located, spreading and flowing down the valleys.

Warm-based glaciers: glaciers that are over or at a freezing temperature in the interface and warmer at the base.