Stable isotopic composition of top consumers in Arctic cryoconite holes: revealing different position in supraglacial trophic network

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Abstract. Cryoconite holes are ecosystems on the glacier surface characterized by dynamic nature and truncated food webs. It is acknowledged that cryoconite holes play an important role being biodiversity hot-spots and factories for organic matter on glaciers. The most common cryoconite apex consumers are the cosmopolitan invertebrates – tardigrades and rotifers. Several studies have highlighted the relevance of cryoconite tardigrades and rotifers to cryoconite holes' ecosystem functioning. However, due to the dominant occurrence of prokaryotes on glaciers, these consumers are usually out of the major scope of most studies aiming at biological processes on glaciers. The aim of this study is to present data about isotopic composition of tardigrades, rotifers and cryoconite from three High Arctic glaciers in Svalbard and discuss their potential trophic relations. We found that tardigrades have lower $\delta^{15}N$ values than rotifers, which indicates different food requirements of both consumers. The $\delta^{13}C$ values revealed similarities among the consumers from the same glaciers and differences between consumers and cryoconite among glaciers. The resulted $\delta^{13}C$ values point to similar carbon requirement of consumers within a glacier but differences in carbon input between glaciers. The results comprise the first observation of cryoconite holes' consumers through stable isotopic analyses using an improved method of cryoconite sample processing and pave the way for further studies of the supraglacial trophic network.

1   Introduction

Supraglacial habitat, the environment on the glacier surface, forms the most biologically active and productive part of glaciers all around the world (Hodson et al., 2008). The flat parts of glacier surface accumulate sediment (so called cryoconite, Nordenskiöld (1875)) which – due to its dark colour – reduces albedo and creates water-filled depressions called cryoconite holes (Cook et al., 2016; Takeuchi et al., 2001). At the bottom of these holes, cryoconite forms aggregates composed of bacteria, organic and inorganic matter (Takeuchi et al., 2001). As biodiversity hot-spots of the glacier surface, cryoconite holes host a myriad of organisms ranging from bacteria, algae and fungi to metazoans such as tardigrades (phylum Tardigrada) and rotifers (phylum Rotifera) (Cook et al., 2016; Kaczmarek et al., 2016; Zawierucha et al., 2015). Tardigrades and rotifers are cosmopolitan microscopic invertebrates contributing to multiple aquatic and terrestrial trophic levels as carnivorous, herbivorous, omnivorous and microbivorous species (Guidetti et al., 2012; Guil and Sanchez-Moreno, 2013; Hallas and Yeates, 1972; Kutikova, 2003). Due to their ability to survive various extreme conditions (Guidetti et al., 2011; Ricci, 2001),

40   these animals represent a large component of microfauna in polar and high mountain regions and are the dominant
metazoans inhabiting cryoconite holes (Klekowski and Opaliński, 1986; Zawierucha et al., 2019a).

As the top consumers of Arctic cryoconite holes, tardigrades and rotifers presumably play an important role in
controlling the populations of primary producers and thus contribute to the local community structure (Vonnahme
et al., 2016; Zawierucha et al., 2015; Zawierucha et al., 2018). Previous research on cryoconite holes' metazoans

45   from Svalbard archipelago revealed that the size distribution of algae, particularly Zygnematales and
Chlorococcales, correlates with the community structure of consumers represented by tardigrades and rotifers
(Vonnahme et al., 2016). Nevertheless, other studies from the margin of the Greenland ice sheet indicate a lack of
quantitative relations between numbers of top consumers and potential food such as cyanobacteria and algae
(Zawierucha et al., 2018), which demonstrates the variability influenced by multiple factors occurring on various

50   glaciers (Porazinska et al., 2004). As described by Střítecká and Devetter (2015), tardigrades and rotifers are
efficient filtrators and especially rotifers reveal high filtration rates in cryoconite. The feeding behaviour and
morphology of the feeding apparatus indicate that cryoconite species consume mostly algae, bacteria and detritus
(Devetter, 2009; Iakovenko et al., 2015; Zawierucha et al., 2016). However, their diet in various environments
differs interspecifically (De Smet and Van Rompu, 1994; Guidetti et al., 2012; Guil and Sanchez-Moreno, 2013;

55   Hallas and Yeates, 1972; Kutikova, 2003; Mialet et al., 2013; Wallace and Snell, 2010; Zawierucha et al., 2016).

Analyses of stable isotopes are a well-developed tool which enables us to uncover the trophic interactions of
organisms within various systems (McCutchan et al., 2003; O'Reilly et al., 2003; Wada, 2009; Yoshii et al., 1999).
Because of the differences in isotopic fractionation, $\delta^{13}C$ ($^{13}C/^{12}C$) and $\delta^{15}N$ ($^{15}N/^{14}N$) isotopic ratios of organisms
and their potential food can reflect their possible mutual relationships and position within the food web (Michener

60   and Lajtha, 2008). Isotopic fractionation is caused by physical or biochemical processes which prefer or
discriminate heavier or lighter isotopes (Michener and Lajtha, 2008). The $\delta^{13}C$ value reflects the diet of the
organism and is similar or slightly higher within the animal compared to its food (Peterson and Fry, 1987). The
slight increase between organismal $\delta^{13}C$ and the $\delta^{13}C$ values of its diet is caused by higher assimilation of ^{13}C
supported by preferential ^{12}C depletion (of CO_2) during respiration (Blair et al., 1985; DeNiro and Epstein, 1978;

65   Ekblad and Högberg, 2000; Wada, 2009). Therefore, the process of consumption and growth generally tends to
increase the heavier isotope (^{13}C) value within the consumer's body compared to its diet. Larger variations in
values are however balanced by a higher release of ^{13}C during excretion (DeNiro and Epstein, 1978). The $\delta^{15}N$
values reflect the nitrogen isotopic composition of the organism's diet and point to the position of organisms in a
food chain (DeNiro and Epstein, 1981). The $\delta^{15}N$ value is usually higher in the animal body compared to its diet

70   and increases with the trophic level (DeNiro and Epstein, 1981; Kling et al., 1992; Zah et al., 2001). This increase
is mostly caused by a higher proportion of proteins within the diet and subsequent preferential excretion of $\delta^{14}N$
during protein metabolism (Kling et al., 1992; McCutchan et al., 2003). Furthermore, if the environment is limited
by a nutrient (the biogenic element), the consumer's body fractionates isotopes differently than in case of no
nutrient limitation (Michener and Lajtha, 2008; Šantrůček et al., 2018). Stable isotopes of carbon and nitrogen are

75   the most common food web tracers used in ecological studies (Michener and Lajtha, 2008). In case of invertebrates,
many studies focus on aquatic or soil food webs where producers and consumers can be easily collected and
prepared, and their body size enables us to create a required number of analyses with a sufficient number of
individuals (e.g. Ponsard and Arditi, 2000; Wada, 2009). Several studies have also focused on carbon and nitrogen

stable isotopes in polar systems. However, none of them on glaciers, which are an essential part of polar
80   ecosystems.

Glaciers are one of the key indicators in observation of climate changes and more importantly, they are dynamic
biogeochemical reactors capable of altering processes in downstream deglaciated areas as well as in coastal marine
ecosystems (Bardgett et al., 2007; Foreman et al., 2007; Hodson et al., 2008; Hood et al., 2009; Williams and
Ferrigno, 2012). The primary producers such as cyanobacteria and algae are an important factor reflecting
85   differences in the nutrient input on the glacier surface contributing to the glacial ecosystem functioning (Hodson
et al., 2008; Stibal et al., 2012; Vonnahme et al., 2016). Studies focusing on the role of top consumers in cryoconite
holes are however lacking, which may hinder our understanding of cryoconite holes' ecology and glacial
ecosystem's functioning. This study is based on data from three High Arctic inland glaciers, all three located in a
different geographical and geological context. We examined carbon and nitrogen stable isotopic composition of
90   cryoconite, tardigrades and rotifers, and made a synthesis of their potential relationships in the cryoconite hole
trophic food web.

2   Material and Methods

2.1   Study site and sampling

Samples of cryoconite were collected from three glaciers (Ebbabreen, Nordenskiöldbreen and Svenbreen; *breen*
95   means glacier in Norwegian) located at Central Svalbard (78° N and 14–17° E) during July and August 2016.
Svenbreen is a representative of small glaciers in the geologically older part of Billefjorden Fault Zone. Ebbabreen
and Nordenskiöldbreen are larger valley glaciers within a geologically younger zone. On each glacier,
representative cryoconite holes (varied in shape, size and depth) were sampled in the upper (close to the
equilibrium line) and the lower part (closer to the glacier terminus) of the ablation zone around the main axis of
100   the glacier. 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. (2016). Sampled cryoconite from each part of the ablation
zone was poured together and put into sterile Whirl-Pak® (Nasco, Fort Atkinson, WI). Water pH was measured
during the sampling by a Hanna Instrument (HI 98130). Data about the air temperature were provided by the
105   meteorological station at Bertilbreen which is a glacier adjacent to the examined Svenbreen. After sampling,
cryoconite was stored on ice in a field refrigerator (a plastic barrel entrenched into permafrost) and subsequently
frozen at −20 °C and kept frozen until analysis.

2.2   Preparation of samples for isotopic analyses

For each replicate, a part of cryoconite (~ 2–4 cm³) was separately melted by dropping distilled water through the
110   sample into a glass beaker, transferred into a falcon tube and stored in a cooling box. Animals were collected under
a light microscope (Olympus CX31 and Leica DM750) using a glass Pasteur pipette. All work was performed in
nitrile gloves to avoid carbon contamination. Every individual specimen was cleaned from alien particles and
transferred at least once to a drop of clean distilled water before transferring into an Eppendorf tube. The Eppendorf
tubes were also continuously cooled by a cooling pad. The collected individuals were stored in a freezer at –20 °C
115   until lyophilization and further processing started. After at least 300 individuals of both taxa (tardigrades and

rotifers) were collected from each sample, the Eppendorf tubes were thawed and all individuals from each sample were transferred into a pre-weighted tin capsule (Costech 41077, 5 × 9 mm). If the water content in the capsule exceeded ½ of the volume, capsules were dried inside a desiccator with silica gel (0.5–2.5 h) until the water inside the capsules was reduced to 1/3 of the volume. The samples were consequently frozen at –20 °C and at least half

120 an hour before the lyophilization stored at –80 °C. The duration of the lyophilization was 4 hours. Thereafter, samples were weighed (Mettler Toledo Excellence Plus XP6, linearity = 0.0004 mg), the capsules were closed and wrapped, and analysed immediately or stored in a desiccator until the analyses were performed. The average weight of a dry sample of invertebrates was ~ 29.5 μg. Four replicates of tardigrades, rotifers and cryoconite from Svenbreen, five replicates of tardigrades, four replicates of rotifers and three replicates of cryoconite from

125 Nordenskiöldbreen, and three replicates of tardigrades, two replicates of rotifer and two replicates of cryoconite from Ebbabreen were collected for the isotopic analyses.

Cryoconite intended for the isotopic analyses was cleaned from tardigrades and rotifers, which were collected in parallel for isotopic analyses. After the collection, cryoconite was stored in Eppendorf tubes at –20 °C. When all samples were prepared, cryoconite was homogenised using an agate pestle and mortar and dried in a thin layer on

130 a Petri dish at 45 °C. The duration of drying was 8 hours.

For the analyses of $\delta^{15}N$ in organic matter (OM), cryoconite was transferred without any other preparation into pre-weighed tin capsules (Costech 41077, 5 × 9 mm) and weighed. The average amount of cryoconite used for analyses was ~ 31 mg. For the analyses of $\delta^{13}C$ in organic matter, 11–12 mg of cryoconite was transferred into pre-weighed silver capsules (Elemental Analyses, 8 × 5 mm, D2008) and carbonates (e.g. calcite, dolomite) were

135 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, samples were left drying at 50 °C for 17 hours. After drying, silver capsules were inserted into tin capsules and put into a desiccator for 10–20 days.

2.3 Stable isotopes analyses

140 The $\delta^{13}C$ and $\delta^{15}N$ values in all samples were analysed using a Flash 2000 elemental analyser (ThermoFisher Scientific). Released gasses (NO_x, CO_2) separated in a GC column were transferred to an isotope-ratio mass spectrometer Delta V Advantage (ThermoFisher Scientific) through a capillary by Continuous Flow IV system (ThermoFisher Scientific). The stable isotope 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

145 normalized to a calibration curve based on international standards IAEA-CH-6, IAEA-CH-3, IAEA 600 for carbon and IAEA-N-2, IAEA-N-1, IAEA-NO-3 for nitrogen. The calibration curve for analyses of cryoconite was based on the international standard ST-Soil Standard (Peaty) and ST-Soil Standard (SSclay). Analytical precision as a long reproducibility for standards was within ±0.03 ‰ for $\delta^{13}C$ and ±0.02 ‰ for $\delta^{15}N$.

The isotopic values of nitrogen in OM as well as organic carbon (decarbonized cryoconite) in cryoconite were

150 used as reference to isotopic composition of potential food source for invertebrates.

2.4 X-Ray Diffraction

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 an 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.

2.5 Community structure

For the species identification, at least 10 cm^3 of cryoconite was used from each sample. Tardigrades were collected using a glass Pasteur pipette and the first observation was made under a stereomicroscope (Olympus SZ 51). Immediately after collecting, clean tardigrades were transferred on glass slides and mounted in a small drop of the Hoyer's medium (Anderson, 1954; Ramazzotti and Maucci, 1983). After one day of drying in 56 °C, tardigrades were identified under a light microscope with phase contrast (Olympus BX53) associated with a digital camera ARTCAM 500. Tardigrades were classified to the dominant feeding behaviour groups according to Guidetti et al. (2012), Guil and Sanchez-Moreno (2013), Hallas and Yeates (1972) and Kosztyła et al. (2016). 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 of feeding behaviour of rotifers was primarily conducted following the monography by Doner (1965). For the identification of eukaryotic primary producers, small drops of thawed and well-mixed cryoconite were placed on the mount. Afterwards, algae and cyanobacteria were identified using a light microscope Olympus BX51 equipped with Nomarski interference contrast and the digital camera Olympus EOS 700D. Identification was based on publications by Starmach (1966), Ettl and Gärtner (2014) and Wehr et al. (2015).

2.6 Statistical Analysis

All statistical analyses were conducted in R version 3.5.3 (R Development Core Team, 2018). To test the differences between δ^{15}N isotopic values of tardigrades and rotifers, Kruskal−Wallis rank sum test was used. Before the correlation coefficient tests were applied, Shapiro−Wilk test was used to test the normal distribution of the data. Therefore, Spearman's rank correlation coefficient and Pearson's product-moment correlation coefficient were counted between isotopic values (δ^{13}C and δ^{15}N) of cryoconite and isotopic values of tardigrades and rotifers. Correlation coefficients using Shannon−Wiener Index of Diversity were used to reveal differences between species composition and isotopic values (δ^{15}N, δ^{13}C) of tardigrades. To compare isotopic values of tardigrades, rotifers and cryoconite from each sampling site, One-Way ANOVA and Tukey multiple comparisons of means were applied. For the purpose of statistical analyses, all replicates from the same sampling campaigns were averaged.

3 Results

3.1 Isotopic signatures

The isotopic signature of nitrogen showed significant differences in δ^{15}N between tardigrades and rotifers in all samples (Kruskal−Wallis chi-squared = 12.685, *df* = 1, n = 22, *p*-value = 0.00037). All measured δ^{15}N values of tardigrades revealed lower δ^{15}N values than rotifers as shown in Fig. 1 and Table 1. Furthermore, we measured

$\delta^{15}N$ values of nitrogen in organic matter from cryoconite, but there was no significant relation with $\delta^{15}N$ values of tardigrades and rotifers found.
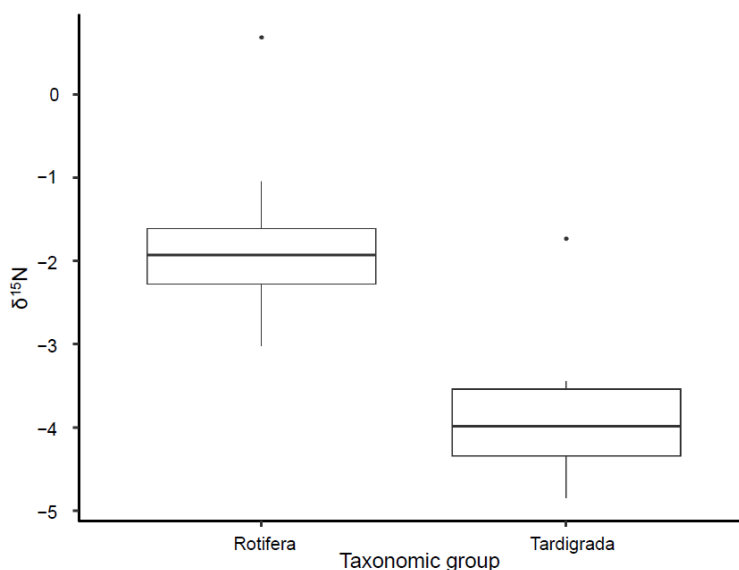
Figure 1. Differences in $\delta^{15}N$ between tardigrades and rotifers analysed by Kruskal−Wallis rank sum test. The diagram displays medians and distribution of measured $\delta^{15}N$ values. The whiskers represent the lowest and highest measured values. The outliers represent $\delta^{15}N$ values from one replicate from Ebbabreen.

Table 1. Description of samples and isotopic values ($\delta^{13}C$, $\delta^{15}N$) 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. The T and R letters in Dominance column signify: T (dominance of tardigrades), R (dominance of rotifers), T = R (equal proportion of tardigrades and rotifers) in particular samples collected during sampling campaigns.

Glacier	Tardigrades		Rotifers		Cryoconite		Dominances
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C*$	phylum
Sven	−3.55	−23.75	−1.57	−27.09	−2.20	−22.65	T
	−4.04	−26.36	−1.81	−26.69	−2.17	−22.08	R
	−3.52	−26.33	−2.04	−31.16	−2.24	−24.09	R
	−4.39	−26.45	−1.04	−28.53	−1.64	−23.25	R
Nordenskiöld	−4.39	−22.91	−2.20	−23.36	−3.58	−21.56	T = R
	−3.45	−23.30	−1.72	−23.25	−2.30	−20.56	T = R
	−3.76	−22.58	−2.30	−23.02	−2.98	−22.52	T = R
	−4.15	−24.78	−3.02	−25.19			
	−3.93	−25.11					
Ebba	−1.73	−24.19	0.69	−26.32	−2.30	−22.24	R
	−4.85	−25.22	−2.38	−25.13	−4.29	−23.31	T
	−4.33	−25.15					

Regarding the isotopic signatures of carbon, we found a positive correlation of the δ^{13}C values of decarbonized cryoconite and the δ^{13}C of rotifers (r = 0.83, n = 19, *p*-value = 0.006) (Fig. 2). The respective relationship among tardigrades was not significant (n = 21, *p*-value > 0.05).
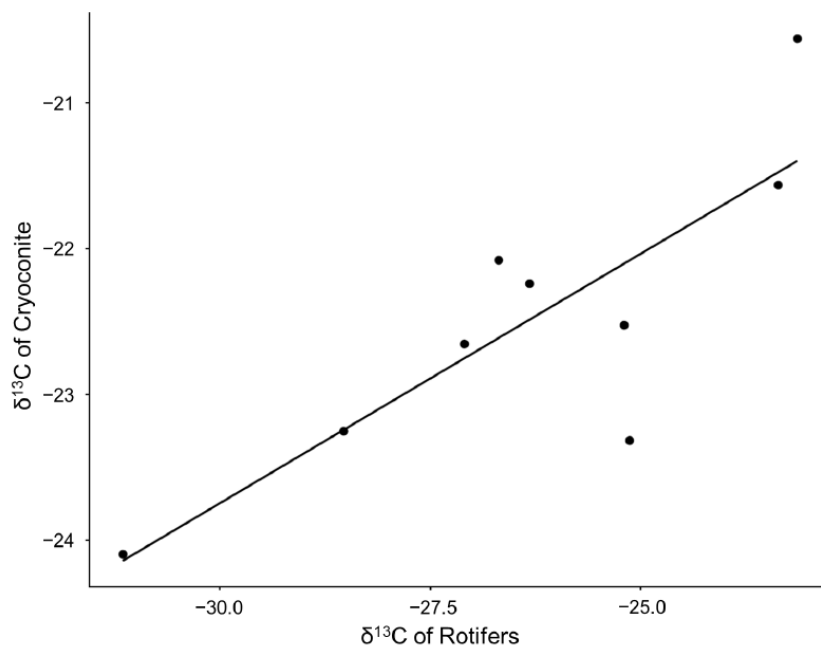
Figure 2. Correlation between δ^{13}C of rotifers and cryoconite with the linear regression line.

In all samples, differences in δ^{13}C and δ^{15}N of tardigrades and rotifers among glaciers were tested using ANOVA test with the mean values of δ^{13}C and δ^{15}N and the Tukey multiple comparisons of means. These analyses showed a significant difference in δ^{13}C values of rotifers between glaciers (*p*-value = 0.029) (Fig. 3a), mostly between Nordenskiöldbreen and Svenbreen (*p*-value = 0.025). All other tests did not reveal any significant pattern (Fig. 3).
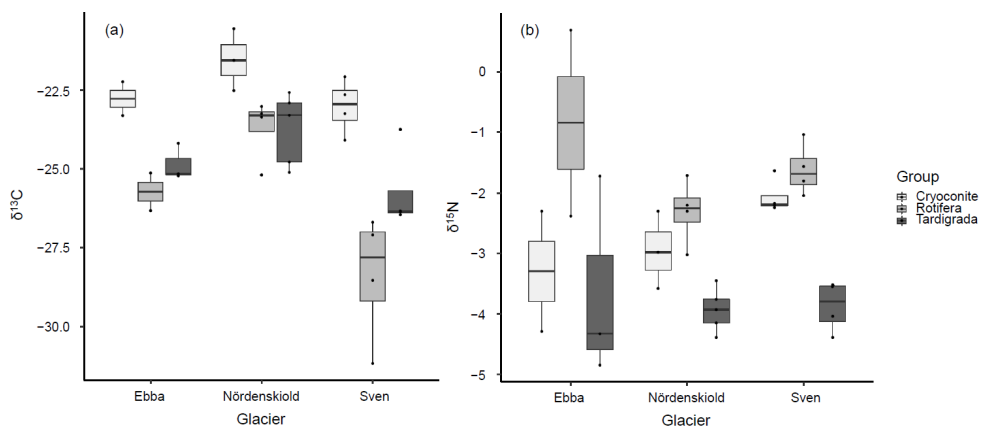
Figure 3. Distribution of δ^{13}C **(a)** and δ^{15}N **(b)** isotopic values in tardigrades (n = 12), rotifers (n = 10) and cryoconite (n = 9) among glaciers.

3.2 Composition of cryoconite

X-Ray diffraction of cryoconite showed that the glaciers differ in mineral composition. Svenbreen has a low
210   amount of dolomite and amphibole which are dominantly found within the metamorphic basement rocks around
Ebbabreen and Nordenskiöldbreen. The distribution of minerals within each glacier is shown in Table A1
(Appendices). The ANOVA analysis applied on the mean $\delta^{13}C$ values of OC in cryoconite did not reveal any
significant difference between glaciers. Due to the logistical issues, pH in cryoconite holes was measured only on
Svenbreen and Nordenskiöldbreen with values pH < 7.

215   ### 3.3 Community structure

During the collection of animals for isotopic analyses, we also counted the proportion in abundances of tardigrades
and rotifers within all replicates. A difference in relative abundance lower than 5 % was considered an equal
proportion (Table 1).

Regarding the species composition of primary producers, we identified representatives of algae and cyanobacteria
220   from all samples. In case of algae, we observed mostly Zygnematales (*Ancylonema* sp., *Mesotaenium* sp.).
Oscillatoriales (*Phormidium* sp.), Nostocales (*Nostoc*) and Synechococcales (*Leptolyngbya* sp.) were observed as
the most abundant cyanobacteria. Quantification of primary producers was not done due to the preservation by
freezing which presumably had a taxon-specific effect on the survival of cells of the phototrophs.

During the division of consumers into trophic groups, only tardigrades were identified in a sufficient number for
225   analyses. Species composition of rotifers was not analysed because of the majority of individuals occurring in a
dormant stage, which made it impossible to identify the required number for analyses. Rotifers found within the
samples were identified as *Macrotrachella* sp. and *Adineta* sp. Regarding tardigrades, we identified 1117
individuals which were divided into three trophic groups: *Pilatobius* sp. as microbivorous (41.24 %), hypsibids as
herbivorous (53.33 %) and isohypsibids as omnivorous (5.43 %). As shown in Fig. 4, the composition of tardigrade
230   trophic groups is not equal among glaciers.

Correlations between trophic groups of tardigrades and isotopic values ($\delta^{15}N$, $\delta^{13}C$) of tardigrades and
decarbonized cryoconite did not reveal any significant relationship.
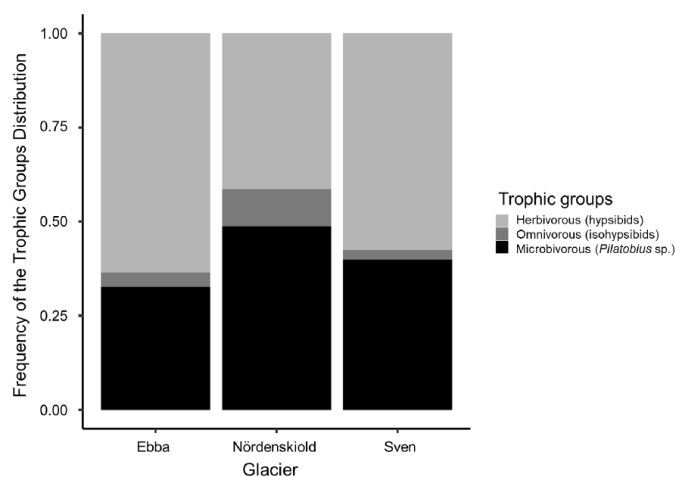
Figure 4. Bar plot visualization of tardigrades' trophic groups relative distribution among glaciers.

4 Discussion

4.1 Isotopic signatures and trophic relations of cryoconite consumers

There are only three studies focusing on the isotopic composition of polar tardigrades and rotifers, all conducted in Antarctica: the research of Almela et al. (2019) and Velázquez et al. (2017), who focused on microbial mats in the non-glaciated part of Byers Peninsula and the research of Shaw et al. (2018), who focused on the non-glaciated part of the Taylor Valley. Isotopic composition of tardigrades and rotifers from Taylor Valley was nearly the same reflecting similarities in composition of their diet. On the contrary, in the study of Almela et al. (2019), tardigrades reflected lower values of $\delta^{15}N$ and lower values of $\delta^{13}C$ compared to rotifers, and in the study of Velázquez et al. (2017) tardigrades reflected lower $\delta^{15}N$ and higher values of $\delta^{13}C$ than rotifers. Moreover, in both studies from Byers Peninsula, the $\delta^{15}N$ values of proposed food was always higher than the $\delta^{15}N$ values of consumers. It is known that the variability in absolute isotopic composition among systems has various reasons, for example differences in the isotopic composition of the nutrient pool (Montoya et al., 1990). The isotopic values of consumers can also vary based on the seasonal variability in isotopic values of their food (Zah et al., 2001). In cryoconite holes, the input of nutrients as well as changes in the community structure of microbes vary during the season (Säwström et al., 2002; Stibal et al., 2008). Therefore, the variability in $\delta^{15}N$ and $\delta^{13}C$ values as well as variation in the distribution of isotopic composition as described in Antarctic studies was expected.

Our results showed that rotifers revealed higher values of ^{15}N isotope compared to tardigrades suggesting potential differences in $\delta^{15}N$ composition of their diet. Predominantly, higher values of $\delta^{15}N$ usually indicate a higher trophic level (Kling et al., 1992; Wada, 2009). However, based on the observed food preferences of tardigrades and rotifers from cryoconite (Střítecká and Devetter, 2015; Zawierucha et al., 2016) we cannot assume a strict trophic division of their food. Primarily, tardigrades found in samples were identified as probably microbivorous (*Pilatobius* sp.), herbivorous (hypsibids) and omnivorous (isohypsibids) species. Nevertheless, we cannot exclude that *Pilatobius* sp. with its ventrally located mouth does consume algae during scratching biofilms from the surface of granules. The same applies to isohypsibids, which have a relatively wide buccal tube and can utilise more food sources including algae, protozoans and other small invertebrates. Moreover, in the laboratory cultures, all studied groups of tardigrades feed on algae (Kosztyła et al., 2016). Rotifers were mostly identified as filter feeders (*Macrotrachella* sp.) or scrapers (*Adineta* sp.) (Herzig et al., 2006). However, *Adineta* sp. never exceed 10 % of their total amount and the living specimens can be well distinguished from *Macrotrachella* sp. during collecting. The differences in $\delta^{15}N$ between both consumers could suggest $\delta^{15}N$ enrichment in food for rotifers caused by consumption of bacteria or DOM enriched in $\delta^{15}N$. This assumption is supported by Nagarkar et al. (2004) and Kohler et al. (2018), who described that cyanobacteria have higher content of proteins and a higher $\delta^{15}N$ values typical for nitrogen fixing organisms. The other potential explanation of the observed pattern is tardigrades' consumption of algae, which can vary in $\delta^{15}N$ depending on their C:N ratio (Adams and Sterner, 2000). In cryoconite holes, consumers are probably highly limited by the lack of nutrients and the small size of food. Therefore, the ingested food composition may shift from its optimum.

270   Generally, the δ¹³C values of tardigrades and rotifers in our study were lower than δ¹³C values in decarbonized cryoconite. This difference is similar as described by Almela et al. (2019) and Velázquez et al. (2017) in Antarctic microbial mats. Nevertheless, it contrasts with the fundamental literature (Peterson and Fry, 1987; Wada, 2009) as well as with the study of Shaw et al. (2018) focusing on Antarctic soil microbial mats, which presented δ¹³C values of tardigrades and rotifers similar or slightly higher than δ¹³C values of their potential diet. The differences

275   between these results could point to differences in carbon fractionation on glacier surface or within tardigrades and rotifers in comparison with freshwater zooplankton and soil microfauna. The observed correlation between cryoconite and rotifers in δ¹³C could also suggests that rotifer food (suspended bacteria) is the representative for much of the cryoconite organic carbon. On the other hand, such correlation in tardigrades is not significant, because they feed on primary producers (algae and cyanobacteria) related mostly to air CO_2, which has the same δ¹³C

280   everywhere. It may therefore serve as indirect evidence for the bacterivory of rotifers and the algivory of tardigrades in cryoconite holes.

4.2   Variations in isotopic signatures among glaciers

As shown in the results, the isotopic composition of tardigrades, rotifers and cryoconite between the replicates and between the glaciers differ. Furthermore, we observed variations in the proportional representation of tardigrades and rotifers, and in the community structure of tardigrades among the glaciers and the replicates as well.

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The variability in δ¹³C values could indicate specific nutrient requirements of primary producers affected by the variability in spatial characteristics of the glacier surroundings and consequent variations in the nutrient input onto glacier surface (Bagshaw et al., 2013; Hagen et al., 1993). As presented by Post (2002), who focused on freshwater food webs, larger studied lakes evinced higher δ¹³C values than small lakes suggesting higher occurrence of

290   autochthonous carbon input favouring heavier ¹³C isotope signature of the food web. It is highly possible that due to its smaller size, Svenbreen presumably has a higher allochthonous input of nutrients in the form of organic matter from adjacent habitats, which causes depletion of ¹³C in isotopic signature because of a longer chain of fractionations favouring lighter ¹²C typical for allochthonous source of carbon (Peterson and Fry, 1987; Post, 2002). Consequently, the depletion in ¹³C of consumers from Svenbreen could signify consumption of DOM from

295   the primary production or detritus (Abelson and Hoering, 1961; Iakovenko et al., 2015; Macko and Estep, 1984). Oppositely, tardigrades and rotifers from Ebbabreen and Nordenskiöldbreen had higher δ¹³C than consumers from Svenbreen. We assume that this increase was caused by a larger size of these glaciers and a potential larger component of autochthonous production (Stibal et al., 2010) which uses "heavier" carbon from atmospheric CO_2 (Post, 2002) and has a shorter chain of transformations and discriminations against δ¹³C during the assimilation of

300   inorganic matter (Michener and Lajtha, 2008). However, the observed variations in δ¹³C among glaciers could also reflect a different proportional representation of herbivorous and other consumers (DeNiro and Epstein, 1978; Michener and Lajtha, 2008), or a dynamical character of sudden processes occurring on the glacial surface including changes in the input of organic and inorganic matter (Chandler et al., 2015; Telling et al., 2012; Wagenbach et al., 1996; Zah et al., 2001). Therefore, further investigations are essential.

305   Considering the close δ¹³C isotopic values of consumers from Ebbabreen and Nordenskiöldbreen, the observed pattern could signify an overlap in food preferences of both consumers (Almela et al., 2019; DeNiro and Epstein, 1978; Mialet et al., 2013; Ricci, 1984; Velázquez et al., 2017). The non-strict division of consumed food between

tardigrades and rotifers was apparent in various feeding experiments (Mialet et al., 2013; Ricci, 1984; Střítecká and Devetter, 2015) even though Almela et al. (2019) demonstrated that tardigrades were related to larger particles

310 (e.g. algae) and rotifers mostly to smaller ones (e.g. POM). Similarities in distribution of $\delta^{13}C$ in comparison with differences in $\delta^{15}N$ between tardigrades and rotifers could also indicate that rotifers consume DOC originating from extracellular exudates of algae or cyanobacteria, but the source of nitrogen (e.g. bacteria, cyanobacteria and organic detritus) is different.

Regarding differences in $\delta^{15}N$, some samples evinced high presence of cyanobacteria *Leptolyngbya* sp., and the

315 high $\delta^{15}N$ could refer to a higher content of ^{15}N in cyanobacteria populations (Darby and Neher, 2012). However, the observed variation could also be a result of different mineral composition of cryoconite among glaciers located in different parts of Billefjorden Fault Zone. Especially in samples from Svenbreen, we detected a very low amount of amphibole and dolomite which were common on Ebbabreen and Nordenskiöldbreen. Considering higher potential solubility of minerals due to acidic pH of cryoconite holes (4.48−5.9) and differences in mineral

320 composition of cryoconite aggregates among glaciers, the differences in the community structure of microbial communities and consequent isotopic signatures could also be related to the variability in composition of available minerals released by biogeochemical weathering (Barker and Banfield, 1998; Carson et al., 2007; Roberts et al., 2004; Zawierucha et al., 2019b). Moreover, upper parts of Svenbreen were covered by snow during sampling, whereas before and during sampling of Ebbabreen, the air temperature increased to 8.8 °C (according to the

325 meteorological station at Bertilbreen). Therefore, the higher content of $\delta^{15}N$ in these samples could also be caused by presence of NO_3^- in the meltwater (Hodson et al., 2005).

5   Conclusions

This study presents the first description of $\delta^{13}C$ and $\delta^{15}N$ isotopic composition of cryoconite consumers and their potential food. Despite the variability in distribution of isotopic values, we showed that $\delta^{15}N$ differs among

330 tardigrades and rotifers. The $\delta^{13}C$ values reveal variability in their distribution among the taxa as well as glaciers. In particular, the $\delta^{15}N$ values present evidence of differences in feeding behaviour between both groups. The $\delta^{13}C$ values provide evidence that the input and source of carbon among glaciers may differ and these differences can influence the isotopic composition of $\delta^{13}C$ in cryoconite as well as in animals. We also revealed a significant correlation between organic carbon from cryoconite and rotifers, which indicates that rotifers may be related

335 more to cryoconite carbon from bacteria than tardigrades, which are considered to be more herbivorous. Nevertheless, further research is required to elucidate the cryoconite trophic levels, the entire diet of the consumers and their contribution to supraglacial nutrient pathways. Another outcome of this study is the introduction of modified technique of sample preparation avoiding procedures such as sugar centrifugation or oven drying.

340

6 Appendices

Table A1. Mineral composition in particular samples analysed by X-Ray diffraction. X letter means presence of the mineral, XX means high presence of the mineral. The sign (−) means that the mineral was not detected.

Sample	Quartz	Plagioclase	K-Feldspar	Amphibole	Dolomite	Muscovite/Illite	Chlorite
SL1	XX	X	X	−	−	XX	X
SU1	XX	X	X	−	−	X	X
SL2	XX	X	X	−	−	XX	XX
SU2	XX	X	X	X	X	XX	XX
NL1	XX	X	X	X	X	XX	XX
NU1	XX	X	X	X	X	XX	XX
NL2	XX	X	X	X	X	XX	XX
EL2	X	X	X	X	XX	XX	XX
EU2	XX	X	X	X	X	XX	XX

345   *Code availability*. All codes related to figures and analyses were made in R (version 3. 5. 1.) and are available upon request of the corresponding author.

Data availability. All data about isotopic composition, trophic groups composition and mineral composition are available upon request to the corresponding author. Meteorological data from Bertilbreen were kindly provided by Associate Professor Kamil Láska and all requests must be sent to him.

350   *Author contributions*. JDŽ, TJ, JT and KZ developed the study design. The field sampling was conducted by TJ and JDŽ. The stable isotopes analyses were conducted by TJ, JT and LV. The identification of trophic groups of tardigrades were conducted by TJ and KZ. The identification of rotifers was conducted by MD. TJ compiled and processed all presented data and prepared the manuscript contributing revisions from all co-authors.

Competing interests. The authors declare that they have no conflict of interest.

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