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Separation and Elemental Composition of Tardigrades and Rotifers from Cryoconite in Svalbard

Separace a prvkové složení želvušek a vířníků v kryokonitu na Svalbardu

Diploma Thesis

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Statement:

I hereby state that I have completed this thesis by myself and that I have properly cited all literature and other information sources I have used. Neither this thesis nor its parts have been submitted to achieve any other academic title(s).

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Tereza Jaroměřská

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ABSTRACT

For years considered pristine, glacial ecosystems are attracting more attention of biologists from various branches in last decades. Moreover, they are currently considered to be ecosystems forming the coldest biome on the Earth. The life on glaciers ranges from viruses and bacteria to highest consumers such as few groups of microinvertebrates. The most common are tardigrades (phylum Tardigrada) and rotifers (phylum Rotifera) which inhabit cryoconite holes on the glacial surface. Several studies highlight the importance of the role of these consumers. However, due to the dominance of prokaryotes on the glacial surface, these microinvertebrates are usually out of the major scope of most studies aiming at biological processes. The present study shows pioneering results on the isotopic composition of tardigrades and rotifers, which are the top consumers in cryoconite ecosystems, and is a foundation for the exploration of trophic pathways and interactions within cryoconite holes using elemental and stable isotopic analyses. It also presents information about the species composition of tardigrades and rotifers on different glaciers and in different parts of the ablation zone. We identified 5 species of tardigrades (*Hypsibius* sp., *Hypsibius* cf. *dujardini*, *Pilatobius* sp., *Isohypsibius* sp. and *Cryoconicus kaczmareki*) and 2 species of rotifers (*Macrotrachella* sp. and *Adineta vaga*) from three glaciers in Billefjorden (Svalbard). Furthermore, we found differences in species composition, body size and buccal tube length of tardigrades between glaciers and between different parts of the ablation zone. As a major part of the project, we analysed isotopic and elemental composition of tardigrades, rotifers and organic matter from cryoconite with a modified method of separation of animals from the sediment which excluded additional chemicals. Measured isotopic values showed that tardigrades have significantly lower $\delta^{15}N$ values than rotifers. The $\delta^{13}C$ values showed similarities between animals from similar glaciers and similar parts of the ablation zone. We also measured the $\delta^{13}C$ and $\delta^{15}N$ values of organic matter within cryoconite (potential food for consumers) which revealed that the dominant component of cryoconite organic matter can be a food for rotifers but not for tardigrades.

Keywords: glacial ecosystems, High Arctic, elemental composition, ecological stoichiometry, stable isotopes.

ABSTRAKT

Ledovcové ekosystémy, dlouhou dobu považované za nedotčené, přitahují v posledních dekádách stále větší pozornost biologů nejrůznějšího zaměření. Vedle toho jsou též považovány za ekosystémy tvořící nejchladnější biom na Zemi. Životní formy na ledovcích zahrnují organismy od virů a bakterií až po vrcholové konzumenty, mezi které patří několik skupin drobných bezobratlých živočichů. Nejčastějšími z nich jsou želvušky (kmen Tardigrada) a vířníci (kmen Rotifera), kteří obývají kryokonitové jamky na povrchu ledovců. Několik studií již vyzdvihlo význam těchto konzumentů, přesto kvůli dominanci prokaryotních organismů v daném prostředí většinou stojí mimo hlavní zájem většiny vědeckých prací věnovaných biologickým procesům. Předkládaná diplomová práce přináší svého druhu první výsledky o izotopickém složení želvušek a vířníků, kteří jsou vrcholovými konzumenty v ekosystému kryokonitových jamek. Zároveň jde o prvotní snahu o detailní výzkum trofických vztahů v kryokonitových jamkách za využití prvkových analýz a analýz poměrů stabilních izotopů. Prezentovány jsou dále údaje o druhovém složení želvušek a vířníků na různých ledovcích a v různých částech jejich ablačních zón. Ve výsledku jsme na třech ledovcích situovaných v oblasti Billefjorden (Svalbard) doložili přítomnost 5 druhů želvušek (*Hypsibius* sp., *Hypsibius* cf. *dujardini*, *Pilatobius* sp., *Isohypsibius* sp. a *Cryoconicus kaczmareki*) a 2 druhů vířníků (*Macrotrachella* sp. a *Adineta vaga*). Kromě toho jsme nalezli rozdíly v druhovém složení, velikosti těla a délce bukálního aparátu želvušek mezi těmito ledovci a mezi různými částmi jejich ablačních zón. V rámci nejzásadnější části projektu jsme vyhodnotili izotopické a prvkové složení želvušek, vířníků a organické hmoty obsažené v kryokonitu, a to za využití uzpůsobené metody separace živočichů z kryokonitu, která není založena na přídavku chemikálií. Získané výsledky ukazují signifikantně nižší hodnoty $\delta^{15}N$ u želvušek než u vířníků. Hodnoty $\delta^{13}C$ jsou u obou skupin u konkrétních ledovců a u konkrétních částí ablačních zón podobné. Dále jsme změřili hodnoty $\delta^{13}C$ and $\delta^{15}N$ u organické hmoty obsažené v kryokonitu, která je potenciálním zdrojem potravy uvedených konzumentů. Z výsledků vyplývá, že dominantní složka organické hmoty v kryokonitu může být zdrojem potravy vířníků, nikoliv však želvušek.

Klíčová slova: ledovcové ekosystémy, Arktida, prvkové složení, ekologická stechiometrie, stabilní izotopy.

1 General Introduction

1.1 Introduction to Ecology of Glacial Ecosystems

Glacial systems are probably one of the most rapidly changing systems on our planet (Oerlemans 2005). Due to the dynamic character of these ice masses, they are one of the key indicators in observations of climate changes (Williams and Ferrigno 2012). Moreover, they host various forms of organisms, especially within cryoconite holes (Hodson et al. 2008, Zawierucha et al. 2015a). As biogeochemical reactors, glaciers are capable of altering processes in recently deglaciated areas in glacier fore-fields and in downstream coastal and marine ecosystems (Bardgett 2007, Foreman et al. 2007, Hodson et al. 2008, Hood et al. 2009). In this study we focus on glacial microinvertebrates, which were recently recognized to be an important factor influencing the nutrient pathways and community structures among cryoconite holes of various glaciers (Vonnahme et al. 2016, Zawierucha et al. 2018a).

Glaciers are oligotrophic ecosystems with an input of nutrients restricted to atmospheric deposition, weathering of mineral dust, aeolian deposition, autochthonous recycling and locally also bird guano deposition (Benassai et al. 2005, Hodson et al. 2005, Stibal et al. 2008a, Anesio et al. 2009, Xu et al. 2010, Telling et al. 2011, Edwards et al. 2014, Vonnahme et al. 2016). The variability of sources can be amplified by strong seasonal changes (Stibal et al. 2008a, Telling et al. 2011). Adjacent areas of glaciers can vary a lot in terms of geology, vegetation and stage of soil development. Thus, the proportion of allochthonous matter brought to the glacial surface can cause differences in the composition and structure of material and biota (Marshall and Chalmers 1997, Stibal et al. 2008a, Grzesiak et al. 2015).

Cryoconite holes (Fig. 1) are small ponds formed by sediment (hereafter cryoconite – from Greek *krýos* – icy cold and *kónis* – dust, named by Nordenskiöld (1875)) which melt into the glacial surface due to the low albedo and differences in albedo of surrounding ice surface under radiative weather conditions (Takeuchi et al. 2001a). The diameter and depth of holes are noticeably variable, but both usually range from a few centimetres to tens of centimetres (Gerdel and Drouet 1960, Fountain et al. 2004, Zawierucha et al. 2019b). Cryoconite consists of inorganic (mineral particles) and organic matter (Takeuchi et al. 2001a, Takeuchi et al. 2001b). The organic component of the sediment contains bacteria, cyanobacteria and algae, which provide a source of nutrients for consumers, mostly tardigrades and rotifers (De Smet and Van Rompu 1994, Takeuchi et al. 2001b, Porazinska et al. 2004, Devetter 2009, Zawierucha et al. 2015a). Cryoconite also harbours a rich pool of dead organic matter of allochthonous (e.g. wind-blown plant fragments) and autochthonous (mostly microbial) origin (Takeuchi et al. 2001a). Therefore, cryoconite holes typically form the most nutrient-rich and biologically active habitats within the supraglacial environment (Stibal et al. 2006, Hodson et

al. 2008, Cook et al. 2016, Nicholes et al. 2019) and, due to the truncated food webs and restricted sources, appear to be a good model in studies of the ecological relations.

The elemental composition (C:N:P, carbon:nitrogen:phosphorus) of food sources (primary producers, allochthonous organic matter), consumers and decomposers are strongly linked to each other (Sterner and Elser 2002). As an illustration of possible interactions (neglecting the complexity of ecosystem's interactions), we can use a simple planktonic community where consumers elemental composition can have either high N:P or low N:P. If high N:P consumers are dominant, phytoplankton tends to be N depleted, if low N:P consumers are dominant, phytoplankton tends to be P depleted (Sterner and Elser 2002). Similarly, bacterial communities which utilize detritus are affected by the stoichiometry (C:N:P) of the detritus. When the detritus is N depleted, bacterial communities retain N, discharge more P and vice versa (Sterner and Elser 2002). Cryoconite holes represent a great habitat for studying these ecological relationships due to their truncated food webs and simplified system proxies compared to temperate habitats (Säwström et al. 2002, Cook et al. 2016). However, it is necessary to take into consideration that cryoconite holes (as well as other systems) probably have multiple limiting factors and are also affected by co-limitations of nutrients and abiotic factors (Mueller and Pollard 2004, Säwström et al. 2007)

1.2 Tardigrades and Rotifers from Polar Ecosystems

Tardigrades and rotifers are microscopic invertebrates commonly found in terrestrial (e.g. mosses, soil, lichens) as well as aquatic habitats (e.g. marine and freshwater sediments, littoral zone with submerged plants and biofilms). As a result of the their evolutionary history, high tolerance of unfavourable conditions, small sizes and long-range dispersal capability (Jenkins and Underwood 1998, Jørgensen et al. 2007, Fontaneto et al. 2008, Nelson et al. 2015, Fontaneto 2019), they inhabit a variety of environments, even those with extreme conditions such as deep-sea sediments, polar deserts, nunataks or glaciers (Dastych 1985, Ayres et al. 2007, Zawierucha et al. 2013, Zeppilli et al. 2013). Tardigrades and rotifers play an important role at multiple trophic level as carnivorous, herbivorous, omnivorous and microbivorous species (Hallas and Yeates 1972, Kutikova 2003, Guidetti et al. 2012, Guil and Sanchez-Moreno 2013). In polar ecosystems (e.g. tundra soils, glaciers or on nunataks) tardigrades and rotifers are dominant, or even the only metazoans and representatives of the highest trophic consumers (Convey and McInnes 2005, Zawierucha et al. 2015a, Zawierucha et al. 2016a). Moreover, their species diversity in these systems is surprisingly high (Maucci 1996, Garey et al. 2008, Fontaneto et al. 2015, Zawierucha et al. 2015a). However, information elucidating their occurrence in polar systems remains poor. There are two major opinions discussing their distribution and colonization of polar regions: 1. they are migrants which reached polar habitats by wind and adapted to polar terrestrial and freshwater ecosystems after ice recession; 2. they survived in refugia and expanded to surrounding habitats (Coulson et al. 2014).

To survive low temperatures, periodic freezing and high exposition to UV radiation, tardigrades and rotifers use a mechanism known as cryptobiosis (ametabolic dormant state) which includes protection against low temperatures (cryobiosis) and lack of water (anhydrobiosis), plus they are also resistant to starvation (Sømme 1996, Ricci 2001, Bertolani et al. 2004, Watanabe 2006, Altiero et al. 2011, Guidetti et al. 2011, Bonifacio et al. 2012, Marotta et al. 2012, Zawierucha et al. 2015a, Giovannini et al. 2018). Furthermore, they have resistant resting stages and eggs which help them to survive when water is absent or frozen (Dumont 1983, Nelson et al. 2015).

In polar ecosystems, tardigrades and rotifers represent a large component of microfauna in tundra and are the dominant metazoans inhabiting cryoconite holes (Klekowski and Opaliński 1986, Zawierucha et al. 2015a). However, due to the strong selective pressure caused by various abiotic factors including low nutrient concentrations, the glacier surface is inhabited by a limited number of tardigrade and rotifer species (Zawierucha et al. 2015a, Zawierucha et al. 2016a, De Smet and Van Rompu 1994). As the top consumers of cryoconite holes, tardigrades and rotifers play a significant role in nutrient recycling, contributing to the local community structure (Vonnahme et al. 2016, Zawierucha et al. 2018a). Data on the elemental and isotopic composition of these invertebrates, which are crucial for the description of their trophic position and interactions within the food web in the cryoconite hole's ecosystem, are however lacking.

1.3 Study Area

The Svalbard archipelago is one of the northernmost territories in the Northern Hemisphere. Approximately 60 % of dry land is covered by glaciers and the climate is relatively mild due to the warm ocean currents from the North Atlantic Ocean. Svalbard is also known to have higher annual precipitation compared to other High Arctic habitats (Hagen et al. 1993).

Due to the high latitude, Svalbard has a low diversity of terrestrial plants, invertebrates and vertebrates (Harland 1997). Low temperatures and consequently low inputs of available forms of nutrients from dead biomass and chemical weathering cause terrestrial species to rely on nutrient fluxes from marine ecosystem (Keller et al. 2007, Sistla et al. 2012, Zwolicki et al. 2016). During the summer, when the sea ice melts, the marine ecosystem provides a rich source of food for various marine and terrestrial animals and has a crucial role in the functioning of food chains around the whole Arctic region (Castro and Huber 2015).

Birds are the most numerous representatives of vertebrates within the Arctic terrestrial ecosystem (Harland 1997). Despite many important environmental functions, they are a significant factor in fertilization of soil for plant growth because low decomposition connected to low temperatures causes permafrost to not release as many nutrients and minerals as would be optimal (Billings 1987, Wainright et al. 1998). Moreover, guano from bird colonies can affect abundance, relative density, trophic group structure and even the body size of tardigrades from tundra habitats as well (Zawierucha et al. 2019a). As an implication of these extreme

conditions, all organisms show adaptations to low temperatures, low nutrients, high radiation during summer and a short reproductive season (Pielou 2012).

Samples of cryoconite were collected on Svenbreen, Ebbabreen and Nordenskiöldbreen (breen = glacier), hereinafter also referred to as Sven, Ebba and Nordenskiöld without their glacier endings. These glaciers are located inside Petuniabukta (bukta = bay), a part of Billefjorden (fjorden = fjord), the central fjord of Isfjorden (Fig. 2). This fjord is mostly known because of the Billefjorden Fault Zone (Fig. 2), a geological boundary between Devonian clastic rocks on the west and Carboniferous sequences in the east (Szczuciński and Rachlewicz 2007). The glaciers were selected based on different spatial characteristics such as size or character of their adjacent areas.

Figure 1. Cryoconite holes at Nordenskiöldbreen (photo by author).

Figure 2. Location of Petuniabukta (left) and the Billefjorden Fault Zone (right) (photo by author). Map of Petuniabukta was adapted from https://toposvalbard.npolar.no/ and modified.

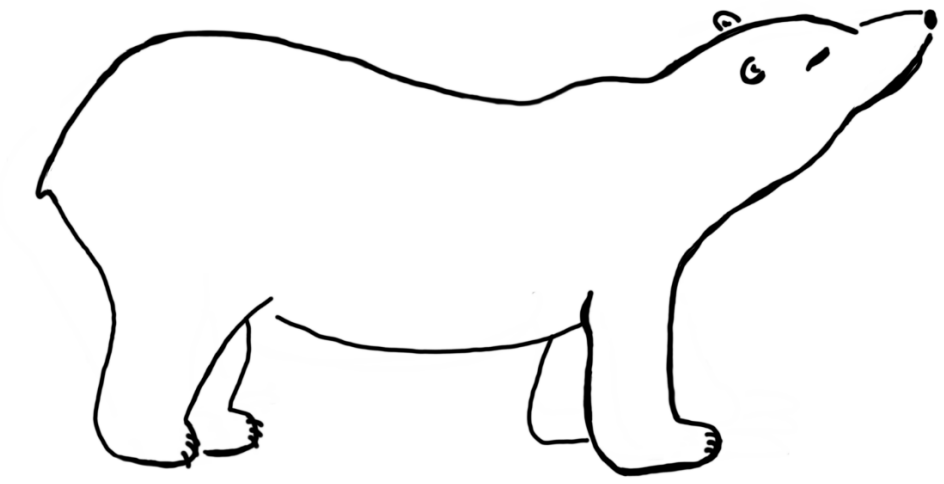
1.4 Aims of the Thesis

1. The first aim of the thesis is to describe the species composition, food preferences and body size distribution among the three studied glaciers. We assume that spatial characteristics of the glaciers could influence the structure and diversity of invertebrates inhabiting cryoconite holes. We hypothesise that the body size of tardigrades increases with decreasing altitude along the ablation zone (the area with a negative mass balance) because of the preferential transport of bigger species. The data from the first part of the thesis provides a foundation for the second part, which focuses on elemental and isotopic analyses.

2. The second and major aim of the thesis is to elucidate the elemental (C:N:P) and isotopic ($\delta^{13}C$, $\delta^{15}N$) composition of tardigrades, rotifers and sediment from cryoconite holes from the three studied glaciers. We hypothesise that the elemental composition of tardigrades and rotifers reflects their diet and therefore shapes in nutrient utilization influencing the whole cryoconite ecosystem.

The text of this diploma thesis is designed as two projects which have much in common, but they are not absolutely connected. Therefore, it is structured as one compact unit containing two reciprocal parts focusing on the ecological interactions of cryoconite's tardigrades and rotifers from different points of views. The main intention of this structure is to create a basis for two papers which will be completed during the following doctoral studies.

2 Species Composition of Cryoconite Consumers Considering Environmental Variability on High Arctic Glaciers



2.1 Introduction

As diversity hotspots of the glacial surface, cryoconite holes form a suitable habitat for various species of bacteria, cyanobacteria, algae and few species of invertebrates (Stibal et al. 2006, Zawierucha et al. 2015a). Depending on conditions and input of nutrients during the season, organismal activity within cryoconite holes results in high photosynthetic and nitrogen fixation rates despite low phosphorus concentrations (Säwström et al. 2002, Anesio et al. 2009, Stibal et al. 2009, Segawa et al. 2014). Thus, cryoconite systems play a non-negligible role in nutrient cycling on the glacial surface.

Tardigrades living within cryoconite holes belong to the class Eutardigrades (Zawierucha et al. 2015a). As suspension feeders and grazers, cryoconite tardigrades consume mostly algae, but some species can be detritovorous or microbivorous (Zawierucha et al. 2016a). Despite the high diversity of tardigrades in tundra (Coulson et al. 2014), there is evidence of only 5 species living on glacial surface at Svalbard (Zawierucha et al. 2016a, Zawierucha in prep.). Tardigrades commonly found on Svalbard glaciers are *Pilatobius* sp., *Hypsibius* sp., *Hypsibius* cf. *dujardini*, *Isohypsibius* sp. and very rarely *Cryoconicus kaczmareki* (Zawierucha et al. 2018b).

Rotifers are invertebrates which require moist environment with adequate water to enable filtration, mostly of bacteria (Devetter 2009, Wallace and Snell 2010, Nielsen et al. 2012). As reported in Nielsen et al. (2012), who focused on wet and dry areas across Antarctic polar deserts, tardigrades were found in both areas, but rotifers were present only within moist areas. Accounts of the diversity of rotifers inhabiting cryoconite holes remain scarce due to difficulties associated with species identification (Kaya et al. 2010, Zawierucha et al. 2015a). The most abundant group are bdelloid rotifers. However, according to recent evidence, they have lower species richness in cryoconite holes than monogonts (De Smet and Van Rompu 1994, Zawierucha et al. 2015a). In cryoconite holes on Svalbard, the most dominant bdelloid rotifers are *Macrotrachella* sp., *Adineta vaga* and rarely members of a monogont genus *Encentrum* (Zawierucha et al. 2015a, Vonnahme et al. 2016, Vondrák unpublished data).

As top consumers with various feeding strategies, tardigrades and rotifers are an important component in nutrient cycling and trophic networks within cryoconite holes (De Smet and Van Rompu 1994, Zawierucha et al. 2016a). As evident from Vonnahme et al. (2015), there are correlations between species composition of primary producers and consumers (tardigrades and rotifers) connected with environmental as well as ecological controls. Further data showing the importance of their role are still lacking, however (Devetter 2009, Vonnahme et al. 2016, Zawierucha et al. 2018c).

Melting processes on the glacier contribute to the weathering of minerals and nutrients from the surrounding rocks (Aciego et al. 2015, Hawkings et al. 2015, Hawkings et al. 2016). These processes are mostly caused by seasonal warming connected to melting, rainfall or warm wind occurrence (e.g. föhn events) (Marsh and Woo 1981, Vieli et al. 2004). Moreover, the

geological setting (acidic or alkaline bedrock) has the ability to modify chemistry of local surface waters. Therefore, geology may have an indirect impact on tardigrade species composition and abundances (Dastych 1985). Nevertheless, Dastych (1985) examined only tardigrades from tundra and did not consider the effect of bird guano which can affect pH and thus community structure as found by Zawierucha et al. (2016b).

Organic and inorganic material brought onto glacier surface by melting or other factors (e.g. atmospheric deposition or guano input from bird colonies), have a crucial role for biogeochemical processes and the functioning of biota within cryoconite holes (Hodson et al. 2010, Stibal et al. 2012, Boetius et al. 2015, Vonnahme et al. 2016). As examined by Bagshaw et al. (2013) on Dry Valley glaciers in Antarctica, the highest proportion of nutrients was found within cryoconite holes on small glaciers. On the contrary, larger glacier had a higher concentration of DIC (dissolved inorganic carbon), SO_4^{2-}, Ca^{2+} and Mg^{2+}, as a probable result of longer residence times and the related more efficient release of minerals from sediment within the holes. Cryoconite holes on large glaciers also had low photosynthetic rates suggesting low biological activity and consequently low uptake by microbial communities. Porazinska et al. (2004) and Zawierucha et al. (2016a) described that higher concentrations of cations Mg^{2+}, K^{+} and Ca^{2+} and NH^{4+} are positively correlated with tardigrades occurrence, suggesting that mineral composition may play an important role on the species composition of a cryoconite hole. Higher nutrient inputs can also result in greater body size and thus increase reproduction rates and survival ability during unfavourable conditions (Zawierucha et al. 2015b). Ca^{2+} in a form of calcium carbonate ($CaCO_3$) is a crucial element for the synthesis of a new buccal-pharyngeal apparatus of tardigrades during moulting (Guidetti et al. 2012). Calcium was also found within solid structures of bdelloid rotifers (Koehler and Hayes 1969).

Species of cyanobacteria and algae found within cryoconite holes correspond with the species found in surrounding terrestrial and aquatic habitats (Säwström et al. 2002). Moreover, recent studies show greater abundances and activity of prokaryotic and eukaryotic organisms within the sediment than within the water column of cryoconite holes (Säwström et al. 2002, Sommers et al. 2019). Consumers living inside cryoconite prefer greater size and higher amount of cryoconite aggregates instead of fine sediment due to their higher stability and higher variability of food sources (Porazinska et al. 2004, Zawierucha et al. 2018a). A higher stability of cryoconite aggregates as a consequence of a non-frequent flushing by melted ice water was also mentioned in Mueller and Pollard (2004) who suggested that this stability is one of the factors influencing the cryoconite community structure. All presented information implies that spatial characteristics and size of the glacier may affect primary producers (Hodson et al. 2008, Sommers et al. 2019) and consequently the species composition of consumers.

The aim of this part of the thesis is to describe differences in species composition and body size distribution among three glaciers with different spatial characteristics. Focus has been placed

on the environmental proxies of these glaciers and on the potential impact on the release of minerals and nutrients. We hypothesise that sediment composition and location of cryoconite holes on the glacier surface can influence the community of grazers and their feeding strategies. We also hypothesise that body size varies between the lower and upper parts of the ablation zone due to the preferential transport of bigger individuals which have a greater surface area, creating a sensitivity to removal. Moreover, bigger species cannot be sufficiently hidden or retained in the sediment.

2.2 Methods

2.2.1 Study Sites and Sampling

Samples of cryoconite were collected from Ebbabreen, Nordenskiöldbreen and Svenbreen (Fig. 3) located at the Petuniabukta and Adolfbukta (76–80° 300 N and 10–35° E) on Spitsbergen island in Svalbard during July and August 2016 (Tab. 1).

Due to the Billefjorden Fault Zone, Petuniabukta forms a very geologically heterogeneous area with various rocks of different ages and origins (Dallmann et al. 2004). The Devonian clastic rocks in the western part are mostly composed from carbonates, coal, mudstones and sandstones. The Carboniferous sequences in the eastern part consist of various sedimentary rocks such as conglomerates, sandstones, mudstones, limestones, coal, gypsum, anhydride and dolomite (Dallmann et al. 2004, Szczuciński and Rachlewicz 2007).

Ebbabreen is a polythermal partially valley glacier connected to the Mittag-Leffler glacier (Kostrzewski et al. 1989, Rachlewicz et al. 2007, Małecki 2015). Ebbabreen is also the largest glacier in the Dickson Land (genetically related to the Olav V Land icefields) located in the east part of Petuniabukta (Dallmann et al. 2004, Małecki 2015). The theoretical steady-state equilibrium line is at an altitude of 580 m a. s. l. However, the accumulation zone is probably higher (Małecki 2015). Svenbreen is a polythermal valley glacier located in the western part of the Petuniabukta (Rachlewicz et al. 2007). Svenbreen is the only glacier in our study which is on the geologically older left side of the Billefjorden Fault Zone. The theoretical steady-state equilibrium line altitude of Svenbreen is about 420 m a. s. l. (Małecki 2014). Nordenskiöldbreen is a polythermal outlet glacier of Lomonosovfonna (fonna – ice field/ice cap), the highest ice field on Svalbard (Isaksson et al. 2001, Ewertowski et al. 2016). This glacier closes the Adolfbukta, the eastern bay of Billefjorden (Macheret 1981, Dallmann et al. 2004). In comparison with Svenbreen and Ebbabreen, Nordenskiöldbreen is the only tidewater glacier in Billefjorden (Dallmann et al. 2004). As a tidewater glacier, Nordenskiöldbreen has the highest retreat rates in comparison with other glaciers in Billefjorden (Rachlewicz et al. 2007). The theoretical steady-state equilibrium line altitude of Nordenskiöldbreen is around 550 m a. s. l. (Bamber et al. 2005).

Sampling was conducted twice from each glacier (within the interval of approximately one week between each sampling) using a high density polyethylene (HDPE) bottle with two siphons according to Mueller et al. (2001) with modifications after Vonnahme et al. (2015). On each glacier an area in the upper and lower part of the ablation zone (hereinafter also referred to as upper or lower part) close to the central line with the selection of representative cryoconite holes (small, bigger, shallow, deeper) was sampled. On Ebbabreen, 4 samples were collected. Samples from the first Ebbabreen sampling were lost during the transport to the Czech Republic. On Svenbreen 4 samples were collected and on Nordenskiöldbreen only 3 samples were collected, the fourth sample was not collected because of a polar bear occurrence. Water pH and conductivity (µS) were measured during the sampling by a Hanna Instrument (HI 98130). Samples were stored on ice in a field refrigerator (a plastic barrel entrenched into permafrost). The samples were subsequently transported to a freezer in Longyearbyen within the duration of 18–48 hours after the sampling.

Figure 3. Location of the sampling sites (Svenbreen – blue, Ebbabreen – orange and Nordenskiöldbreen – green). The map is adapted from https://toposvalbard.npolar.no/.

Table 1. General information about samples and sampling. Samples from Ebbabreen with the star mark (*) were lost during the transport.

SAMPLE CODE	GLACIER	ABLATION ZONE (PART)	ALTITUDE (m a. s. l.)	SAMPLING DATE
SL1	Sven	lower	382	30. 7. 2016
SU1	Sven	upper	637	30. 7. 2016
SL2	Sven	lower	421	6. 8. 2016
SU2	Sven	upper	663	6. 8. 2016
NL1	Nordenskiöld	lower	152	29. 7. 2016
NU1	Nordenskiöld	upper	211	29. 7. 2016
NL2	Nordenskiöld	lower	152	1. 9. 2016
EL1*	Ebba	lower	152	27. 7. 2016
EU1*	Ebba	upper	457	27. 7. 2016
EL2	Ebba	lower	158	2. 8. 2016
EU2	Ebba	upper	459	2. 8. 2016

2.2.2 Determination of Tardigrade Species, Buccal-Pharyngeal Apparatus and Body Size Measurements

Part of cryoconite (~10 cm^3) from each sample was separately melted by dropping distilled water through the sample into a glass beaker to minimize the number of freeze-thaw cycles of the whole sample. Tardigrades were collected using a glass Pasteur pipette into distilled water with one drop of EtOH under a stereo microscope (Olympus SZ 51). Immediately after the collection, clean animals were transferred on glass slides and mounted in a small drop of the permanent mounting Hoyer's medium (Anderson 1954, Ramazzotti and Maucci 1983).

After one day of drying in 56 °C, specimens were measured under a light microscope with phase contrast (Olympus BX53) associated with a digital camera ARTCAM 500. Measurements were conducted using a QuickPHOTO microscope software calibrated to the magnification of microscope. Every tardigrade was identified by the buccal-pharyngeal apparatus (hereinafter referred to as buccal tube length) and claw morphology according to Guidetti et al. (2012), Guil and Sanchez-Moreno (2013) and Nelson et al. (2015). Species identification followed Zawierucha et al. (2016a). The best method for the estimation of Eutardigrade body size is the measurement of buccal tube length (e.g. Bartels et al. 2011). In our specimens the buccal tubes were not always arranged in one plane and it was difficult to obtain the correct angle for morphometry. Therefore, it was decided to measure directly body length within suitably orientated specimens as well. The measurements were conducted from the anterior to the posterior part of the body, excluding the hind legs. Measurements of buccal tube followed Pilato (1981). Particular taxa were also classified to the dominant feeding behaviours groups according to Guidetti et al. (2012) and Guil and Sanchez-Moreno (2013).

2.2.3 Determination of Rotifer Species

Part of the cryoconite was melted as described above and separately put on slides. Specimens of bdelloid rotifers were identified using a compound light microscope when moving (identification is performed using the morphology of their cirri and trophi). Identification was primarily conducted following the monography by Doner (1965).

2.2.4 Determination of Eukaryotic Primary Producers

Small drop of thawed and well mixed cryoconite was placed on the mount. Afterwards, algae and cyanobacteria were examined in Nomarski interference contrast, using light microscope Olympus BX51 associated with the digital camera Olympus EOS 700D. The specimens were identified by Assoc. Prof. Pavel Škaloud, Ph.D. from the Department of Botany, Faculty of Science, Charles University.

2.2.5 Statistical Procedures

The differences between body size, buccal apparatus size and species composition of tardigrades were tested using R version 3.5.3 (R Development Core Team 2018).

Boxplots were made to show the intraspecific differences between body size and buccal tube length between glaciers and different parts of the ablation zone (Appendix 2). For testing these differences, the Kruskal-Wallis test and ANOVA (Lmer) were used. All tardigrades smaller than 190 μm were excluded from the statistical analyses due to the larger buccal apparatus compared to body size in young instars (Kosztyła et al. 2016), which could overestimate results. The correlation between body size and buccal tube length was also tested.

To compare species composition of tardigrades from different glaciers, Shannon-Wiener Index (hereinafter Shannon Index) of Diversity and Simpson Index of Diversity 1 – D (hereinafter Simpson Index) were examined.

2.3 Results

We determined 998 individuals of tardigrades (order Eutardigrada) from three glaciers in Billefjorden, namely Ebbabreen (186 identified tardigrades), Svenbreen (407 identified tardigrades) and Nordenskiöldbreen (405 identified tardigrades). From the total number of individuals, 977 were suitable for measurements of buccal apparatus, body length (proxy for the body size) or both. The tardigrades found within the samples were identified as *Pilatobius* sp., *Hypsibius* sp., *Hypsibius* cf. *dujardini*, *Isohypsibius* sp. and *Cryoconicus kaczmareki*. Tardigrades introduced without a specific name are new for science. Rotifers found within samples were identified as *Macrotrachella* sp. and *Adineta vaga*. There were only approximately 20 individuals of rotifers available for determination, however. The abundances of collected animals and species are shown in the Table 2. The tardigrades used for

determination of species composition were prepared by different methodology than the tardigrades for elemental and isotopic analyses (see the chapter 3), therefore, the total numbers of tardigrades and rotifers did not correspond with the numbers of identified species. The most abundant species were *Pilatobius* sp. and *Hypsibius* sp., lower abundances were observed in *Hypsibius* cf. *dujardini* and *Isohypsibius* sp. Tardigrade *Cryoconicus kaczmareki* was so rare (only few individuals were found) that we did not include it into any analyses in this part of the study. The distribution of species with the particular abundances is shown in the Figure 4.

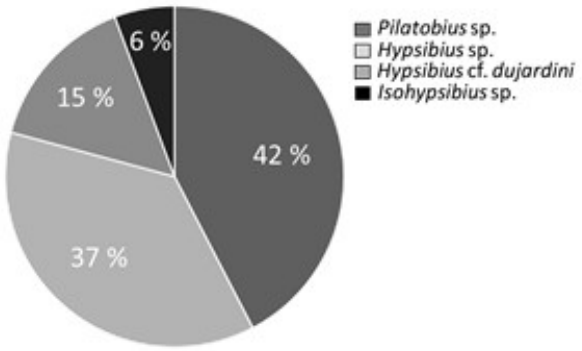


Figure 4. Distribution of tardigrade species expressed as relative abundance (%).

Table 2. Information about total number of collected animals and number of collected and identified species. Total tardigrades and rotifers numbers were counted from the animals collected for isotopic analyses. The symbol of (>) marks samples with an abundance superior to the calculated amount.

Sample	**SL1**	**SU1**	**SL2**	**SU2**	**NL1**	**NU1**	**NL2**	**EL2**	**EU2**
Rotifers total	490	> 1310	> 538	1071	764	846	806	> 569	340
Tardigrades total	776	828	58	820	856	847	847	345	> 1503
Hypsibius sp.	66	82	3	83	20	12	42	40	18
Hypsibius cf. *dujardini*	14	2	2	0	62	9	24	7	34
Pilatobius sp.	32	58	18	35	27	99	70	6	76
Isohypsibius sp.	7	2	2	1	26	1	13	4	1

To test the species richness and diversity of tardigrades between glaciers and parts of the ablation zone, we chose Shannon Index and Simpson Index. Shannon Index showed significant differences between tardigrades from upper and lower parts of the ablation zone from all glaciers together (p-value = 0.019) (Fig. 6). Shannon and Simpson Indexes used for establishing the dominances of various species between glaciers did not show any significant differences (Tab. 3). However, there is a distinguishable lower diversity at Svenbreen (Fig. 5). The proportions of species between lower and upper part of the ablation zone are shown in the Figure 7. Species composition of rotifers was not analysed due to the low number of identified individuals.

Table 3. Shannon and Simpson Indexes results for the biodiversity between glaciers and parts of the ablation zone.

Examined Subjects	Estimate (Shannon)	*p*-values (Shannon)	Estimate (Simpson)	*p*-values (Simpson)
Ebbabreen vs. Nordenskiöldbreen	0.068	0.958	0.027	0.981
Svenbreen vs. Nordenskiöldbreen	−0.160	0.722	−0.051	0.896
Svenbreen vs. Ebbabreen	−0.092	0.922	−0.025	0.982
Lower part vs. upper part	−0.342	0.019	−0.133	0.128

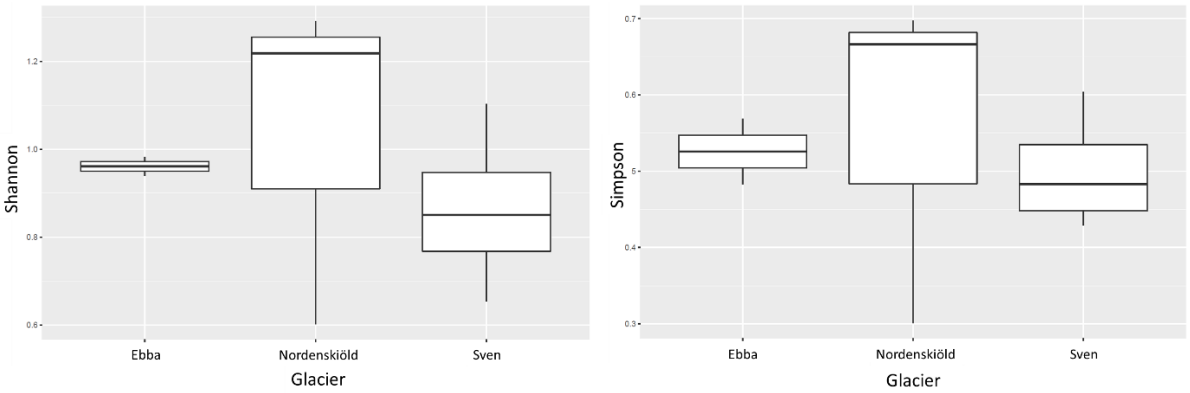


Figure 5. Shannon and Simpson Indexes of Diversity comparing diversity of tardigrades at each glacier.

Figure 6. Shannon index comparing diversity of tardigrades at upper and lower part of the ablation zone of sampled glaciers.

As expected, all species showed positive correlation of buccal tube length and body size (Appendix 1), *Pilatobius* sp. ($r^2 = 0.680$), *Hypsibius* sp. ($r^2 = 0.787$), *Hypsibius* cf. *dujardini* ($r^2 = 0.715$), *Isohypsibius* sp. ($r^2 = 0.867$).

The Kruskal-Wallis test and ANOVA (Lmer) revealed differences in buccal tube length between different parts of the ablation zone glaciers in *Hypsibius* sp. (*p*-value = 0.0009) and *Pilatobius* sp. (*p*-value = 0.0335). *Hypsibius* sp. had also significant differences in Kruskal-Wallis test comparing buccal tube length between lower and upper part of the ablation zone among all glaciers (*p*-value = 0.0160) and in body size between lower and upper part of the ablation zone on Ebbabreen (*p*-value = 0.0440). *Hypsibius* cf. *dujardini* revealed significant differences in body size between lower and upper part of the ablation zone among all glaciers tested by Kruskal-Wallis test (*p*-value = 0.0471) and by ANOVA (Lmer, *p*-value = 0.0209). *Hypsibius* cf. *dujardini* had also differences in buccal tube length between upper and lower part of the ablation zone of Ebbabreen (*p*-value = 0.0267). However, all species revealing significant differences had low number of individuals in some samples reducing the statistical significance of the results. All above mentioned results are in the Table 4.

Table 4. Results of Kruskal-Wallis (i, iv) and ANOVA (Lmer) (ii, iii) tests comparing intraspecific buccal tube length (BT) and body size (BS) between glaciers and between parts of the ablation zone. The *p*-values > 0.05 are highlighted in bold.

i) Measured *p*-values of Kruskal-Wallis tests among all glaciers.

Species	BS All Glaciers	BT All Glaciers	BS lower/upper	BT lower/upper
Hypsibius cf. *dujardini*	0.1229	0.4646	**0.0471**	0.4674
Hypsibius sp.	0.8691	**0.0009**	0.1454	**0.0160**
Isohypsibius sp.	0.7120	0.1303	0.9881	0.5884
Pilatobius sp.	0.1999	**0.0335**	0.6838	0.8922

ii) Measured *p*-values of ANOVA (Lmer) among all glaciers.

Species	BS All Glaciers	BT All Glaciers	BS lower/upper	BT lower/upper
Hypsibius cf. *dujardini*	0.0678	0.4524	**0.0209**	0.5189
Hypsibius sp.	0.5044	**0.0102**	0.0900	0.3102
Isohypsibius sp.	0.5426	0.1332	0.4877	0.2886
Pilatobius sp.	0.3592	**0.0472**	0.3554	0.6442

iii) Measured *p*-values of ANOVA (Lmer) of body size and buccal tube length between parts of the ablation zone without measurements from Sven for *Hypsibius* cf. *dujardini* (there were no *Hypsibius* cf. *dujardini* present in the sample Sven upper from 2nd sampling).

	Hypsibius cf. *dujardini*
BS (*p*-value)	0.0689
BT (*p*-value)	0.2501

iv) Results of Kruskal-Wallis comparing intraspecific body size and buccal tube length between lower and upper part of the ablation zone of each glacier.

Species	**BS Ebba**	**BS Nordenskiöld**	**BS Sven**
Hypsibius cf. *dujardini*	0.2748	0.1520	0.3082
Hypsibius sp.	**0.0440**	0.0861	0.7638
Isohypsibius sp.	0.1573	0.3985	0.5688
Pilatobius sp.	0.9908	0.2022	0.6435

Species	**BT Ebba**	**BT Nordenskiöld**	**BT Sven**
Hypsibius cf. *dujardini*	**0.0267**	0.8010	0.2012
Hypsibius sp.	0.0890	0.8948	0.4989
Isohypsibius sp.	0.1797	0.1186	0.7697
Pilatobius sp.	0.4271	0.0743	0.3883

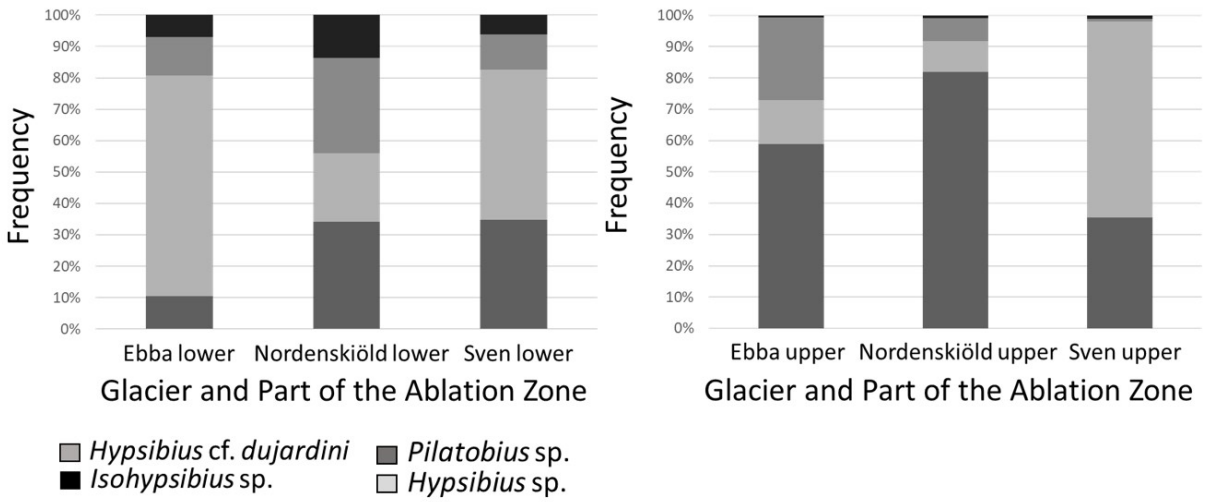


Figure 7. Differences in species composition of tardigrades at different parts of ablation zone.

Algae and cyanobacteria were found in all samples. We identified: *Leptolyngbya* sp., Nostocales, *Phormidium*, *Mesoteanium*, *Ancylonema*, *Chlamydomonas* sp., *Chloromonas* sp. and *Vischeria* sp. *Leptolyngbya* sp. dominated in the sample SL2 (Svenbreen lower part, 2nd

sampling) and EL2 (Ebbabreen lower part, 2nd sampling). Dominance of a green algae *Mesoteanium* was observed only within EL2 (Ebbabreen lower part, 2nd sampling). Other samples were more or less similar with higher abundance of cyanobacteria compared to algae.

2.4 Discussion

We examined the community structure on three glaciers located in Billefjorden and found differences in species composition of tardigrades. Rotifers were present but they were not determined in a representative number for statistical analyses. We also found some trends in the intraspecific differences in buccal tube length and body size between glaciers and between parts of the ablation zone in *Hypsibius* sp., *Pilatobius* sp. and *Hypsibius* cf. *dujardini*. Even though these tardigrades revealed some trends, it is necessary to enhance the number of examined individuals to corroborate or disprove our hypothesis that bigger individuals are more abundant within the lower parts of the ablation zone. Further examination is also required because body size differences between genera could influence the grazing pressure and utilization of resources in places with higher occurrence of bigger (*Isohypsibius* sp.) or smaller (*Pilatobius* sp.) species.

Svenbreen is a small valley glacier located in the western part of Petuniabukta in a geologically older part of Billefjorden Fault zone (Rachlewicz et al. 2007, Szczuciński and Rachlewicz 2007). We expect that due to the smaller size of the glacier and highly vegetated surrounding, Svenbreen should be much more supported by organic matter than Ebbabreen and Nordenskiöldbreen and thus affected by bacterial decomposition and occurrence of microbivorous species. From the total number of collected animals, we have found that Svenbreen has a dominance of rotifers within all samples excluding one sample taken from the lower part of the ablation zone during the first sampling session (SL1). Based on microscopic observations, this SL1 sample was dominated by algae, mostly *Mesotaenium*. However, the sample from the second sampling session (SL2) was full of cyanobacteria (*Leptolyngbya* sp.) with the lowest number of tardigrades from all analysed samples. The SL2 sample was different probably due to a weather event which altered the cryoconite structure and disturbed the formed cryoconite aggregates (Zawierucha et al. 2019b). We assume that the higher occurrence of rotifers should relate to higher abundances of bacteria, however, we do not have any data about bacterial biomass to support these assumptions.

The upper parts of Svenbreen (samples SU1 and SU2) and the lower parts of Ebbabreen (sample EL2) were also dominated by rotifers. Based on our observations, it is evident that these samples have higher numbers of *Hypsibius* sp. compared to *Pilatobius* sp. what could point to the competitive pressure with rotifers. There is not any evident pattern between species composition of tardigrades in samples with the dominance of tardigrades. However, it is complicated to estimate such relations in very fragile and dynamic system such as cryoconite holes, which can often disappear within few days (Zawierucha et al. 2019b). Cryoconite holes

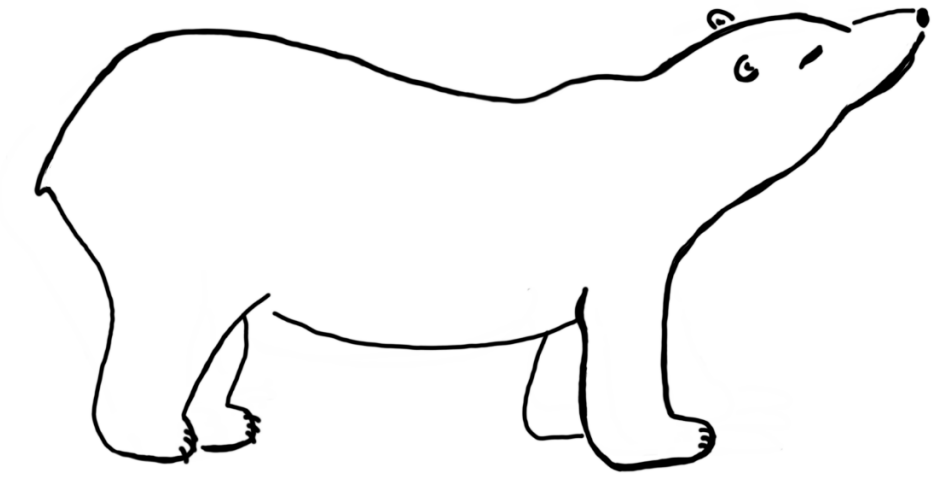
from Nordenskiöldbreen had almost equal numbers of tardigrades and rotifers among all samples with observed dominance of *Nostocales*.

In the case of tardigrade species composition, we documented higher relative abundance of *Pilatobius* sp. compared to *Isohypsibius* sp. and both species of *Hypsibius* in the upper parts of Ebbabreen and Nordenskiöldbreen. The lower parts were mostly dominated by *Hypsibius* sp. and *Hypsibius* cf. *dujardini*. The abundance of *Isohypsibius* sp. was also higher in lower parts. These findings could suggest that lower parts offer higher variability of sources supporting higher biodiversity. Cryoconite holes on Svenbreen have almost similar species composition at both parts with a dominance of *Hypsibius* sp. However, it is necessary to take into consideration that samples from the second sampling of the lower part of Svenbreen (SL2) and second sampling from the lower part of Ebbabreen (EL2) had a low occurrence of tardigrades which do not allow to evaluate the results properly.

2.5 Conclusions

In this part of the thesis we demonstrated that even though cryoconite holes have similar temperature and structural characteristics, the community structure of tardigrades differs among the three examined glaciers. We also found that samples with the dominance of rotifers show lower abundances of mostly microbivorous *Pilatobius* sp. and higher abundances of mostly algivorous genus *Hypsibius* suggesting the division of feeding niches. The differences in size distribution between different parts of the ablation zone were found in *Hypsibius* sp. and *Hypsibius* cf. *dujardini.* However, to corroborate our hypothesis about differences in size distribution between different parts of the ablation zone it is necessary to enhance the number of examined individuals. It is also necessary to enhance the number of identified rotifers in further examination.

3 Elemental and Isotopic Composition of Tardigrades and Rotifers Inhabiting Cryoconite Holes of Three High Arctic Glaciers



3.1 Introduction

According to current knowledge, viruses, bacteria and other autotrophic and heterotrophic organisms play an important role in nutrient utilization and nutrient pathways within glacial ecosystems (Anesio and Laybourn-Parry 2012, Stibal et al. 2012). There are many studies focusing on biogeochemical and microbiological processes within the glacier ecosystems in recent literature (Barrett et al. 2007, Macdonell et al. 2012, Telling et al. 2012, Edwards et al. 2014). However, information about the role of tardigrades and rotifers, which are the top consumers there, are lacking (Zawierucha et al. 2018a, Vonnahme et al. 2016). These invertebrates consume mostly algae, bacteria and detritus. Rotifers are mostly bacterivorous, while tardigrades feed on algae as well as bacteria (De Smet and Van Rompu 1994, Wallace and Snell 2010, Mialet et al. 2013, Zawierucha et al. 2016a). We expect that these different feeding strategies cause differences in isotopic composition and trophic position due to differences in the uptake of nutrients by bacteria, cyanobacteria and algae. We also expect that the isotopic composition of tardigrades and rotifers between examined glaciers will differ due to different spatial characteristics influencing cryoconite holes and consequently their biota as well.

In temperate freshwater systems, Sterner (1986) described that grazers can affect population of phytoplankton by two means – directly by grazing and indirectly by affecting the ratio of nutrients by their release after the consumption called nutrient recycling (Sterner and Elser 2002). Trophic networks in Arctic ponds show lower complexity than ponds in temperate habitats and most of the primary production comes from the benthic primary producers (Rautio et al. 2011). Vonnahme et al. (2016) has found that the size distribution of algae, particularly Zygnemales and Chlorococcales, positively correlate with the distribution of grazers. Larger Zygnemales positively correlates with tardigrades occurrence, and Zygnemales and smaller Chlorococcales with rotifers occurrence. The other important finding of that work was that Cyanobacteria had no correlations with the abundance of grazers but were found in higher proportion further away from the glacier margins. In soil habitats, tardigrades may react as r-strategists with increasing feeding rate when the prey had high densities and environment had favourable conditions (Sánchez-Moreno et al. 2008). This information suggests that tardigrades and rotifers are probably an important factor shaping the community of primary producers by grazing and nutrient recycling, and together with microbes, should have an impact on the community structure and the nutrient pathways within cryoconite holes.

The distribution of nutrients across the glacial surface is very unequal (Stibal et al. 2012). Available forms of nutrients in cryoconite holes are provided by melted snow (Hodson et al. 2005), ice (Brown 2002), long-range and local atmospheric deposition (Stibal et al. 2008a, Stibal et al. 2012) and by activity of heterotrophic microbial communities which utilize the inorganic matter or decompose the organic matter (Tranter et al. 2004, Anesio et al. 2010). The

importance of autochthonous organic matter formed by organisms increases with decreasing distance from other sources (Stibal et al. 2010).

Carbon, nitrogen and phosphorus are the major components of organic matter and essential elements for growth and functioning of biota on the glacial surface (Hodson et al. 2005, Anesio et al. 2009, Telling et al. 2011, Bagshaw et al. 2013). Due to the different characteristics of cryoconite holes, the ratios and concentrations of these major nutrients can be very distinct from the rest of the glacier surface (Tranter et al. 2004, Stibal et al. 2008b) and between glaciers (Edwards et al. 2011).

Dissolved carbon occurs in two major forms available for organisms on the glacial surface − as dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) (Tranter et al. 2004). The ratio of DIC:DOC in ice-lidded cryoconite holes studied by Tranter et al. (2004) was calculated as 2:1, while the measurements of Stibal et al. (2008a) done on open holes showed higher content of DOC compared to DIC. In that study, proportion of total carbon (TC = organic carbon + inorganic carbon) remained approximately equal during the summer season (there were observed decrease in TC only at the beginning of the season). Stibal et al. (2008a) also concluded that the majority of organic carbon originated from production out of the studied glacier, prevailing the content of organic carbon from annual autochthonous production of the cryoconite associated organisms. This indicates the importance of the allochthonous input of organic carbon to the cryoconite hole ecosystem. Within the open holes, photosynthesis was found higher than respiration causing positive carbon fixation rates on the glacial surface (Anesio et al. 2009). However, Stibal et al. (2012) summarized the balance between photosynthesis and respiration on the glacier surface and found that ice sheet margins and small glaciers can act as CO_2 source instead of inner parts of ice sheets and bigger glaciers which can act more as CO_2 sinks. In any case, the total photosynthetic rates on the glacier surface remains low due to the high radiation levels (Säwström et al. 2002).

The input of reactive species of nitrogen into cryoconite holes is mostly restricted to meltwater from the snow cover, rain, snow precipitation and nitrogen fixation by cyanobacteria (Telling et al. 2011). Input from bird colonies which are abundant around some glaciers seems to have spatially limited but significant impact as well (Vonnahme et al. 2016). On Svalbard glaciers, the C:N ratio of biomass and inorganic forms of NO_3^- and NH_4^+ decrease during the summer season. It suggests that cryoconite system can become nitrogen limited during the period of organismal activity (Tranter et al. 2004, Stibal et al. 2008a). This deficiency can lead to the dominance of cyanobacteria (Vonnahme et al. 2016). However, the study of Pittino et al. (2018) suggested an opposite pattern when the cyanobacteria occur during the beginning of the melting season, participating on cryoconite aggregates formation and increasing the organic carbon (OC) content, and disappear lately due to the increasing dominance of heterotrophs. Because of the high energetic costs and sufficient input of inorganic nitrogen (IN) from atmosphere,

nitrogen fixation is a minor source of nitrogen for cryoconite communities. However, it provides an important source of this essential nutrient while other supplies are depleted during the melting season (Telling et al. 2011).

Due to the indispensability of phosphorus for the growth of living organisms along with its biologically inaccessible forms occurring under oxic conditions, it is typically the most limiting nutrient in aquatic ecosystems (Karl 2000, Kalff 2002). High TDN:TDP (TDN – total dissolved nitrogen, TDP – total dissolved phosphorus) ratios and decreasing phosphorus concentrations with increasing carbon uptake show, that there is a strong trend for cryoconite holes to be, as other freshwater habitats, mainly phosphorus limited (Tranter et al. 2004, Stibal and Tranter 2007, Stibal et al. 2009). The main source of inorganic phosphorus are rocks (e.g. apatite) adjacent to the glacier body losing its sediment and releasing IP (inorganic phosphorus) by weathering (Hodson et al. 2005, Hawkings et al. 2016). Stibal et al. (2008b) demonstrated that total phosphorus content in cryoconite included about 57 % of OP (organic phosphorus), mostly from allochthonous origin. Conversion of inorganic phosphorus to its organic forms was found as minor process in cryoconite holes. Bioavailable phosphorus (e.g. P_2O_5, DOP – dissolved organic phosphorus) then occurs in low, but sufficient concentration within cryoconite and it is controlled mostly by physical and geochemical processes (Stibal et al. 2008b).

Stable isotopes are energetically stable and non-radioactive atoms which have been used for the description of food webs and trophic pathways in many ecological studies in the past decades (e.g. DeNiro and Epstein 1978, DeNiro and Epstein 1981, Michener and Lajtha 2008). For the analyses of differences in trophic levels and sources of nutrients, $\delta^{15}N$, $\delta^{13}C$ values and $\delta^{15}N/\delta^{14}N$, $\delta^{13}C/\delta^{12}C$ ratios are mostly used (Yoshii et al. 1999, Michener and Lajtha 2008, Wada 2009). Autotrophs absorb carbon and nitrogen from inorganic matter which has a different isotopic composition than the consumer's body. The process which causes these differences between ingested matter and body composition is called isotopic fractionation. Fractionation is caused by physical or biochemical processes which prefer or discriminate heavier or lighter isotopes (Michener and Lajtha 2008). The isotopic ratio of the sample is calculated by using the equation: $\delta\ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$. The resulting ratio or value shows the amount of heavier isotope related to the isotopic composition of the international standards which are PeeDee Belemnite for carbon and atmospheric nitrogen (N_2) for nitrogen. Higher delta values (δ) indicate a higher content of the heavier isotope within the sample (Craig 1953, Michener and Lajtha 2008).

The $\delta^{13}C$ value reflects the diet of the organism and is approximately similar or slightly higher within the animal compared to its food. The differences between organismal $\delta^{13}C/\delta^{12}C$ and the $\delta^{13}C/\delta^{12}C$ ratios of its diet are caused by higher assimilation of ^{13}C supported by preferential ^{12}C depletion (of CO_2) during respiration (DeNiro and Epstein 1978, Blair et al. 1985, Ekblad

and Högberg 2000, Wada 2009). These differences are balanced by a higher release of ^{13}C during excretion (DeNiro and Epstein 1978).

The $\delta^{15}N$ value towards the $\delta^{14}N$ value is also higher in animal body compared to its diet (DeNiro and Epstein 1981). The equations describing the relation between isotopic composition of organisms and their diet are:

1. For carbon
   $\Delta_{ANIMAL-DIET} = \delta^{13}C_{ANIMAL} - \delta^{13}C_{DIET}$ (DeNiro and Epstein 1978)

2. For nitrogen
   $\Delta_{ANIMAL-DIET} = \delta^{15}N_{ANIMAL} - \delta^{15}N_{DIET}$ (DeNiro and Epstein 1981)

Moreover, $\delta^{15}N$ values increase with the trophic level (Kling et al. 1992, Zah et al. 2001). This increase is mostly caused by higher proportion of proteins within the diet and subsequent preferential excretion of $\delta^{14}N$ during the protein metabolism (Kling et al. 1992, McCutchan et al. 2003).

Nitrogen isotopic composition of primary producers and subsequently consumers can vary due to various environmental factors. When the environment is limited by some nutrient, in this case nitrogen, the consumer's body fractionates differently than in case where there is no nutrient limitation (Michener and Lajtha 2008, Šantrůček et al. 2018). Therefore, it is always necessary to measure isotopic composition of food to get reliable estimate of trophic relationships within the ecosystem.

The only study focused on isotopic composition of polar tardigrades and rotifers was done by Shaw et al. (2018) in non-glaciated part of the Taylor Valley, Antarctica. Significant differences were found in $\delta^{15}N$ values between primary producers (cyanobacteria) and consumers (rotifers, tardigrades and nematods) which divided soil fauna into three main trophic levels. The isotopic value of the first established trophic level (cyanobacterial mat) was $\delta^{15}N$ –5.36 ± 0.96 ‰. The $\delta^{15}N$ of the secondary trophic level was enriched +2 ‰ for the nematode *Plectus murrayi* and +4 ‰ for tardigrades and rotifers. The third trophic level consisting of nematode *Eudorylaimus antarcticus* was enriched +2.25 ‰ relatively to tardigrades and rotifers and +4 ‰ relatively to *Plectus murrayi.*

Based on the previous findings, our intention is to measure isotopic composition ($\delta^{13}C$, $\delta^{15}N$) of the substrate, biomass of primary producers, and biomass of consumers from various cryoconite holes and obtain data for the description of trophic interactions and trophic levels within these systems.

The aim of this part of the thesis is to develop and test the modified methodology and analyse the elemental and isotopic composition of tardigrades and rotifers as well as to analyse the composition of organic and inorganic matter from cryoconite. Results will provide data for the

description of cryoconite grazers and their trophic relations which are non-negligible factors in cryoconite hole's nutrient utilization.

3.2 Methods

3.2.1 Study Sites and Sampling

See the section 2.2.1.

3.2.2 Separation of Tardigrades and Rotifers from Cryoconite

Part of cryoconite (~2–4 cm^3) from each sample was separately melted by dropping distilled water through the sample into a glass beaker to minimize the number of freeze-thaw cycles of the whole sample. The rest of the cryoconite sample was immediately returned into to the freezer. After the melted material was settled on the bottom of the beaker, as many cryoconite aggregates and big sediment particles as possible were removed by plastic spoon and transported into a separate Falcon tube. The fine particles which were left within the beaker were then transported into another Falcon tube. Animals were collected from both Falcon tubes. The size separation of sediment particles accelerated collecting of tardigrades and rotifers due to the higher numbers of animals within the fine sediment.

Animals were then collected under a light microscope (Olympus CX31) using a Pasteur pipette. Every single individual was transferred at least once to a drop of clean distilled water before it was transferred into an Eppendorf tube. Eppendorf tubes for clean animals as well as samples of sediment were continuously cooled by a frozen cooling pad. The collected individuals were stored in separate Eppendorf tubes in a freezer at –20 °C until the lyophilization and further processing started. The total number of collected animals were used for the estimation of frequencies of each taxon within a particular sample.

3.2.3 Elemental and Isotopic Analyses of Invertebrates

3.2.3.1 Carbon and Nitrogen

At least 300 individuals of both taxa were collected from each sample for the elemental and isotopic analyses. When the required number of individuals was prepared, samples of tardigrades and rotifers were thawed and once again washed through distilled water and separately put into pre-weighted tin capsules (Costech 41077, 5 × 9 mm). Filled capsules were put into a desiccator with silica gel and left drying from 0.5 to 2.5 hours (the differences in the duration of drying were caused by differences in water content within the capsules). After the water inside the capsules was reduced to 1/3 of the volume, the samples were stored at –20 °C and at least half an hour at –80 °C before the lyophilization started. The duration of the lyophilization was 4 hours. Thereafter, samples were weighted (Mettler Toledo Excellence Plus

XP6), the capsules were closed and wrapped and immediately analysed or stored in a desiccator until the analyses were performed.

The samples were combusted and total carbon (TC), total nitrogen (TN) and $\delta^{13}C/\delta^{12}C$ and $\delta^{15}N/\delta^{14}N$ isotopes were analysed using a Flash 2000 elemental analyser (ThermoFisher Scientific). Released gasses (NO_x, CO_2) separated in a GC column were transferred to the source of an isotope-ratio mass spectrometer Delta V Advantage (ThermoFisher Scientific) source through a capillary by Continuous Flow IV system (ThermoFisher Scientific). Stable isotopes results are expressed in standard delta notation (δ), with samples measured relatively to Pee Dee Belemnite for carbon isotopes and atmospheric N_2 for nitrogen isotopes and normalized to a calibration curve based on international standards IAEA-CH-6, IAEA-CH-3 and IAEA 600 for carbon and IAEA-N-2, IAEA-N-1 and IAEA-NO-3 for nitrogen. Analytical precision as a long reproducibility for standards was within ±0.03 ‰ for $\delta^{13}C$ and ±0.02 ‰ for $\delta^{15}N$.

The data about TC and TN were not included into the study due to the low number of samples which had the sufficient amount of CO_x and NO_x. Therefore, C:N:P ratios were not established.

3.2.3.2 Phosphorus

Samples for phosphorus concentration analyses were prepared using membrane filters (MF-Millipore ™, 0,45 μm). The procedure was done under a light microscope (Olympus CX31). Collected samples were melted, cleaned through deionized water and placed to clean filters which were put on a Petri dish (the aluminium foil was put under the filters to avoid sticking during the drying process). The number of animals given to the filters varied between 71 and 120 individuals. The filters were left drying at 50 °C for approximately 8 hours. All filters were placed into laboratory drying oven and covered by aluminium foil to avoid contamination.

After all filters were dried, samples were dissolved in Savillex bottles with 0.5 ml of concentrated HNO_3. To speed up the process, all bottles were put on a hot plate (125 °C) and boiled for a few minutes. When all filters were dissolved, the samples were evaporated to half of its volume to achieve the final concentration of the solution approximately 2.5 %. Consequently, all dissolved samples were poured into test tubes and deionized water (diH_2O) was added to refill the volume to 10 ml. All samples were then gently stirred and refrigerated (~4 °C) until they were analysed.

Analyses were performed by a spectrometer ICP-MS iCAP 6500 radial (ThermoFisher Scientific). The results were not included into the study, because all concentrations were on or below detection limits for analyses.

3.2.4 Elemental and Isotopic Analyses of Cryoconite

Cryoconite intended for analyses was cleaned from tardigrades and rotifers which were collected in parallel for elemental analyses. After the collection, cryoconite was stored at – 20 °C. When all samples were prepared, cryoconite was homogenised using agate pestle and a mortar. The sediment was subsequently dried in a drying oven at 45 °C for about 7 hours.

3.2.4.1 Analyses of Carbon and Nitrogen in Cryoconite with Carbonates

Dried cryoconite was transferred without any other preparation into pre-weighted open tin capsules (Costech 41077, 5 × 9 mm) and weighted. The amount of cryoconite varied from 29 mg to 41 mg. Weighted tin capsules with cryoconite were closed and wrapped and analysed for TC, TN, $\delta^{13}C/\delta^{12}C$ and $\delta^{15}N/\delta^{14}N$ using an elemental analyser EA Flash 2000 (ThermoFisher Scientific) with IRMS Delta V Advantage (ThermoFisher Scientific, see the section 3.2.3.1 for details).

3.2.4.2 Analyses of Carbon and Nitrogen in Cryoconite without Carbonates

The dried cryoconite was transferred into pre-weighted silver capsules (Elemental Analyses, 8 × 5 mm, D2008). Approximately 12 mg of cryoconite were used for analyses. Carbonates (e.g. calcite, dolomite) were then dissolved using 10% HCl moistened with diH_2O. The acid was pipetted into the capsules followed by additions of 10, 20, 30, 50 and 100 mL with drying after each addition according to Brodie et al. (2011) with the modification after Vindušková et al. (2019).

After the last acid addition (100 mL), the sediment was left drying at 50 °C for 17 hours. When the sediment was dry, the silver capsules were closed, wrapped, inserted into tin capsules and put into a desiccator for 20 days.

Even though there were not any "Champagne" reaction observed during the acid addition, the residue of acid salts occurred in two wells on an immune pad where the samples were stored.

Tin capsules with sediment were then wrapped and analysed for TC, TN, $\delta^{13}C/\delta^{12}C$ and $\delta^{15}N/\delta^{14}N$ using an elemental analyser EA Flash 2000 (Thermo) with IRMS Delta V Advantage. The calibration curve was based on the international standard ST-Soil Standard (Peaty). See the section 3.2.3.1 for details.

The sediment was examined for isotopic and elemental composition of OC, IC and nitrogen within the organic fraction. Unfortunately, not all $\delta^{13}C$ values of OC were measured due to an error in dilution during analyses. The proportion of carbon and nitrogen within cryoconite were also not included due an error during the measurements.

3.2.5 X-Ray Diffraction of Cryoconite

To reveal the differences in geological composition of sediment among the three glaciers, mineral phases of homogenized sediment were determined by an X-Ray diffraction analysis on the PANalytical X`PertPro (PW3040/60) with a X`Celerator detector. The measurements were conducted under following conditions: radiation – CuKα, 40 kV, 30 mA, angular range –3–70° 2θ, step 0.02°/150 s. The results were evaluated using a X`Pert HighScore Plus software 1.0d program with a JCPDS PDF-2 (ICDD, 2002) database.

3.3 Results

3.3.1 Elemental and Isotopic Composition of Tardigrades and Rotifers

Significant differences in isotopic composition ($\delta^{13}C$, $\delta^{15}N$) between tardigrades and rotifers were found in all samples (Kruskal-Wallis chi-squared = 14.153, *df* = 1, *p*-value = 0.0001685). All values are shown in the Table 5. Some of the samples did not have a sufficient amount of gas (CO_x and NO_x) to keep the linearity of the sample. These samples were removed from statistical analyses. Tardigrades revealed significantly lower values of $\delta^{15}N$ isotope than rotifers (Fig. 8). The isotopic values for tardigrades range between −3.45 ‰ and −4.85 ‰ and for rotifers between −3.02 ‰ and −1.04 ‰.

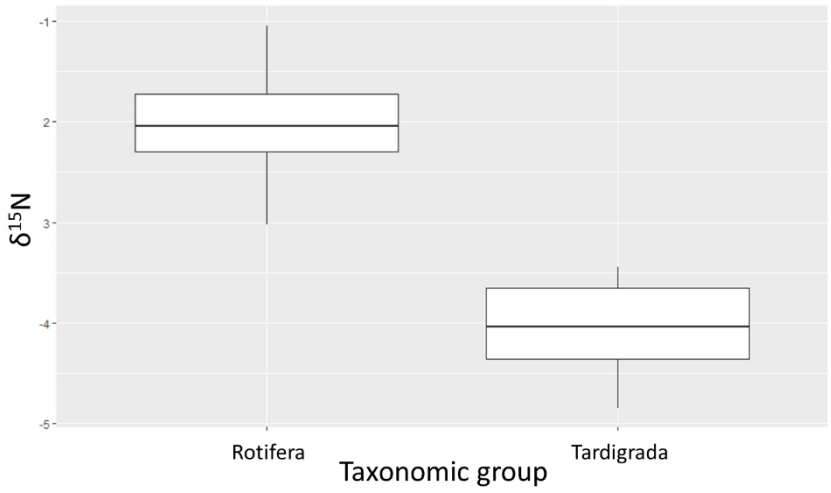


Figure 8. Differences in $\delta^{15}N$ between tardigrades and Rotifera.

According to $\delta^{13}C$ values of tardigrades and rotifers, the results divide animals from each glacier into groups with the highest $\delta^{13}C$ values at the Nordenskiöldbreen upper part (NU) and the lowest at the Svenbreen excluding value of tardigrades from SL1 which are close to the samples from Nordenskiöldbreen. Isotopic values of samples from Ebbabreen are ranging close to −25 ‰ for tardigrades as well as for rotifers. Animals from Nordenskiöldbreen are divided into two groups with the highest values of $\delta^{13}C$ (around −23 ‰) for samples from the first sampling session and values close to −25 ‰ for samples from the second sampling session. Similarities between the sampling sites and the glaciers are shown in the Figure 9.

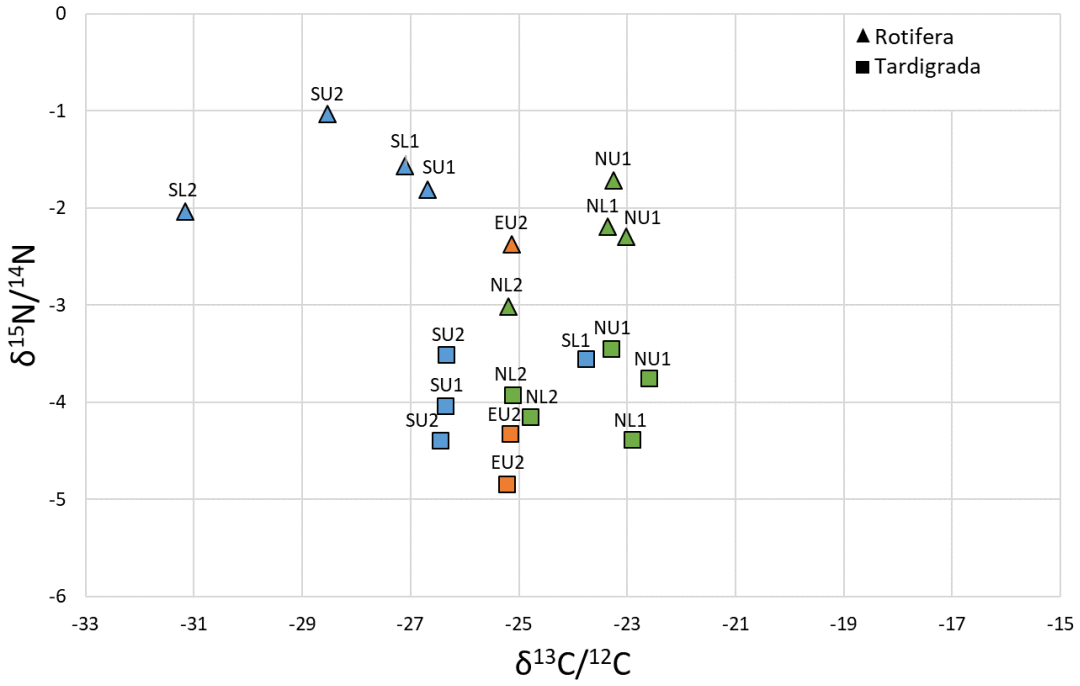


Figure 9. Isotopic signature of tardigrades and rotifers from different parts of the ablation zone (L – lower part, U – upper part) and different sampling session (1 – first sampling, 2 – second sampling) at Ebbabreen (red), Nordenskiöldbreen (green) and Svenbreen (blue). Data are presented in ‰ related to PDB and atmospheric N_2 standards.

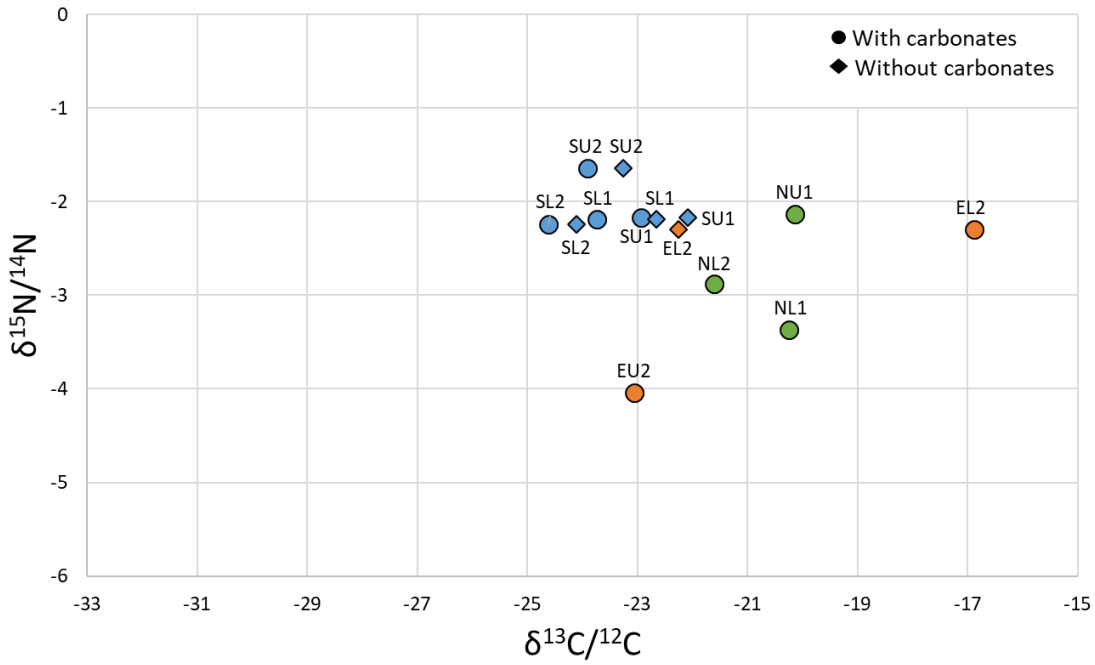


Figure 10. Isotopic values of cryoconite with carbonates and without carbonates from different parts of the ablation zone (L – lower part, U – upper part) and different sampling session (1 – first sampling, 2 – second sampling) at Ebbabreen (red), Nordenskiöldbreen (green) and Svenbreen (blue). Data are presented in ‰ related to PDB and atmospheric N_2 standards.

Table 5. Description of samples and values of isotopic ratios ($\delta^{13}C$, $\delta^{15}N$) of tardigrades, rotifers and cryoconite. Isotopic values are presented as ‰ and related to the international standards Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen. The $\delta^{13}C^*$ are values of cryoconite after carbonate removal. Conductivity (σ) and pH were measured in the field. Conductivity values are given in µS. In case that two measurements have been made, both values are listed. The sign (−) means that the measurement was not conducted.

	Tardigrades		Rotifers		Cryoconite			Parameters	
Sample	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{13}C^*$**	**pH**	**σ**
SL1	−3.55	−23.75	−1.57	−27.09	−2.20	−23.72	−22.65	5	1
SU1	−4.04	−26.36	−1.81	−26.69	−2.17	−22.92	−22.08	5.57	69
SL2	–	–	−2.04	−31.16	−2.24	−24.61	−24.09	–	–
SU2	−3.52 −4.39	−26.33 −26.45	−1.04	−28.53	−1.64	−23.89	−23.25	–	–
NL1	−4.39	−22.91	−2.20	−23.36	−3.37	−20.23	–	4.48	4
NU1	−3.45 −3.76	−23.30 −22.58	−1.72 −2.30	−23.25 −23.02	−2.14	−20.13	–	5.9	3
NL2	−4.15 −3.93	−24.78 −25.11	−3.02	−25.19	−2.88	−21.59	–	–	–
EL2	–	–	–	−27.39	−2.30	−16.86	−22.24	–	–
EU2	−4.85 −4.33	−25.22 −25.15	−2.38	−25.13	−4.05	−23.05	–	–	–

3.3.2 Isotopic Composition of Cryoconite and its Differences between the Sampling Sites

Isotopic analyses of cryoconite with removed tardigrades and rotifers showed differences between samples from Svenbreen as a distinct from Ebbabreen and Nordenskiöldbreen (Fig. 10). The highest value of $\delta^{15}N$ (−1.64 ‰) was detected in the sample from Svenbreen. The lowest $\delta^{15}N$ was detected in the sample from the upper part of Ebbabreen with the value −4.05 ‰. Svenbreen is a representative of small glaciers in the geologically older part of Billefjorden Fault Zone. Ebbabreen and Nordenskiöldbreen are larger valley glaciers within the geologically younger zone. X-Ray diffraction of the cryoconite showed that glaciers differ in mineral composition. Svenbreen has the absence of dolomite and amphibole which are dominantly found within metamorphic basement rocks around Ebba and Nordenskiöld. No phosphate minerals such as apatite were detected. Svenbreen also showed lower values of $\delta^{13}C$ in TC fraction compared to Ebbabreen and Nordenskiöldbreen. Nevertheless, we were not able to prove these differences with $\delta^{13}C$ values of OC due to the lack of data from Nordenskiöldbreen and the upper part of Ebbabreen. The measured samples showed that cryoconite after carbonate removal (relieved from the inorganic carbon) from Svenbreen has higher values of $\delta^{13}C$ in OC in comparison with $\delta^{13}C$ of TC. Oppositely, decarbonated cryoconite from the lower part of Ebbabreen (EL2) shows a decrease in $\delta^{13}C$ value in

comparison with $\delta^{13}C$ of TC. All the above-mentioned results are summarized in the Table 5. The comparison of $\delta^{15}N$ values between rotifers, tardigrades and organic matter within cryoconite is demonstrated in the Figure 11.

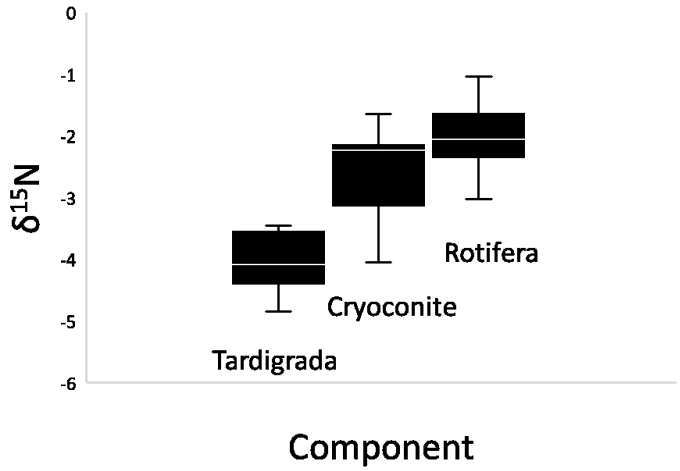


Figure 11. The boxplots comparing $\delta^{15}N$ values of tardigrades, rotifers and organic matter within cryoconite. The diagram displays medians and the distribution of measured $\delta^{15}N$ values. The whiskers represent the lowest and highest measured values.

3.4 Discussion

Isotopic analyses showed differences in isotopic composition of rotifers and tardigrades between the phyla and between the glaciers. We have found that rotifers have higher values of ^{15}N isotope compared to tardigrades, suggesting that they have probably different $^{15}N/^{14}N$ composition of the diet. Higher values of $\delta^{15}N$ usually indicate a higher trophic level (Kling et al. 1992, Wada 2009). However, in this case, the higher $\delta^{15}N$ of rotifers can be also caused by their feeding preference for cyanobacteria which have higher content of proteins and higher $\delta^{15}N$ values indicate nitrogen fixation (Nagarkar et al. 2004, Kohler et al. 2018). If we compare $\delta^{15}N$ of nitrogen from organic matter of cryoconite and $\delta^{15}N$ of tardigrades and rotifers, we observe that values of organic matter from cryoconite are within all samples between values of tardigrades and rotifers (with exception of the values of rotifers from NU1 and NL2) (Fig. 11). It suggests that the dominant proportion of organic matter within cryoconite could be possible food for rotifers but not for tardigrades (i.e. $\delta^{15}N$ of food is lower than $\delta^{15}N$ of animal's body). According to determination of primary producers, cyanobacteria were dominant in all samples excluding SU2 and EU2. These data could support our consideration about $\delta^{15}N$ enrichment of cyanobacteria compared to algae. However, the sample with the highest occurrence of algae *Mesotaenium* (SU2) was also the sample with the highest $\delta^{15}N$ values of organic matter within cryoconite, what could oppositely reject our speculation about $\delta^{15}N$ values of cyanobacteria.

Generally, the $\delta^{13}C$ values of animals are often similar or slightly higher than $\delta^{13}C$ values of the animal's diet (Wada 2009). Moreover, animals feeding on a low-protein diet (e.g. herbivores) reflect the bulk isotopic composition of food, whereas animals feeding on high-protein diet (e.g. carnivores) reflect the isotopic composition of fed proteins (DeNiro and Epstein 1978,

Michener and Lajtha 2008). Therefore, the knowledge about isotopic composition of food is necessary for the explanation of observed patterns. In contrast to $\delta^{15}N$ in examined tardigrades and rotifers, $\delta^{13}C$ values shows similar values between animals from the same glaciers across almost all samples. These grouping patterns ought to be a result of specific composition of nutrients occurring within each sampled transect causing similarities in isotopic fractionation of carbon for primary producers, which are a main food for tardigrades and rotifers. It could also be a result of similar or close food requirements of both taxa. As described by DeNiro and Epstein (1978), different species raised on the same diet may reflect the same $\delta^{13}C$ values of their bodies. From feeding experiments (Ricci 1984, Mialet et al. 2013, Devetter unpublished data) it is evident, that rotifers can consume similar food (algae, cyanobacteria) as tardigrades, even though they prefer smaller particles. However, measured $\delta^{15}N$ values suggest that the food composition of tardigrades and rotifers is different. Our results show similarities in $\delta^{13}C$ values of tardigrades and rotifers but differences in $\delta^{15}N$ values. This can also indicate that rotifers consume DOC originating from extracellular exudates of algae but the source of nitrogen (e.g. bacteria, cyanobacteria and organic detritus) is different. Besides predation, osmotrophy of invertebrates was described in Chaetognatha (arrow worms) as an alternative feeding strategy (Casanova et al. 2012), but this option remains controversial in rotifers in the light of the recent knowledge.

The differences in $\delta^{13}C$ values of tardigrades and rotifers between different sampling sites are probably caused by various interconnected agents such as different nutrient inputs, community structure, surrounding and morphology of the glacier. Moreover, the measured differences between samples from the first and second sampling with a one-week interval between them suggest a dynamical character of processes occurring on the glacial surface, including changes in the input of organic and inorganic matter. It is also highly probable that due to the smaller size, Svenbreen, which has the majority of the samples with low $\delta^{13}C$ values, responds to these environmental changes faster than the larger glaciers.

The small size of Svenbreen compared to Ebbabreen and Nordenskiöldbreen also presumably results in higher allochthonous input of nutrients in the form of organic matter from surrounding habitats. Furthermore, it is possible that the depletion in $\delta^{13}C$ is caused by longer chains of fractionations against $\delta^{13}C$ within microbial communities (possible food for invertebrates) decomposing OM. However, for the better understanding of its functioning it is necessary to obtain data from other trophic levels within the system. Tardigrades and rotifers from Ebbabreen and Nordenskiöldbreen have higher $\delta^{13}C$ than animals from Svenbreen. These two glaciers are much larger than Svenbreen and we assume that they have a lager component of autochthonous production and input of inorganic matter (IM) from the atmosphere (Stibal et al. 2010). The high values of $\delta^{13}C$ would then be caused by a shorter chain of transformations and discriminations against $\delta^{13}C$ within organisms by the assimilation of inorganic matter (Michener and Lajtha 2008).

Our results illustrate that rotifers from Svenbreen are very close to the isotopic values of rotifers measured in the study of Shaw et al. (2018). This study focused on Antarctic soils which had $\delta^{15}N$ values slightly below −1 ‰. Rotifers measured in Svenbreen samples ranged between −1.04 ‰ and −2.04 ‰ as it is shown in the Table 5. One the other hand, values of tardigrades in our study are lower than values measured by Shaw et al. (2018). The food presented in Shaw's study were cyanobacterial mats, which had $\delta^{15}N$ values around −5.36 ± 0.96 ‰. Nitrogen from organic matter in our samples had isotopic values ranging from −1.64 ‰ at the upper part of Svenbreen (2nd sampling) to −4.05 ‰ for upper part of Ebbabreen (2nd sampling). Low value of $\delta^{15}N$ at the Ebbabreen upper part, supported by data from Shaw et al. (2018) about cyanobacterial mats isotopic composition, could contribute to the hypothesis that nitrogen fixation plays an important role in case that other sources (e.g. DIN from snowmelt) are depleted (Telling et al. 2011).

In the sample from the lower part of Svenbreen from 2nd sampling, we have found a different composition of cryoconite. This sample had a high content of *Leptolyngbya* sp., and it was dominated by rotifers. *Leptolyngbya* sp. is a cyanobacteria which is known as one of the dominant species forming cryoconite aggregates in Svalbard (Kaštovská et al. 2005). The same pattern with *Leptolyngbya* dominances was found in the lower part of Ebbabreen, again with a dominance of rotifers, but also with a higher abundance of tardigrades compared to Svenbreen. A potential explanation of these differences is the presence of precipitation events preceding the sampling which disturbed the structure of cryoconite aggregates (Zawierucha et al. 2019b) and flushed away tardigrades which cannot stick to the cryoconite or eroded cryoconite and favoured better food for rotifers.

The values of $\delta^{13}C$ of decarbonated cryoconite (thus $\delta^{13}C$ from OC) were slightly higher than those observed in non-decarbonated cryoconite (thus $\delta^{13}C$ of TC). It was an opposite pattern than we expected, because carbonates have usually higher $\delta^{13}C$ values than organic carbon (Karhu and Holland 1996). We suggest that these results could be caused by a low content of ^{13}C within secondary carbonates from early geological events (Hudson 1997). The $\delta^{13}C$ values of OC in our samples showed that the potential food source for tardigrades and rotifers has a higher content of $\delta^{13}C$. It corresponds to the findings of Wada (2009) who examined the food web structure in Lake Baikal. However, our results are not consistent with the findings of Shaw et al. (2018) who examined soil web structure of Taylor Valley in Antarctica and described slightly lower $\delta^{13}C$ values of cyanobacterial mats (possible food) compared to tardigrades and rotifers. The differences between these results could point to differences in carbon fractionation within polar habitats or within tardigrades and rotifers in comparison with freshwater zooplankton. However, we cannot prove this speculation due to the absence of reliable evidence of food requirements and carbon fractionation within microinvertebrates in our samples. We can only state that the distribution of $\delta^{13}C$ values of total carbon among sampling sites are

almost equal to the distribution of $\delta^{13}C$ within animals. We cannot show any other pattern due to the absence of $\delta^{13}C$ values of OC in our study.

In case of $\delta^{15}N$ in cryoconite, we observed the highest values at the upper part of Svenbreen. During the sampling, Svenbreen was covered by snow and had a higher conductivity (69 μS) in cryoconite holes than other sampling sites (1–4 μS). We assume that higher content of $\delta^{15}N$ could be caused by a presence of NO_3^- within the meltwater from snow (Hodson et al. 2005) or by a retention of rainwater due to the position of the sampling site (in the saddle between two valley glaciers). As it was reported by Telling et al. (2011), higher N_2 fixation occurs in habitats with low input of allochthonous ON. The upper parts of Svenbreen as well as the upper parts of Nordenskiöldbreen are not surrounded by slopes which could provide allochthonous OM as the rest of the glacier. Therefore, this could be another explanation why we found the high $\delta^{15}N$ values in these places in comparison with other sampling sites.

The summarization of possible explanations of our results:

- The $\delta^{15}N$ values of rotifers are higher than $\delta^{15}N$ values of tardigrades which could suggest that food for rotifers is enriched in $\delta^{15}N$ in comparison with food for tardigrades. The other possible explanation is that rotifers consume DOM from primary production enriched in $\delta^{15}N$ or that tardigrades consume algae which have low density in cryoconite compared to cyanobacteria and, therefore, we cannot see their isotopic values in our results.
- The $\delta^{13}C$ values are almost similar between tardigrades and rotifers from the same part of the ablation zone of one glacier or from both parts of the ablation zone from one glacier suggesting possible similarities in composition of nutrients for primary producers within the sampling transects or less probable similarities in food of tardigrades and rotifers.
- The $\delta^{13}C$ values of tardigrades and rotifers differ among the samples which could point to the spatial differences between parts of the ablation zone and glaciers influencing input of carbon into cryoconite holes and thus community structure of primary producers which probably differ in length of isotopic fractionation chains thereafter.
- The community structure of primary producers differs between the upper and lower parts of the ablation zone and between the glaciers. Samples with the highest occurrence of cyanobacteria showed dominance of rotifers pointing to possible interconnected agents between nutrient input and species composition of primary producers and grazers within cryoconite holes.
- The $\delta^{15}N$ values of organic matter within cryoconite are mostly between $\delta^{15}N$ values of tardigrades and rotifers. It suggests that the dominant part of organic matter within cryoconite represents food for rotifers.

- The $\delta^{13}C$ values of cryoconite show almost similar distribution as $\delta^{13}C$ values of tardigrades and rotifers. This pattern indicates differences in spatial characteristics of the nutrient input and thus differences in isotopic composition of primary producers and consumers between glaciers.

3.5 Conclusions

Elemental and isotopic compositions of biota and its food are well-known tools for describing ecosystem interactions. In this part of the thesis, we presented the first description of isotopic composition of tardigrades and rotifers from cryoconite holes with the possible causes of differences between phyla, glaciers and parts of ablation zones. The most important findings are the differences in $\delta^{15}N$ values of tardigrades and rotifers and similarities in their $\delta^{13}C$ values among the three examined glaciers. We also introduced a modified technique of sample preparation avoiding procedures such as sugar centrifugation, which can influence the isotopic ratios. Due to the quantity of time demanding procedures, we did not have enough time to repeat analyses and complete the measurements of missing samples. To get reliable results about trophic interactions and trophic levels, it is necessary to obtain data about isotopic composition of each group of primary producers, their species composition at the best supported by analyses of gut content of tardigrades and rotifers (see DeNiro and Epstein 1978 and Yoshii et al. 1999). These shortcomings are planned to be corrected during subsequent research.

4 General Discussion and Conclusions

In the thesis, we present cryoconite holes as simple ecosystems with truncated food webs consisting of tardigrades and rotifers as consumers, algae and cyanobacteria as primary producers and bacteria as decomposers. We did not include protozoa and viruses into this study and there were no predators, such as rotifer *Encentrum* sp. and nematodes, observed within the examined cryoconite holes. As already introduced, the knowledge about trophic interactions and nutrient utilization of tardigrades and rotifers inhabiting cryoconite holes remains very poor. Here, we demonstrate the possibilities and limitations of using stable isotope and elemental analyses in the study of the glacial microinvertebrates and the organic compounds of cryoconite aggregates.

The crucial finding of this thesis is that tardigrades and rotifers differ in $\delta^{15}N$ values, but they are close in $\delta^{13}C$ values among similar sampling sites. These results show that there is (i) a possibility of different isotopic fractionation of nitrogen in each taxon feeding on similar food, (ii) a division of feeding niches causing differences in $\delta^{15}N$ values but similarities in $\delta^{13}C$ values or (iii) a consumption of DOC released from primary producers by rotifers. However, without data about the composition of food and its isotopic values, we cannot establish any reliable explanation of this pattern. Another important finding of present study is that within the examined cryoconite holes, relative abundances of different species of tardigrades and rotifers differ between the sampling sites. Dominances of mostly algivorous tardigrades or mostly bacterivorous rotifers suggest that the community structure of primary producers can be affected by a different grazing pressure. Moreover, different abundances of mostly bacterivorous *Pilatobius* sp. or mostly algivorous *Hypsibius* sp. among the glaciers and parts of the ablation zone can influence the community of primary producers and thus nutrient utilization as well. However, the feeding strategies in both groups are likely more complex.

Based on our data, we conclude that the interactions of consumers and producers, spatial and temporal characteristics of the glaciers and their nutrient pathways are probably strongly linked. However, further elemental and isotopic measurements are necessary for the elucidation of the role of these organisms within the cryoconite food web structure and within the supraglacial environment.

5 References

Aciego S. M., Stevenson E. I. and Arendt C. A. (2015). Climate versus geological controls on glacial meltwater micronutrient production in southern Greenland. *Earth and Planetary Science Letters*, *424*, 51-58.

Altiero T., Guidetti R., Caselli V., Cesari M. and Rebecchi L. (2011). Ultraviolet radiation tolerance in hydrated and desiccated eutardigrades. *Journal of Zoological Systematics and Evolutionary Research*, *49*, 104-110.

Anderson L. E. (1954). Hoyer's solution as a rapid permanent mounting medium for bryophytes. *The Bryologist*, *57*(3), 242-244.

Anesio A. M., Hodson A. J., Fritz A., Psenner R. and Sattler B. (2009). High microbial activity on glaciers: importance to the global carbon cycle. *Global Change Biology*, *15*(4), 955-960.

Anesio A. M., Sattler B., Foreman C., Telling J., Hodson A., Tranter M. and Psenner R. (2010). Carbon fluxes through bacterial communities on glacier surfaces. *Annals of Glaciology*, *51*(56), 32-40.

Anesio A. M. and Laybourn-Parry J. (2012). Glaciers and ice sheets as a biome. *Trends in Ecology and Evolution*, *27*(4), 219-225.

Ayres E., Wall D. H., Adams B. J., Barrett J. E. and Virginia R. A. (2007). Unique similarity of faunal communities across aquatic–terrestrial interfaces in a polar desert ecosystem. *Ecosystems*, *10*(4), 523-535.

Bagshaw E. A., Tranter M., Fountain A. G., Welch K., Basagic H. J. and Lyons W. B. (2013). Do cryoconite holes have the potential to be significant sources of C, N, and P to downstream depauperate ecosystems of Taylor Valley, Antarctica? *Arctic, Antarctic, and Alpine Research*, *45*(4), 440-454.

Bamber J. L., Krabill W., Raper V., Dowdeswell J. A. and Oerlemans J. (2005). Elevation changes measured on Svalbard glaciers and ice caps from airborne laser data. *Annals of Glaciology*, *42*, 202-208.

Bardgett R. D. Richter A., Bol R., Garnett M. H., Bäumler R., Xu X., Lopez-Capel E., Manning D. A., Hobbs P. J., Hartley I. R. and Wanek W. (2007). Heterotrophic microbial communities use ancient carbon following glacial retreat. *Biology Letters*, *3*(5), 487-490.

Barrett J. E., Virginia R. A., Lyons W. B., McKnight D. M., Priscu J. C., Doran P. T., Fountain A. G., Wall D. H. and Moorhead D. L. (2007). Biogeochemical stoichiometry of Antarctic dry valley ecosystems. *Journal of Geophysical Research: Biogeosciences*, *112*, G01010.

Bartels P. J., Nelson D. R. and Exline R. P. (2011). Allometry and the removal of body size effects in the morphometric analysis of tardigrades. *Journal of Zoological Systematics and Evolutionary Research*, *49*, 17-25.

Benassai S., Becagli S., Gragnani R., Magand O., Proposito M., Fattori I., Traversi R. and Udisti R. (2005). Sea-spray deposition in Antarctic coastal and plateau areas from ITASE traverses. *Annals of Glaciology*, *41*, 32-40.

Bertolani R., Guidetti R., Jönsson I. K., Altiero T., Boschini D. and Rebecchi L. (2004). Experiences with dormancy in tardigrades. *Journal of Limnology*, 16-25.

Billings W. D. (1987). Constraints to plant growth, reproduction, and establishment in arctic environments. *Arctic and Alpine Research*, *19*(4), 357-365.

Blair N.., Leu A., Muñoz E., Olsen J., Kwong E. and Des Marais D. (1985). Carbon isotopic fractionation in heterotrophic microbial metabolism. *Applied and Environmental Microbiology*, *50*(4), 996-1001.

Boetius A., Anesio A. M., Deming J. W., Mikucki J. A. and Rapp J. Z. (2015). Microbial ecology of the cryosphere: sea ice and glacial habitats. *Nature Reviews Microbiology*, *13*(11), 677.

Bonifacio A., Guidetti R., Altiero T., Sergo V. and Rebecchi L. (2012). Nature, source and function of pigments in tardigrades: in vivo Raman imaging of carotenoids in *Echiniscus blumi*. *PLoS One*, *7*(11), e50162.

Brodie C. R., Leng M. J., Casford J. S., Kendrick C. P., Lloyd J. M., Yongqiang Z. and Bird M. I. (2011). Evidence for bias in C and N concentrations and $\delta^{13}C$ composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods. *Chemical Geology*, *282*(3-4), 67-83.

Brown G. H. (2002). Glacier meltwater hydrochemistry. *Applied Geochemistry*, *17*(7), 855-883.

Burpee B., Saros J. E., Northington R. M. and Simon K. S. (2016). Microbial nutrient limitation in Arctic lakes in a permafrost landscape of southwest Greenland. *Biogeosciences*, *13*, 365-374.

Casanova J. P., Barthelemy R., Duvert M. and Faure E. (2012). Chaetognaths feed primarily on dissolved and fine particulate organic matter, not on prey: implications for marine food webs. *Hypotheses in the Life Sciences*, *2*(1), 20-29.

Castro P. and Huber M. E. (2015). *Marine Biology*. McGraw-Hill Education, New York.

Convey P. and McInnes S. J. (2005). Exceptional tardigrade-dominated ecosystems in Ellsworth Land, Antarctica. *Ecology*, *86*(2), 519-527.

Cook J., Edwards A., Takeuchi N. and Irvine-Fynn T. (2016). Cryoconite: the dark biological secret of the cryosphere. *Progress in Physical Geography*, *40*(1), 66-111.

Coulson S. J., Convey P., Aakra K., Aarvik L., Ávila-Jiménez M. L., Babenko A., Biersma E., Boström S., Brittain J. E., Carlsson A., Christoffersen K. S., De Smet W. H., Ekrem T., Fjellberg A., Füreder L., Gustafsson D., Gwiazdowicz D. J., Hansen L. O., Hullé L., Kaczmarek L., Kolicka M., Kuklin V., Lakka H-K., Lebedeva N., Makarova O., Maraldo K., Melekhina E., Ødegaard F., Pilskog H. E., Simon J. C., Sohlenius B., Solhøy T., Søli G., Stur E., Tanasevitch A., Taskaeva A., Velle G. and Zmudczyńska-Skarbek K. M. (2014). The terrestrial and freshwater invertebrate biodiversity of the archipelagoes of the Barents Sea; Svalbard, Franz Josef Land and Novaya Zemlya. *Soil Biology and Biochemistry*, *68*, 440-470.

Craig H. (1953). The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*, *3*(2-3), 53-92.

Dallmann W. K., Piepjohn K. and Blomeier D. (2004). *Geological map of Billefjorden, Central Spitsbergen, Svalbard: with geological excursion guide*. Norsk Polarinstitutt.

Dastych H. (1985). West Spitsbergen Tardigrada. *Acta Zoologica Cracoviensia*, *28*(2), 2-8.

DeNiro M. J. and Epstein S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, *42*(5), 495-506.

DeNiro M. J. and Epstein S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, *45*(3), 341-351.

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

Devetter M. (2009). Clearance rates of the bdelloid rotifer, *Habrotrocha thienemanni*, a tree-hole inhabitant. *Aquatic Ecology*, *43*(1), 85-89.

Donner J. (1965). *Ordnung Bdelloidea (Rotatoria, Radertiere)*. Akademie-Verlag, Berlin.

Dumont H. J. (1983). Biogeography of rotifers. In *Biology of Rotifers* (pp. 19-30). Springer, Dordrecht.

Edwards A., Anesio A. M., Rassner S. M., Sattler B., Hubbard B., Perkins W. T., Young M. and Griffith G. W. (2011). Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard. *The ISME Journal*, *5*(1), 150.

Edwards A., Mur L. A., Girdwood S. E., Anesio A. M., Stibal M., Rassner S. M., Hell K., Pachebat J. A., Post B., Bussell J. S., Cameron S. J. S., Wyn Griffith G., Hodson A. J. and Sattler B. (2014). Coupled cryoconite ecosystem structure–function relationships are revealed by comparing bacterial communities in alpine and Arctic glaciers. *FEMS Microbiology Ecology*, *89*(2), 222-237.

Ekblad A. and Högberg P. (2000). Analysis of $\delta^{13}C$ of CO_2 distinguishes between microbial respiration of added C4-sucrose and other soil respiration in a C3-ecosystem. *Plant and Soil*, *219*(1-2), 197-209.

Ewertowski M. W., Evans D. J., Roberts D. H. and Tomczyk A. M. (2016). Glacial geomorphology of the terrestrial margins of the tidewater glacier, Nordenskiöldbreen, Svalbard. *Journal of Maps*, *12*(suppl. 1), 476-487.

Fontaneto D., Barraclough T. G., Chen K., Ricci C. and Herniou E. A. (2008). Molecular evidence for broad-scale distributions in bdelloid rotifers: everything is not everywhere but most things are very widespread. *Molecular Ecology*, *17*(13), 3136-3146.

Fontaneto D., Iakovenko N. and De Smet W. H. (2015). Diversity gradients of rotifer species richness in Antarctica. *Hydrobiologia*, *761*(1), 235-248.

Fontaneto D. (2019). Long-distance passive dispersal in microscopic aquatic animals. *Movement Ecology*, *7*(1), 10.

Fountain A. G., Tranter M., Nylen T. H., Lewis K. J. and 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.

Foreman C. M., Sattler B., Mikucki J. A., Porazinska D. L. and Priscu J. C. (2007). Metabolic activity and diversity of cryoconites in the Taylor Valley, Antarctica. *Journal of Geophysical Research: Biogeosciences*, *112*, G04S32.

Garey J. R., McInnes S. J. and Nichols P. B. (2008). Global diversity of tardigrades (Tardigrada) in freshwater. *Hydrobiologia*, *595*(1), 101-106.

Gerdel R. W. and Drouet F. (1960). The cryoconite of the Thule area, Greenland. *Transactions of the American Microscopical Society*, *79*(3), 256-272.

Giovannini I., Altiero T., Guidetti R. and Rebecchi L. (2018). Will the Antarctic tardigrade *Acutuncus antarcticus* be able to withstand environmental stresses related to global climate change? *Journal of Experimental Biology*, *221*(4), jeb160622.

Grzesiak J., Górniak D., Świątecki A., Aleksandrzak-Piekarczyk T., Szatraj K. and Zdanowski M. K. (2015). Microbial community development on the surface of Hans and Werenskiold Glaciers (Svalbard, Arctic): a comparison. *Extremophiles*, *19*(5), 885-897.

Guidetti R., Altiero T. and Rebecchi L. (2011). On dormancy strategies in tardigrades. *Journal of Insect Physiology*, *57*(5), 567-576.

Guidetti R., Altiero T., Marchioro T., Amade L. S., Avdonina A. M., Bertolani R. and Rebecchi L. (2012). Form and function of the feeding apparatus in Eutardigrada (Tardigrada). *Zoomorphology*, *131*(2), 127-148.

Guil N. and Sanchez-Moreno S. (2013). Fine-scale patterns in micrometazoans: tardigrade diversity, community composition and trophic dynamics in leaf litter. *Systematics and Biodiversity*, *11*(2), 181-193.

Hagen J., Liestøl O., Roland K. and Jørgensen T. (1993). *Glacier Atlas of Svalbard and Jan Mayen* (Vol. 129). Oslo: Norsk Polarinstitutt.

Hallas T. E. and Yeates G. W. (1972). Tardigrada of the soil and litter of a Danish beech forest. *Pedobiologia*.

Harland W. B. (1997). Svalbard. *Geological Society, London, Memoirs*, *17*(1), 3-15.

Hawkings J. R., Wadham J. L., Tranter M., Lawson E., Sole A., Cowton T., Tedstone A., Bartholomew I., Nienow P., Chandler D. and Telling J. (2015). The effect of warming climate on nutrient and solute export from the Greenland Ice Sheet. *Geochemical Perspectives Letters*, *1*, 94-104.

Hawkings J., Wadham J., Tranter M., Telling J., Bagshaw E., Beaton A., Sarah-Louise S., Chandler D., Tedstone A. and Nienow P. (2016). The Greenland Ice Sheet as a hot spot of phosphorus weathering and export in the Arctic. *Global Biogeochemical Cycles*, *30*(2), 191-210.

Hodson A. J., Mumford P. N., Kohler J. and Wynn P. M. (2005). The High Arctic glacial ecosystem: new insights from nutrient budgets. *Biogeochemistry*, *72*(2), 233-256.

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

Hodson A., Roberts T. J., Engvall A. C., Holmén K. and Mumford P. (2010). Glacier ecosystem response to episodic nitrogen enrichment in Svalbard, European High Arctic. *Biogeochemistry*, *98*(1-3), 171-184.

Hood E., Fellman J., Spencer R. G., Hernes P. J., Edwards R., D'Amore D. and Scott D. (2009). Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature*, *462*(7276), 1044.

Hudson J. D. (1977). Stable isotopes and limestone lithification. *Journal of the Geological Society*, *133*(6), 637-660.

Isaksson E., Pohjola V., Jauhiainen T., Moore J., Pinglot J. F., Vaikmäe R., Van de Wal R. S. W., Hagen J. O., Ivask J., Karlöf L., Martma T., Meijer H. A. J., Mulvaney R., Thomassen M. and Van den Broeke M. (2001). A new ice-core record from Lomonosovfonna, Svalbard: viewing the 1920–97 data in relation to present climate and environmental conditions. *Journal of Glaciology*, *47*(157), 335-345.

Jenkins D. G. and Underwood M. O. (1998). Zooplankton may not disperse readily in wind, rain, or waterfowl. In *Rotifera VIII: A Comparative Approach* (pp. 15-21). Springer, Dordrecht.

Jørgensen A., Møbjerg N. and Kristensen R. M. (2007). A molecular study of the tardigrade *Echiniscus testudo* (Echiniscidae) reveals low DNA sequence diversity over a large geographical area. *Journal of Limnology*, 77-83.

Kalff J. (2002). *Limnology: Inland Water Ecosystems*. Prentice-Hall, New Jersey.

Karhu J. A. and Holland H. D. (1996). Carbon isotopes and the rise of atmospheric oxygen. *Geology*, *24*(10), 867-870.

Karl D. M. (2000). Aquatic ecology: Phosphorus, the staff of life. *Nature*, *406*(6791), 31.

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

Kaya M., De Smet W. H. and Fontaneto D. (2010). Survey of moss-dwelling bdelloid rotifers from middle Arctic Spitsbergen (Svalbard). *Polar Biology*, *33*(6), 833-842.

Keller K., Blum J. D. and Kling G. W. (2007). Geochemistry of soils and streams on surfaces of varying ages in arctic Alaska. *Arctic, Antarctic, and Alpine Research*, *39*(1), 84-98.

Klekowski R. Z. and Opaliński K. W. (1986). Matter and energy flow in Spitsbergen ornithogenic tundra. *Polar Research*, *4*(2), 187-197.

Kling G. W., Fry B. and O'Brien W. J. (1992). Stable isotopes and planktonic trophic structure in arctic lakes. *Ecology*, *73*(2), 561-566.

Koehler J. K. and Hayes T. L. (1969). The rotifer jaw: A scanning and transmission electron microscope study: I. The trophi of *Philodina acuticornis odiosa*. *Journal of Ultrastructure Research*, *27*(5-6), 402-418.

Kohler T. J., Stanish L. F., Liptzin D., Barrett J. E., and McKnight D. M. (2018). Catch and release: Hyporheic retention and mineralization of N-fixing *Nostoc* sustains downstream microbial mat biomass in two polar desert streams. *Limnology and Oceanography Letters*, *3*(4), 357-364.

Kostrzewski A., Kaniecki A., Kapuściński J., Klimczak R., Stach A. and Zwoliński Z. (1989). The dynamics and rate of denudation of glaciated and non-glaciated catchments, central Spitsbergen. *Polish Polar Research*, *10*(3), 317-367.

Kosztyła P., Stec D., Morek W., Gąsiorek P., Zawierucha K., Michno K., Ufir K., Małek D., Hlebowicz K., Laska A., Dudziak M., Frohme M., Prokop Z. M., Kaczmarek Ł., and Michalczyk Ł. (2016). Experimental taxonomy confirms the environmental stability of morphometric traits in a taxonomically challenging group of microinvertebrates. *Zoological Journal of the Linnean Society*, *178*(4), 765-775.

Kutikova L. A. (2003). Bdelloid rotifers (Rotifera, Bdelloidea) as a component of soil and land biocenoses. *Biology Bulletin of the Russian Academy of Sciences*, *30*(3), 271-274.

Macdonell S. A. and Fitzsimons S. J. (2012). Observations of cryoconite hole system processes on an Antarctic glacier. *Revista Chilena de Historia Natural*, *85*(4), 393-407.

Macheret Y. Y. (1981). Forms of glacial relief of Spitsbergen glaciers. *Annals of Glaciology*, *2*, 45-51.

Małecki J. (2014). Some comments on the flow velocity and thinning of Svenbreen, Dickson Land, Svalbard. *Czech Polar Report*, *4*(1), 1-8.

Małecki J. (2015). Glacio-meteorology of Ebbabreen, Dickson Land, central Svalbard, during 2008–2010 melt seasons. *Polish Polar Research*, *36*(2), 145-161.

Margesin R., Zacke G. and Schinner F. (2002). Characterization of heterotrophic microorganisms in alpine glacier cryoconite. *Arctic, Antarctic, and Alpine Research*, *34*(1), 88-93.

Marotta R., Uggetti A., Ricci C., Leasi F. and Melone G. (2012). Surviving starvation: changes accompanying starvation tolerance in a bdelloid rotifer. *Journal of Morphology*, *273*(1), 1-7.

Marshall W. A., and Chalmers M. O. (1997). Airborne dispersal of Antarctic terrestrial algae and cyanobacteria. *Ecography*, *20*(6), 585-594.

Marsh P. and Woo M. K. (1981). Snowmelt, glacier melt, and high arctic streamflow regimes. *Canadian Journal of Earth Sciences*, *18*(8), 1380-1384.

Maucci W. (1996). Tardigrada of the Arctic tundra with descriptions of two new species. *Zoological Journal of the Linnean Society*, *116*(1-2), 185-204.

McCutchan J. H., Lewis W. M., Kendall C. and McGrath C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, *102*(2), 378-390.

Mialet B., Majdi N., Tackx M., Azémar F. and Buffan-Dubau E. (2013). Selective feeding of bdelloid rotifers in river biofilms. *PLoS One*, *8*(9), e75352.

Michener R. and Lajtha K. (Eds.). (2008). *Stable isotopes in ecology and environmental science*. John Wiley & Sons.

Mueller D. R., Vincent W. F., Pollard W. H. and Fritsen C. H. (2001). Glacial cryoconite ecosystems: a bipolar comparison of algal communities and habitats. *Nova Hedwigia Beiheft*, *123*, 173-198.

Mueller D. R. and Pollard W. H. (2004). Gradient analysis of cryoconite ecosystems from two polar glaciers. *Polar Biology*, *27*(2), 66-74.

Nagarkar S., Williams G. A., Subramanian G. and Saha S. K. (2004). Cyanobacteria-dominated biofilms: a high quality food resource for intertidal grazers. In *Asian Pacific Phycology in the 21st Century: Prospects and Challenges* (pp. 89-95). Springer, Dordrecht.

Nelson D. R., Guidetti R. and Rebecchi L. (2015). Phylum Tardigrada. In *Thorp and Covich's Freshwater Invertebrates*. Academic Press (pp. 347-380).

Nicholes M. J., Williamson C. J., Tranter M., Holland A., Poniecka E., Yallop M. L., The Black and Bloom Group and Anesio A. (2019). Bacterial dynamics in supraglacial habitats of the Greenland Ice Sheet. *Frontiers in Microbiology*, *10*.

Nielsen U. N., Wall D. H., Adams B. J., Virginia R. A., Ball B. A., Gooseff M. N. and McKnight D. M. (2012). The ecology of pulse events: insights from an extreme climatic event in a polar desert ecosystem. *Ecosphere*, *3*(2), 1-15.

Nordenskiöld A. E. (1875). Cryoconite found 1870, July 19th-25th, on the inland ice, east of Auleitsivik Fjord, Disco Bay, Greenland. *Geological Magazine, Decade 2*, *2*, 157-162.

Oerlemans J. (2005). Extracting a climate signal from 169 glacier records. *Science*, *308*(5722), 675-677.

Pielou E. C. (2012). *A Naturalist's Guide to the Arctic*. University of Chicago Press.

Pilato G. (1981). Analisi di nuovi caratteri nello studio degli Eutardigradi. *Animalia*, *8*, 51-57.

Pilato G., Costa G., Conti E., Binda M. G. and Oscar L. I. S. I. (2007). Morphometric analysis of some metric characters of two Macrobiotus species (Eutardigrada, Macrobiotidae). *Journal of Limnology*, 26-32.

Pittino F., Maglio M., Gandolfi I., Azzoni R. S., Diolaiuti G., Ambrosini R. and Franzetti A. (2018). Bacterial communities of cryoconite holes of a temperate alpine glacier show both seasonal trends and year-to-year variability. *Annals of Glaciology*, *59*(77), 1-9.

Porazinska D. L., Fountain A. G., Nylen T. H., Tranter M., Virginia R. A. and 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. and Ewertowski M. (2007). Post-"Little Ice Age" retreat rates of glaciers around Billefjorden in central Spitsbergen, Svalbard. *Polish Polar Research*, *28*(3), 159-186.

R Development Core Team (2018) R: a language and environment for statistical computing. Vienna, Austria. http://www.R-project.org.

Ramazzotti G. & Maucci W. (1983) Il Phylum Tardigradum. Terza edizione riveduta e corretta. *Memorie dell'Istituto Italiano di Idrobiologia Dott.* Marco Marchi, *41*, 1–1012.

Rautio M., Dufresne F., Laurion I., Bonilla S., Vincent W. F. and Christoffersen K. S. (2011). Shallow freshwater ecosystems of the circumpolar Arctic. *Ecoscience*, *18*(3), 204-222.

Ricci C. (1984). Culturing of some bdelloid rotifers. *Hydrobiologia*, *112*(1), 45-51.

Ricci C. (2001). Dormancy patterns in rotifers. *Hydrobiologia*, *446*(1), 1-11.

Sánchez-Moreno S., Ferris H. and Guil N. (2008). Role of tardigrades in the suppressive service of a soil food web. *Agriculture, Ecosystems & Environment*, *124*(3-4), 187-192.

Šantrůček J., Šantrůčková H., Kaštovská E., Květoň J., Tahovská K., Vrábl D. and Vráblová M. (2018). *Stabilní isotopy biogeních prvků: použití v biologii a ekologii.* Academia.

Säwström C., Mumford P., Marshall W., Hodson A. and 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.

Säwström C., Laybourn-Parry J., Granéli W. and Anesio A. M. (2007). Heterotrophic bacterial and viral dynamics in Arctic freshwaters: results from a field study and nutrient-temperature manipulation experiments. *Polar Biology*, *30*(11), 1407-1415.

Segawa T., Ishii S., Ohte N., Akiyoshi A., Yamada A., Maruyama F., Li Z., Hongoh Y. and Takeuchi N. (2014). The nitrogen cycle in cryoconites: naturally occurring nitrification-denitrification granules on a glacier. *Environmental Microbiology*, *16*(10), 3250-3262.

Shaw E. A., Adams B. J., Barrett J. E., Lyons W. B., Virginia R. A. and Wall D. H. (2018). Stable C and N isotope ratios reveal soil food web structure and identify the nematode *Eudorylaimus antarcticus* as an omnivore–predator in Taylor Valley, Antarctica. *Polar Biology*, *41*(5), 1013-1018.

Sistla S. A., Asao S. and Schimel J. P. (2012). Detecting microbial N-limitation in tussock tundra soil: implications for Arctic soil organic carbon cycling. *Soil Biology and Biochemistry*, *55*, 78-84.

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

Sommers P., Darcy J. L., Porazinska D. L., Gendron E. M., Fountain A. G., Zamora F., Vincent K., Cawley K. M., Solon A. J., Vimercati L., Ryder J. and Schmidt S. K. (2019). Comparison of microbial communities in the sediments and water columns of frozen cryoconite holes in the McMurdo Dry Valleys, Antarctica. *Frontiers in Microbiology*, *10,* 65.

Sterner R. W. (1986). Herbivores' direct and indirect effects on algal populations. *Science*, *231*(4738), 605-607.

Sterner R. W. and Elser J. J. (2002). *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton university press.

Stibal M., Šabacká M. and Kaštovská K. (2006). Microbial communities on glacier surfaces in Svalbard: impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. *Microbial Ecology*, *52*(4), 644.

Stibal M. and Tranter M. (2007). Laboratory investigation of inorganic carbon uptake by cryoconite debris from Werenskioldbreen, Svalbard. *Journal of Geophysical Research: Biogeosciences*, *112*, G04S33.

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

Stibal M., Tranter M., Telling J. and Benning L. G. (2008b). Speciation, phase association and potential bioavailability of phosphorus on a Svalbard glacier. *Biogeochemistry*, *90*(1), 1-13.

Stibal M., Anesio A. M., Blues C. J. D. and Tranter M. (2009). Phosphatase activity and organic phosphorus turnover on a high Arctic glacier. *Biogeosciences*, *6*(5), 913-922.

Stibal M., Lawson E. C., Lis G. P., Mak K. M., Wadham J. L. and Anesio A. M. (2010). Organic matter content and quality in supraglacial debris across the ablation zone of the Greenland ice sheet. *Annals of Glaciology*, *51*(56), 1-8.

Stibal M., Šabacká M. and Žárský J. (2012). Biological processes on glacier and ice sheet surfaces. *Nature Geoscience*, *5*(11), 771.

Szczuciński W. and Rachlewicz G. (2007). Geological setting of the Petuniabukta Region. *Landform Analysis*, *5*.

Takeuchi N., Kohshima S. and Seko K. (2001a). 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.

Takeuchi N., Kohshima S., Goto-Azuma K. and Koerner R. M. (2001b). Biological characteristics of dark colored material (cryoconite) on Canadian Arctic glaciers (Devon and Penny ice caps). *Memoirs of National Institute of Polar Research. Special issue*, 54: 495-505.

Telling J., Anesio A. M., Tranter M., Irvine-Fynn T., Hodson A., Butler C. and Wadham J. (2011). Nitrogen fixation on Arctic glaciers, Svalbard. *Journal of Geophysical Research: Biogeosciences*, *116*, G03039.

Telling J., Stibal M., Anesio A. M., Tranter M., Nias I., Cook J., Bellas C., Lis G., Wadham J. L., Sole A., Nienow P. and Hodson A. (2012). Microbial nitrogen cycling on the Greenland Ice Sheet. *Biogeosciences*, *9*(7), 2431-2442.

Tranter M., Fountain A. G., Fritsen C. H., Berry Lyons W., Priscu J. C., Statham P. J. and Welch K. A. (2004). Extreme hydrochemical conditions in natural microcosms entombed within Antarctic ice. *Hydrological Processes*, *18*(2), 379-387.

Vieli A., Jania J., Blatter H. and Funk M. (2004). Short-term velocity variations on Hansbreen, a tidewater glacier in Spitsbergen. *Journal of Glaciology*, *50*(170), 389-398.

Vindušková O., Jandová K. and Frouz J. (2019). Improved method for removing siderite by *in situ* acidification before elemental and isotope analysis of soil organic carbon. *Journal of Plant Nutrition and Soil Science*, *182*(1), 82-91.

Vonnahme T. R., Devetter M., Žárský J. D., Šabacká M. and Elster J. (2016). Controls on microalgal community structures in cryoconite holes upon high-Arctic glaciers, Svalbard. *Biogeosciences*, *13*(3), 659-674.

Wada E. (2009). Stable $\delta^{15}N$ and $\delta^{13}C$ isotope ratios in aquatic ecosystems. *Proceedings of the Japan Academy, Series B*, *85*(3), 98-107.

Wainright S. C., Haney J. C., Kerr C., Golovkin A. N. and Flint M. V. (1998). Utilization of nitrogen derived from seabird guano by terrestrial and marine plants at St. Paul, Pribilof Islands, Bering Sea, Alaska. *Marine Biology*, *131*(1), 63-71.

Wallace R. L. and Snell T. W. (2010). Rotifera. In *Ecology and classification of North American Freshwater Invertebrates* (pp. 173-235). Academic Press.

Watanabe M. (2006). Anhydrobiosis in invertebrates. *Applied Entomology and Zoology*, *41*(1), 15-31.

Williams R. S. Jr. and Ferrigno J. G. (2012). State of the Earth's cryosphere at the beginning of the 21st century: Glaciers, global snow cover, floating ice, and permafrost and periglacial environments. *Director*, *508*, 344-6840.

Xu Y., Simpson A. J., Eyles N. and Simpson M. J. (2010). Sources and molecular composition of cryoconite organic matter from the Athabasca Glacier, Canadian Rocky Mountains. *Organic Geochemistry*, *41*(2), 177-186.

Yoshii K., Melnik N. G., Timoshkin O. A., Bondarenko N. A., Anoshko P. N., Yoshioka T. and Wada E. (1999). Stable isotope analyses of the pelagic food web in Lake Baikal. *Limnology and Oceanography*, *44*(3), 502-511.

Zah R., Burgherr P., Bernasconi S. M. and Uehlinger U. (2001). Stable isotope analysis of macroinvertebrates and their food sources in a glacier stream. *Freshwater Biology*, *46*(7), 871-882.

Zawierucha K., Coulson S. J., Michalczyk Ł. U. and 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. and Kaczmarek Ł. (2015a). What animals can live in cryoconite holes? A faunal review. *Journal of Zoology*, *295*(3), 159-169.

Zawierucha K., Cytan J., Smykla J., Wojczulanis-Jakubas K., Kaczmarek Ł., Kosicki J. Z. and Michalczyk Ł. (2015b). Seabird guano boosts body size of water bears (Tardigrada) inhabiting the Arctic tundra. *Polar Biology*, *38*(4), 579-582.

Zawierucha K., Ostrowska M., Vonnahme T. R., Devetter M., Nawrot A. P., Lehmann S. and Kolicka M. (2016a). Diversity and distribution of Tardigrada in Arctic cryoconite holes. *Journal of Limnology*, *75*(3).

Zawierucha K., Zmudczyńska-Skarbek K., Kaczmarek Ł. and Wojczulanis-Jakubas K. (2016b). The influence of a seabird colony on abundance and species composition of water bears (Tardigrada) in Hornsund (Spitsbergen, Arctic). *Polar Biology*, *39*(4), 713-723.

Zawierucha K., Buda J., Pietryka M., Richter D., Łokas E., Lehmann-Konera S., Makowska N. and Bogdziewicz M. (2018a). Snapshot of micro-animals and associated biotic and abiotic environmental variables on the edge of the south-west Greenland ice sheet. *Limnology*, *19*(1), 141-150.

Zawierucha K., Stec D., Lachowska-Cierlik D., Takeuchi N., Li Z. and Michalczyk Ł. (2018b). High mitochondrial diversity in a new water bear species (Tardigrada: Eutardigrada) from mountain glaciers in central Asia, with the erection of a new genus *Cryoconicus*. In *Annales Zoologici, 68*(1), 179-202.

Zawierucha K., Podkowa P., Marciniak M., Gąsiorek P., Zmudczyńska-Skarbek K., Janko K. and Włodarska-Kowalczuk M. (2018c). Temperature (latitude) and nutrient (seabird guano) effects on limno-terrestrial Tardigrada (*Testechiniscus spitsbergensis* and *Pilatobius recamieri*) body size. *Polar Research*, *37*(1), 1492297.

Zawierucha K., Zmudczyńska-Skarbek K., Guil N. and Bogdziewicz M. (2019a). Seabirds modify trophic groups, while altitude promotes xeric-tolerant species of Tardigrada in the high Arctic tundra (Svalbard archipelago). *Acta Oecologica*, *98*, 50-58.

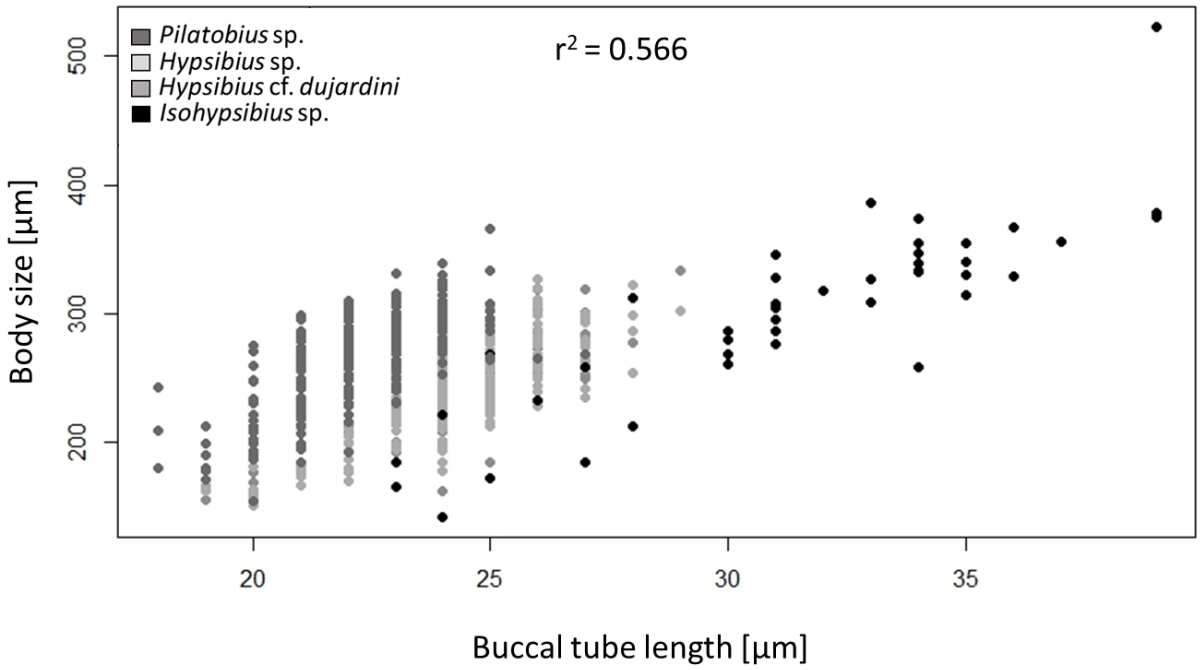
Zawierucha K., Buda J. and Nawrot A. (2019b). Extreme weather event results in the removal of invertebrates from cryoconite holes on an Arctic valley glacier (Longyearbreen, Svalbard). *Ecological Research*, *34*(3), 370-379.

Zeppilli D., Bongiorni L., Cattaneo A., Danovaro R. and Santos R. S. (2013). Meiofauna assemblages of the Condor Seamount (North-East Atlantic Ocean) and adjacent deep-sea sediments. *Deep Sea Research Part II: Topical Studies in Oceanography*, *98*, 87-100.

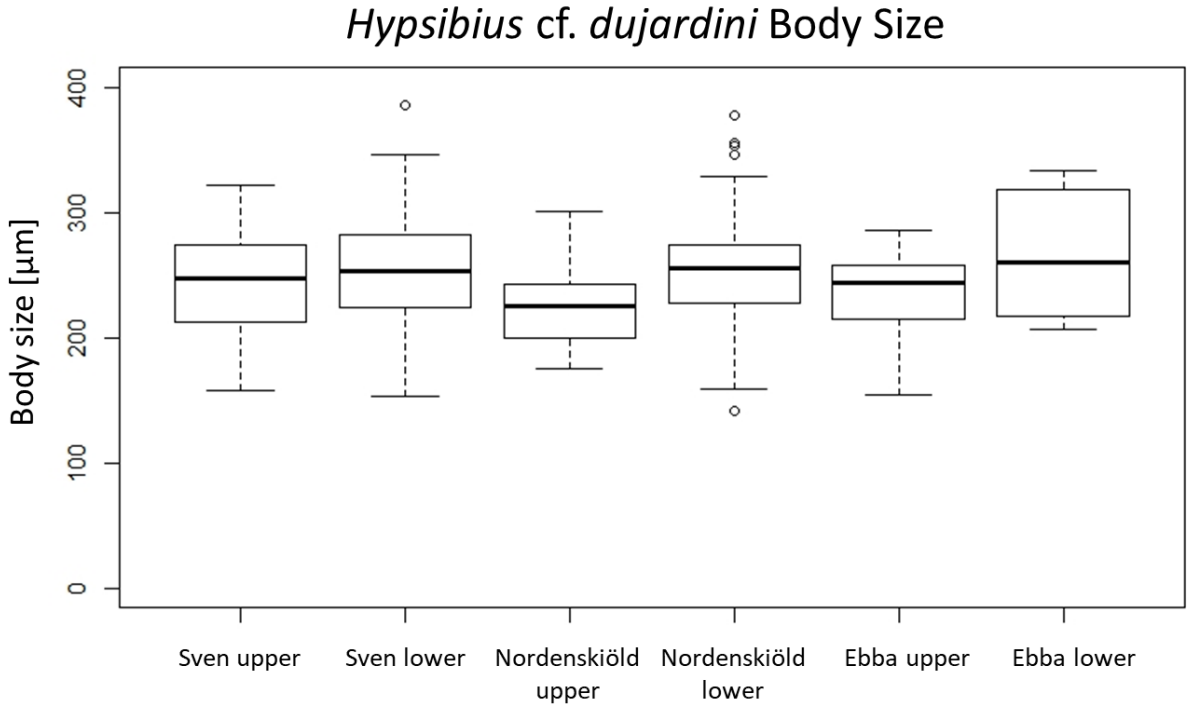
Zwolicki A., Zmudczyńska-Skarbek K., Richard P. and Stempniewicz L. (2016). Importance of marine-derived nutrients supplied by planktivorous seabirds to High Arctic tundra plant communities. *PLoS One*, *11*(5), e0154950.

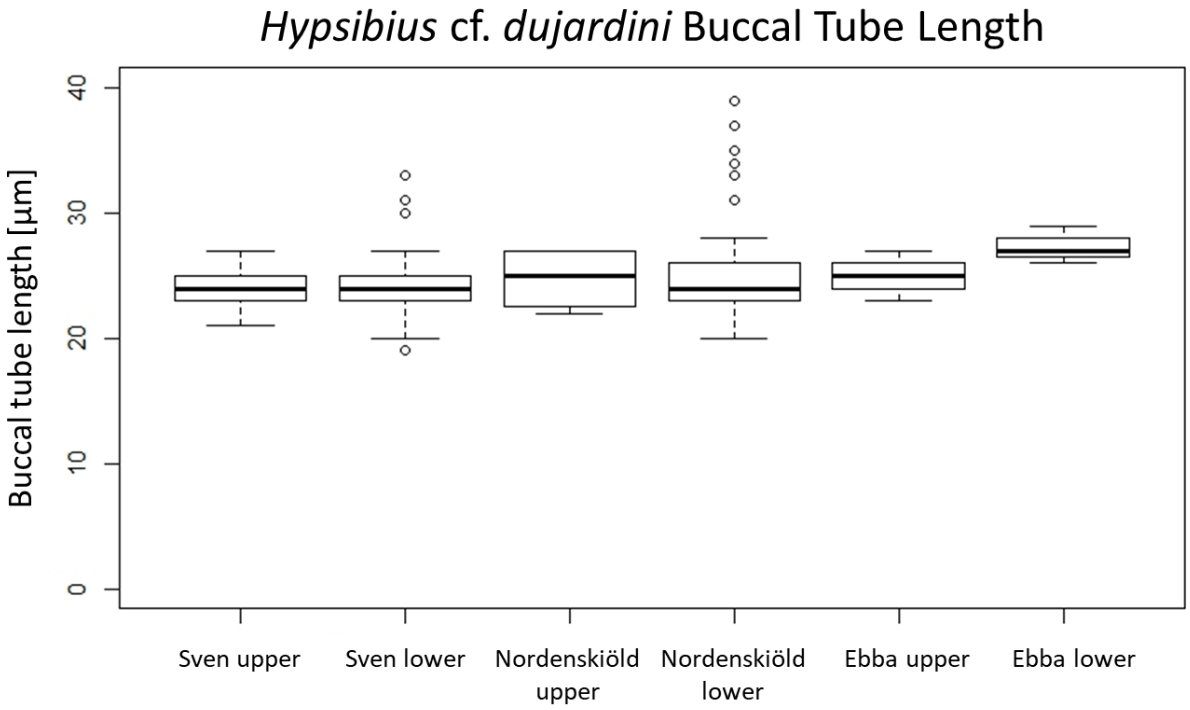
6 Appendices

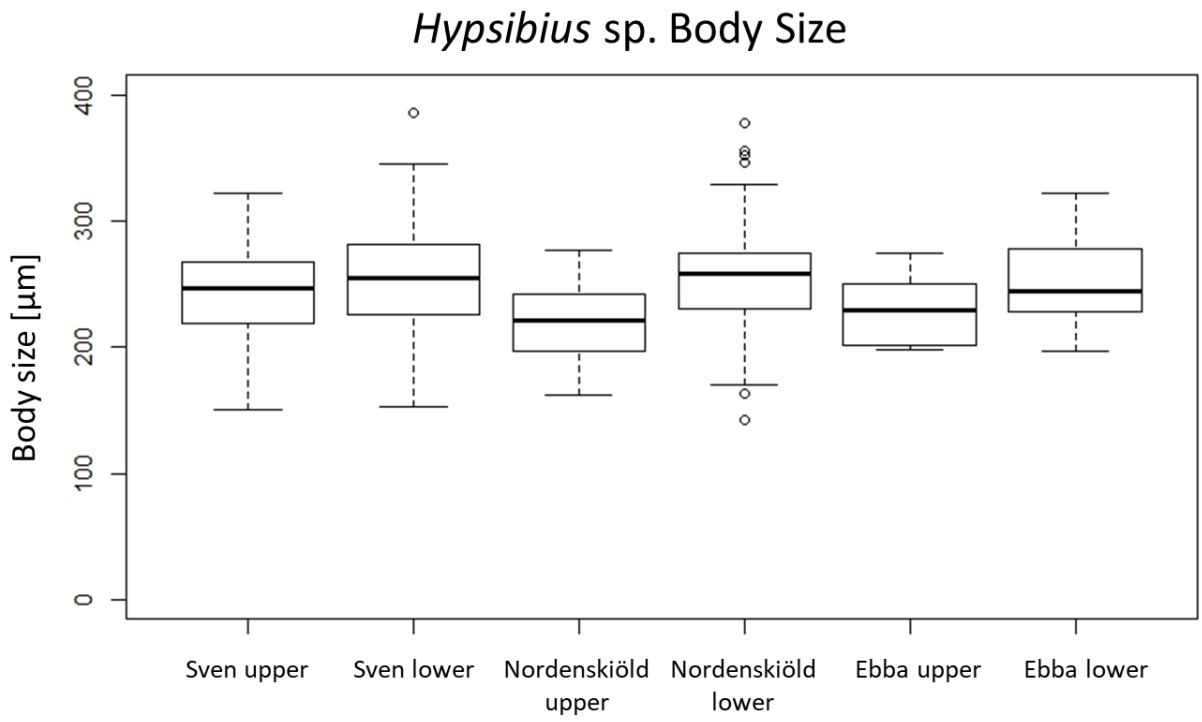
Appendix 1. Table with correlations between buccal tube length and body size in all species and individuals of tardigrades.

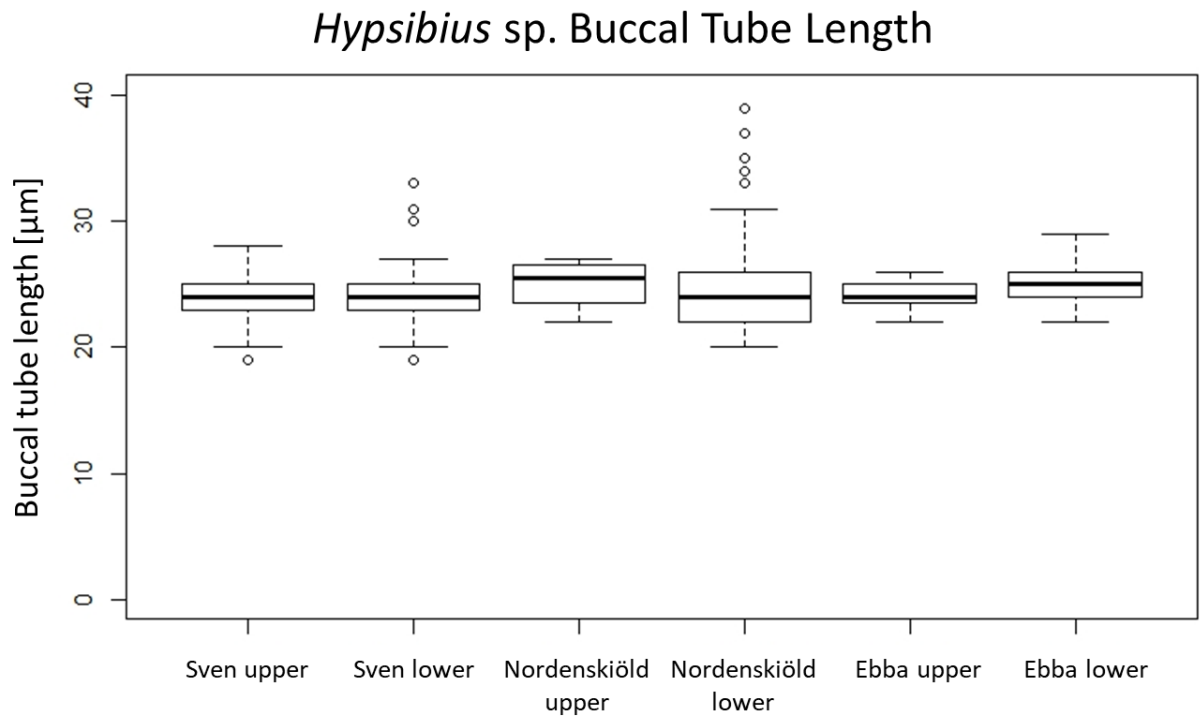


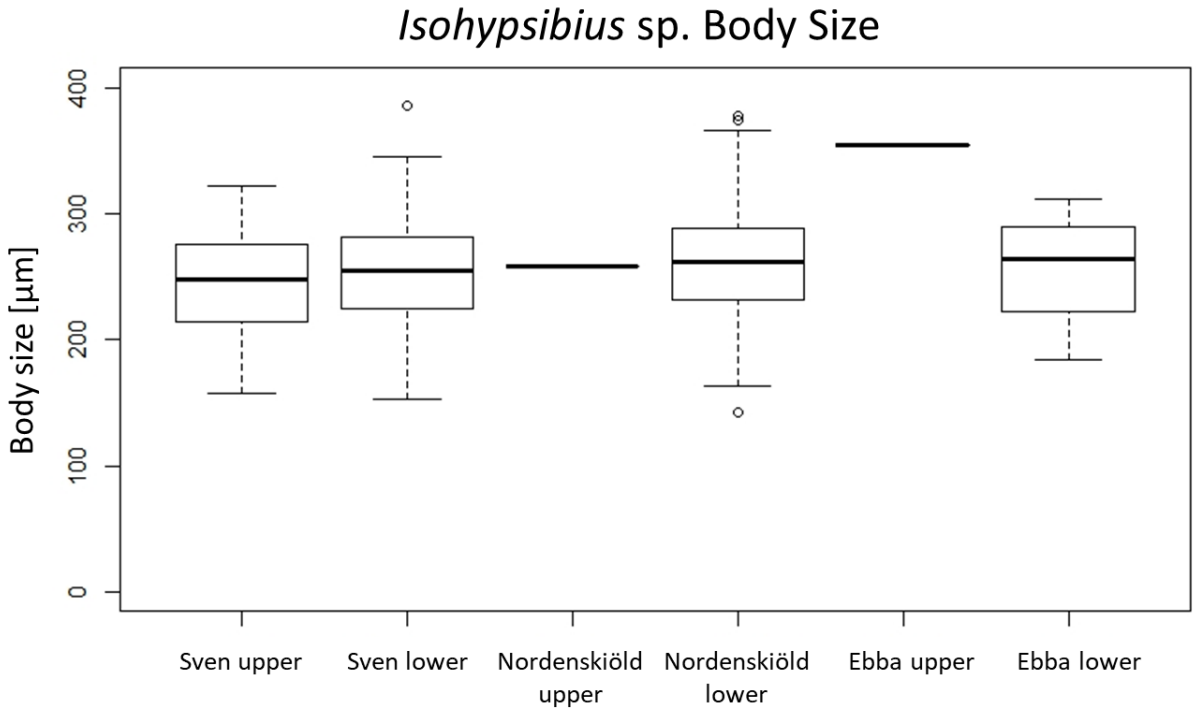
Appendix 2. Boxplots with differences between buccal tube length and body size for each species between glaciers and parts of the ablation zone.

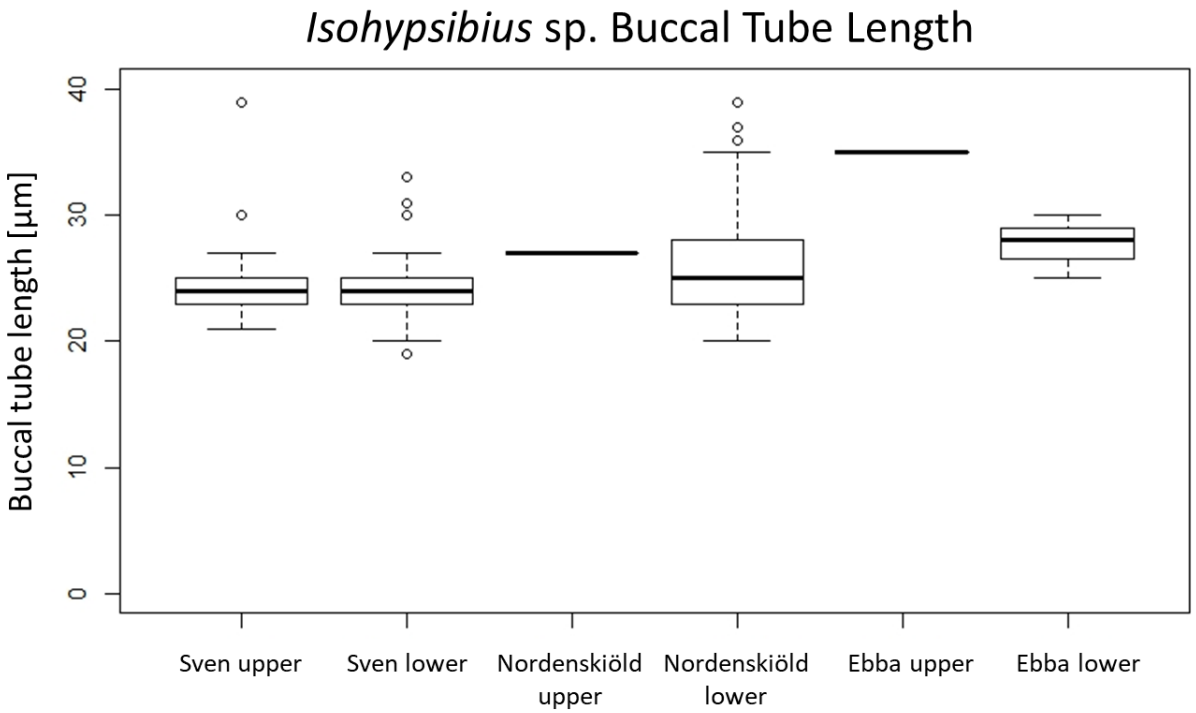


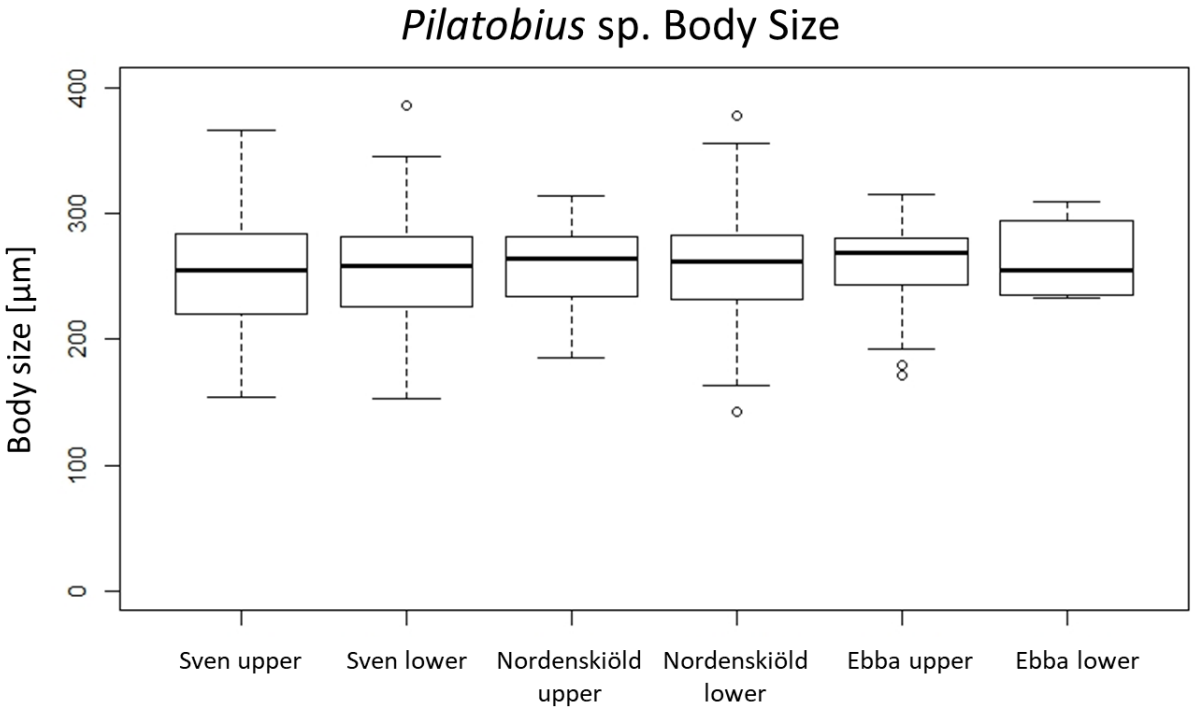


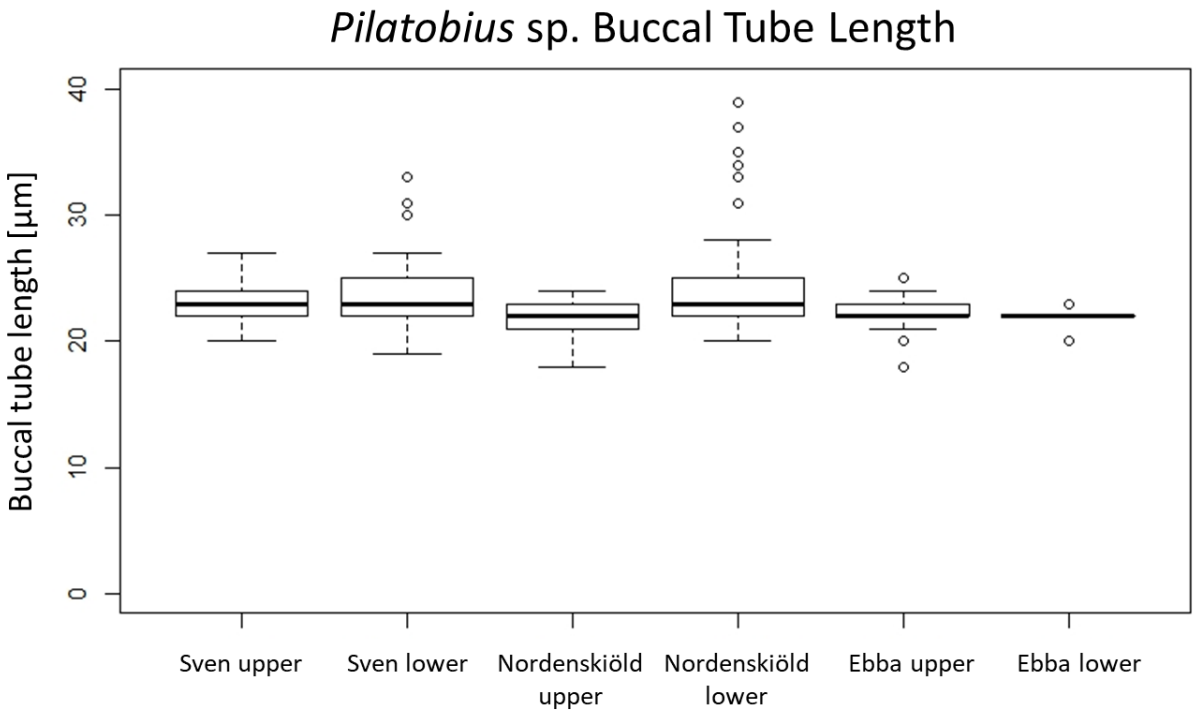

Hypsibius sp. Body Size
Body size [µm]
0
100
200
300
400
Sven upper
Sven lower
Nordenskiöld upper
Nordenskiöld lower
Ebba upper
Ebba lower

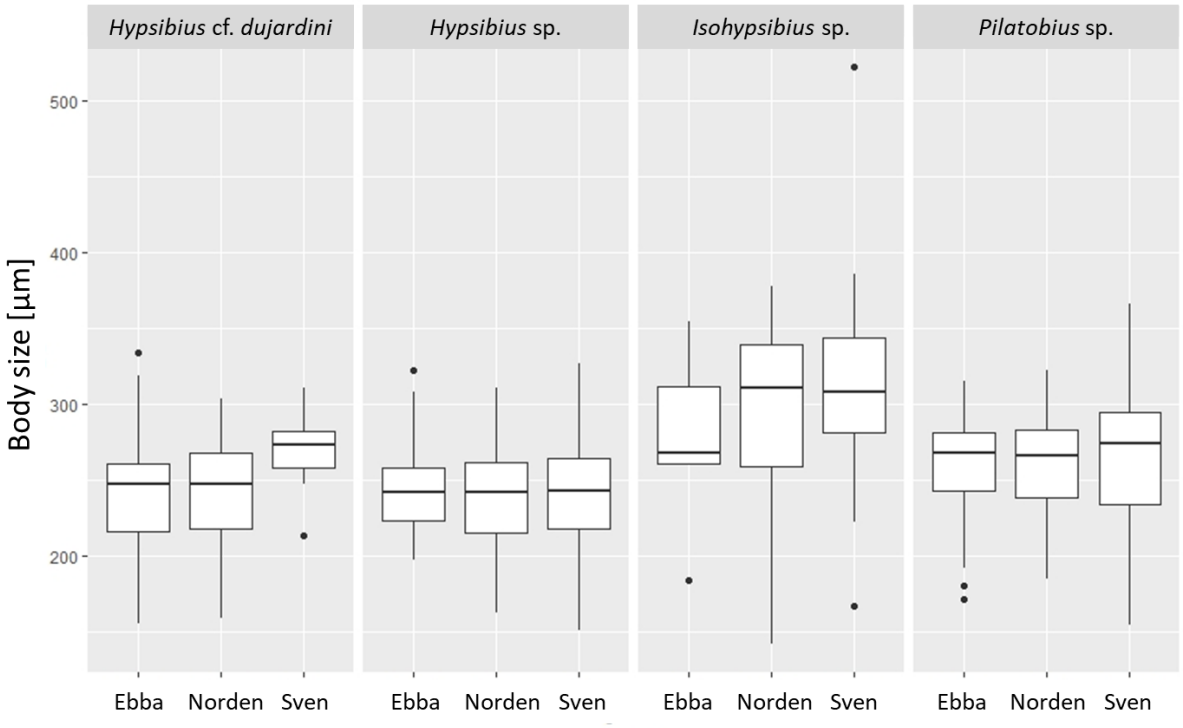

Hypsibius sp. Buccal Tube Length
Buccal tube length [µm]
0
10
20
30
40
Sven upper
Sven lower
Nordenskiöld upper
Nordenskiöld lower
Ebba upper
Ebba lower

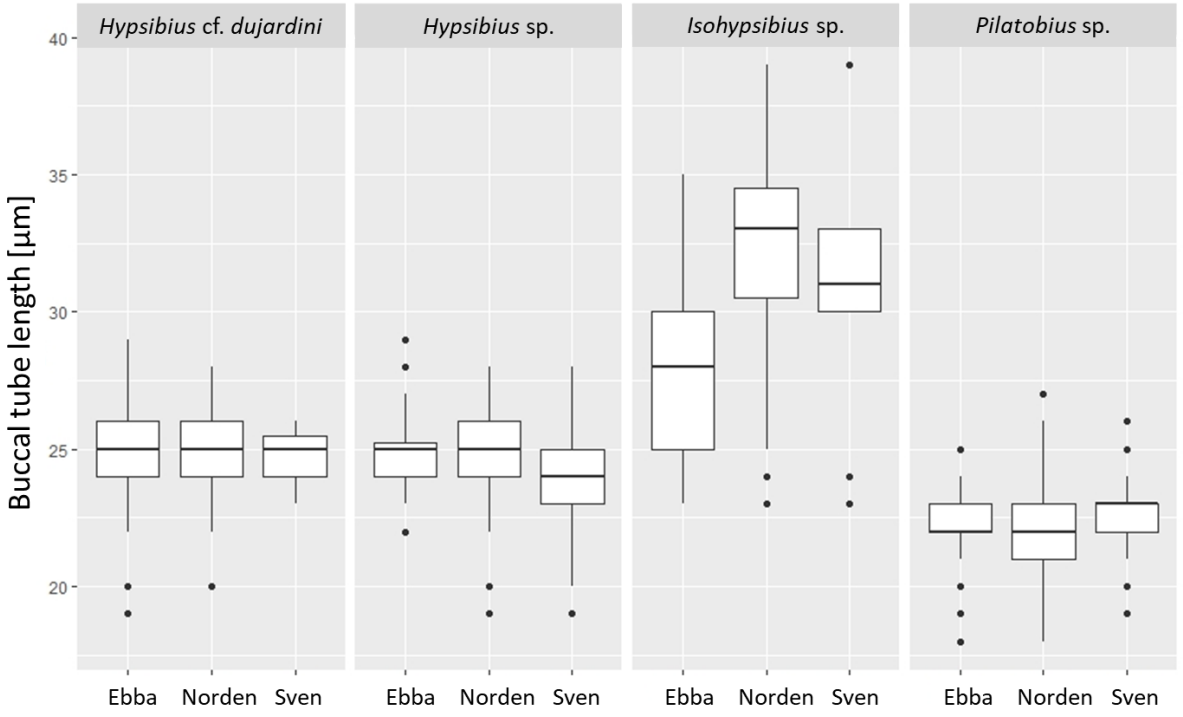

Isohypsibius sp. Body Size
Body size [µm]
0
100
200
300
400
Sven upper
Sven lower
Nordenskiöld upper
Nordenskiöld lower
Ebba upper
Ebba lower


Isohypsibius sp. Buccal Tube Length
Buccal tube length [µm]
0
10
20
30
40
Sven upper
Sven lower
Nordenskiöld upper
Nordenskiöld lower
Ebba upper
Ebba lower


Pilatobius sp. Body Size
Body size [µm]
0
100
200
300
400
Sven upper
Sven lower
Nordenskiöld upper
Nordenskiöld lower
Ebba upper
Ebba lower


Pilatobius sp. Buccal Tube Length
Buccal tube length [µm]
0
10
20
30
40
Sven upper
Sven lower
Nordenskiöld upper
Nordenskiöld lower
Ebba upper
Ebba lower


Hypsibius cf. dujardini
Hypsibius sp.
Isohypsibius sp.
Pilatobius sp.
Body size [µm]
500
400
300
200
Ebba
Norden
Sven


Hypsibius cf. dujardini
Hypsibius sp.
Isohypsibius sp.
Pilatobius sp.
Buccal tube length [µm]
40
35
30
25
20
Ebba
Norden
Sven