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Vertical microzonation of ciliates in cryoconite holes in Ecology Glacier, King George Island

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Abstract: There are hardly any data concerning the vertical micro-distribution of protozoa in water column in cryoconite holes on the glacier surface. Such comparisons can provide insights into the ecology of protozoa. The present research was made on Ecology Glacier (South Shetland Islands, Antarctic); vertical microzonation of ciliates in relation to physical and chemical parameters in cryoconite holes was studied. The density and biomass of protozoans significantly differed between the studied stations (cryoconite holes), with the lowest numbers in the surface water and the highest in the bottom water. The surface waters were dominated by mixotrophic and omnivorous taxa, whereas the deepest sampling level has shown the increase of the proportion of bacterivore species. Ordination analysis indicated that TN and P-PO_4 can strongly regulate the abundance and species composition of protozoa. The redundancy analyses (RDA) showed that the ciliate communities can be separated into two groups. The first group included species associated with surface water: *Halteria grandinella* and *Codonella* sp. The second group included species that are associated with bottom water: *Prorodon* sp., *Holosticha pullaster*, *Stylonychia mytilus*-complex and small scuticociliates.

Key words: Antarctic, cryoconite holes, protozoa, biodiversity.

Introduction

Cryoconite holes are relatively small, shallow, straight-sided holes with concave bottoms. They tend to be not more than 50–60 cm in depth (Säwström *et al.* 2002). Cryoconite holes can cover 0.1–10% of the surface of the glacier (Anesio *et al.* 2009). Cryoconite holes are created when wind-blown sediment preferentially melts at the ice surface due to its relatively low albedo. Over time, the holes become roughly circular and water filled with black cryoconite material at the bottom (Mueller and Pollard 2004; Anesio and Laybourn-Parry 2012). Investigation of the structure and function of glaciers ecology relate mainly to the bacteria (Anesio *et al.* 2007). In cryoconite holes the animal communities, especially meiofauna (e.g. copepods, nematodes and tardigrades), are well known (Anesio *et al.* 2007). However, ecologists have paid little attention to ciliates in cryoconite holes, compared to other habitats. Although these microorganisms are important consumers of pico- and nano- sized producers, as well as nutrient regenerators and important food sources of metazoan (Pierce and Turner 1992), most studies in polar regions paid attention to ciliates of lake ecosystems and soils only (Petz 1997; Roberts *et al.* 2004; Bamforth *et al.* 2005). So far ciliated protozoans were observed in cryoconite holes, usually in sediment, but in most cases no attempt has been made to identify them (Mueller et al. 2001, Porazinska *et al.* 2004). Due to clear diversification of chemical conditions of the water in the micro-vertical arrangement (present study), it seems that a similar differentiation should be expected in case of ciliates. There are hardly no data concerning the vertical micro-distribution of protozoa in water column in the cryoconite holes. These comparisons can provide insights into the ecology of ciliates. So far, researchers have only compared surface and bottom waters in cryoconite holes in respect to species diversity and bacterial abundance in these micro-habitats (Anesio *et al.* 2009). On the other hand, the studies performed by Säwström *et al.* (2007) on ciliate groups in Svalbard glacier cryoconites support a small number of species and a clear domination of haptorid ciliate genus *Monodinium* and oligotrichs *Halteria* spp. and *Strombidium* spp. However, there are still no studies comparing protozoan community composition and the factors conditioning their occurrence in cryoconite hole surface and bottom water micro-habitats. To address these knowledge gaps, we evaluated the following hypotheses: (i) species richness and the abundance of ciliates reveal a distinct vertical microzonation; (ii) the diversification of physical and chemical parameters of surface and bottom waters may have influence on the abundance and the trophic structure of protozoan communities.

Study area

Samples were collected from Ecology Glacier located on the King George Island (South Shetland Islands, West Antarctic) (Fig. 1). Climate of this island can be characterized by a rapid succession of eastward moving low pressure systems, which transport relatively warm, humid air towards the coast of Antarctica (Bintanja 1995; Birkenmajer 2002). This is the cause of the relatively high annual mean temperature (2.0°C) and humidity (82%) at *Arctowski* Station, situated on the south-eastern side of the island (Martianov and Rakusa-Suszczewski 1989).

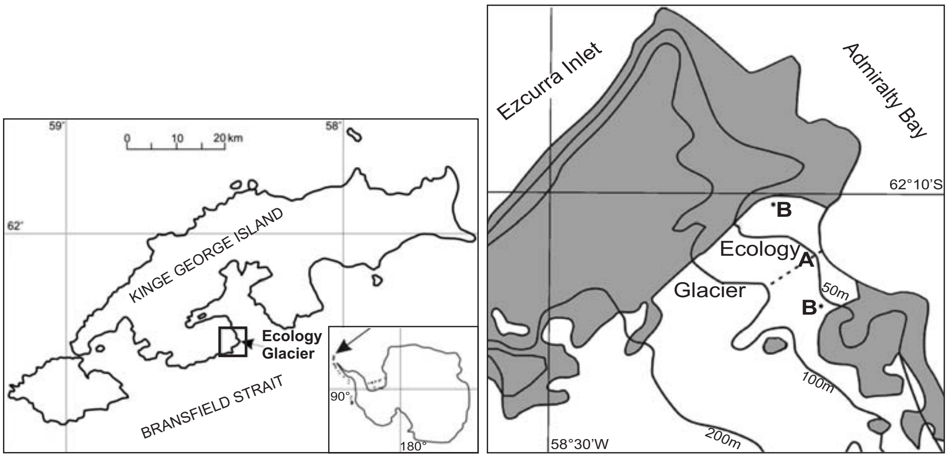

Fig. 1. Location of study area and sampling sites on Ecology Glacier (Admiralty Bay): A, transect with 7 sites; B, sites outside the transect.

During summer, the mean temperature is well above zero. Precipitation varies from 500 mm yr^{-1} at sea-level to approximately 2000 mm yr^{-1} at the summit of the island (Martianov and Rakusa-Suszczewski 1989). The Ecology Glacier drains part of the Warszawa Icefield. During the past decade a rapid retreat of these valley-type tidewater glaciers has been observed. It has been under continuous recession since at least 1956/1957. From 1957 to 1989 it retreated at a rate of 4–4.5 m per year. The retreat rapidly accelerated in the past decade (1989–1999), reaching a rate of up to 30 m per year (Gryziak 2009). Microbial communities were sampled from 62°10.404'S, 58°28.546'W, 85–145 m a.s.l. to 62°10.226'S, 58°28.268'W, 40 m a.s.l. Samples were taken from a zone in the mid-parts of the ablation area.

Material and methods

Protozoan communities were examined in cryoconite holes located on Ecology Glacier (Fig. 1). Sampling was done from 17 January to 24 February 2012. Samples were collected from nine sites. A transect of sampling sites was established along the ablation zone of the glacier (seven cryoconite holes, Fig. 1A), and two additional sites were located in the medial edges of the glacier, approximately half way along the transect (two cryoconite holes, Fig. 1B). In each cryoconite hole two samples were taken from surface water – SW (0–5 cm thick/adjacent water layer) and bottom water – BW (water layer at the bottom surface and/or water between bottom sediments) (Fig. 2). Water and water with sediments was sampled using a plastic pipette (length 15 cm, Ø 50 mm). Three subsamples, about 10 ml each, were poolled into a calibrated vessel to form a composite sample (30 ml) of water or water/sediment. 20 ml of this sample was preserved immediately with Lugol's solution (0.2% final concentration), settled in glass column for over 24 h in the laboratory, and then con-

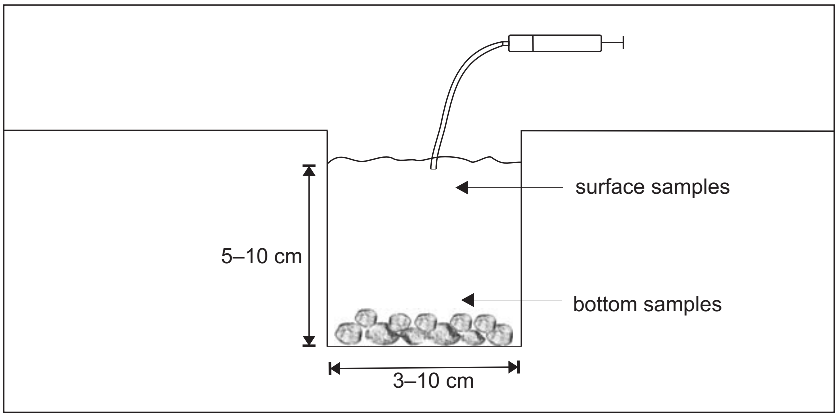

Fig. 2. Scheme of the sampling points in cryoconite hole.

centrated to 10 ml by sedimentation. Ciliates from 0.1 ml of the concentrated sample were counted using a microscope at 400–1000 × magnification. The abundance of microorganisms was calculated in 1 ml of water. The species were determined by means of the following methods: intravital – colouring vacuoles with indifferent red (which can colour the macronucleus) and micro- and macro-nucleus with malachite green (Lee *et al.* 1985); Fernandezo-Galiano method – colouring the cell structures in ammoniacal solution (kinetosomes, micro- and macronucleus) (Fernandezo-Galiano 1994). Trophic identification was done using the method by Foissner and Berger (1996). Ciliate biomass was estimated by multiplying the numerical abundance by the mean cell volume calculated from direct volume measurements using appropriate geometric formulas (Finlay 1982).

Water samples for chemical analyses were taken simultaneously with microbial samples. Temperature, oxygen, pH and conductivity were determined *in situ* using CX-461 multiparameter water quality sonde (Elmetron, Poland), and the remaining variables (N_{tot}, P_{tot}, N-NH_4, P-PO_4) were analysed in the laboratory using a VEGA 400 spectrophotometer equipped with a TR320 thermoreactor Spectroquant (Merck, Germany).

One-way analysis of variance (ANOVA) was used to compare mean abundances and species richness of ciliates between surface water and bottom water of cryoconite. The analysis was performed using STATISTICA 7.0 software.

Ordination techniques were used to describe the relationships between the abundance of ciliates in cryoconite hole surface and bottom water and environmental variables. The length of the gradient indicated by a detrended correspondence analysis of ciliate abundance was 2.08 SD, which suggests that principal component analysis (PCA) and redundancy analysis (RDA) are appropriate method (Ter Braak 1992). PCA was performed in order to specify separation between surface water and bottom water habitats. RDA was used to recognize the most important environmental variables which determine abundances of ciliates in surface water and bottom water of cryoconite. The ordination analyses were performed by means of CANOCO 4.5 for Windows. Diversity analysis applying Shannon-Wiener index was performed using the Multivariate Statistical Package – MVSP.

Results

Physical and chemical parameters, diversity and abundance. — Water temperature among sites and samples ranged from 0 to -0.3±0.2°C. The pH ranged from 8.3 to 8.6±1.3, dissolved oxygen from 8.80 to 11.2 mg l^{-1}, and conductivity fluctuated between 0.29 and 3.4±0.4 µS cm^{-1}. Nutrients level in the water of cryoconites were low, with N_{tot} ranging from 0.9 to 2.0±0.3 mg N l^{-1}, N-NH_4 from 0.045 to 0.125±0.022 mg N l^{-1}, P_{tot} from 0.01 to 0.25±0.01 mg P l^{-1} and P-PO_4 between 0.08 and 0.38±0.04 mg PO_4 l^{-1}. Micro-vertical differences were noted in the conductivity and concentrations of nutrients. Conductivity ranged from 0.30 µS cm^{-1} in surface water to 2.9–3.4 µS.cm^{-1} in bottom water. Nutrients reached the highest values in the bottom water (N_{tot} > 1.0 mg N l^{-1}, N-NH_4 from 0.098 to 0.125 mg N l^{-1}, P_{tot} from 0.22 to 0.25 mg P $l^{-1}$and P-PO_4 between 0.28 and 0.38 mg PO_4 l^{-1}), which were considerably lower in surface water with N_{tot} ranging to 0.9 mg N l^{-1}, N-NH_4 from 0.045 to 0.056 mg N l^{-1}, P_{tot} from 0.01 to 0.15 mg P l^{-1} and P-PO_4 between 0.08 and 0.16 mg PO_4 l^{-1}.

The highest numbers of ciliates taxa occurred in the bottom water (16 taxa) and were much lower in surface water where only five ciliate taxa were identified. Accordingly the diversity analysis revealed a mean Shannon-Wiener diversity index (H) of 2.35. The highest diversity was measured in bottom water ($H = 3.2$), and the lowest diversity was observed in surface water ($H = 1.2$). The most frequent taxa in surface water were *Halteria* sp. and *Prorodon* sp. Three taxa had frequencies < 5%. In bottom water the most frequent were *Prorodon* sp., *Holosticha pullaster*, *Stylonychia mytilus*-complex and scuticociliates (*Cinetochilum margaritaceum*) (Table 1). Mean abundances of ciliates were significantly higher (ANOVA, F = 92.97; $P < 0.001$) in bottom water (21 cells ml^{-1}) than in surface water samples (17 cells ml^{-1}) (Table 1). Similarly, significantly higher biomass (ANOVA, F = 103.95; $P < 0.001$) was observed in bottom water (1.23 µg C ml^{-1}) than in surface water (0.21 µg C ml^{-1}). Distinct micro-vertical differences were noted in the domination structure (% in total numbers of ciliates). Oligotrichs, such as *Codonella cratera* and *Halteria grandinella*, dominated in the surface water. In bottom water the community was predominantly composed of *Prorodon* sp., Hypotrichida (*e.g. Oxytricha* sp. and *Holosticha* sp.) and scuticociliates. Ciliate feeding groups consisted of bacterivores, algae-diatom feeders, mixotrophic ones, predators, and omnivores (Table 1). Algivores and mixotrophic taxa clearly dominated in surface water. In turn, sediments were dominated by bacterivorous and omnivorous ciliates, at 25–30% of the total number.

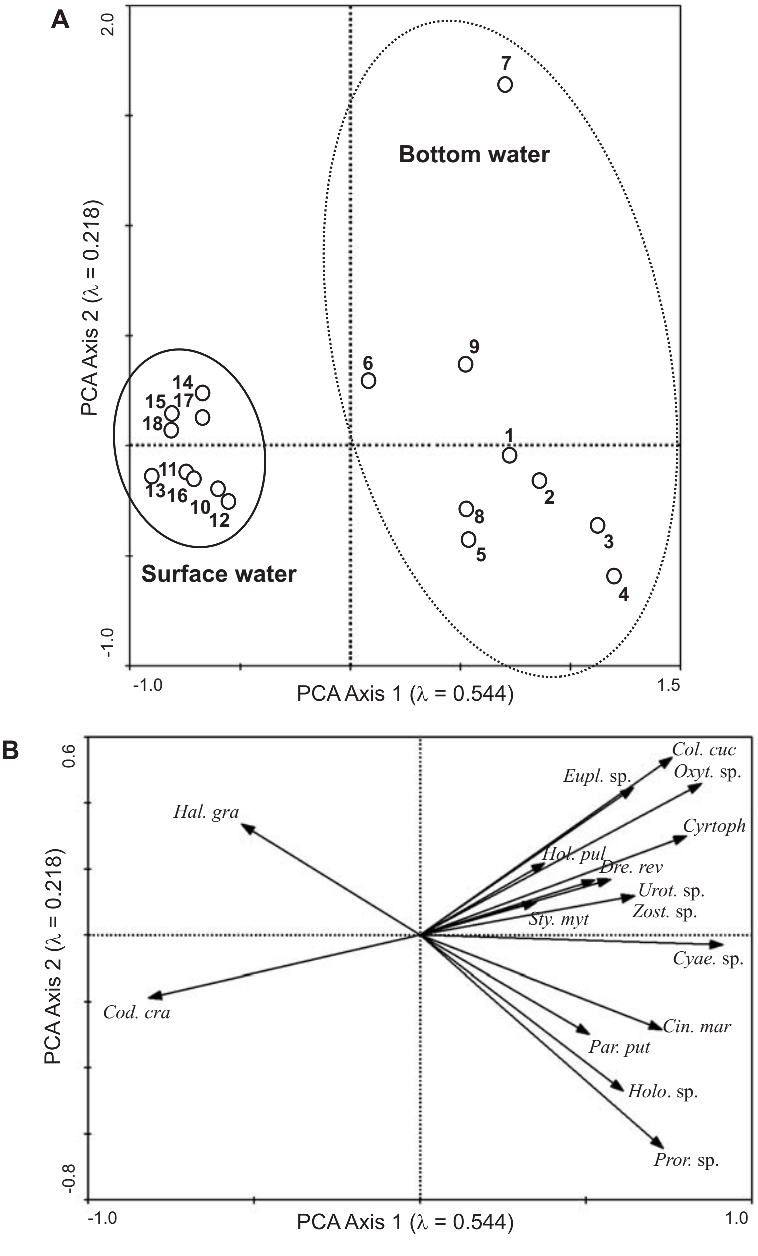

Fig. 3. Principal Components Analysis biplots for axes 1 and 2 showing: **A**, surface and bottom water of crioconite; **B**, ciliates species. Samples collected in studied zones are marked with an Arabic numeral: 1–9 bottom water; 10–18 surface water. (*Cyrtoph. – Cyrtophorida, Cin. mar – Cinetochilum margaritaceum*, *Cod. cra – Codonella cratera*, *Col. cuc – Colpoda cucullus*, *Cyae.* sp. – *Caenomorpha* spp., *Dre. rev. – Drepanomonas revolute*, *Eupl.* sp. – *Euplotes* sp., *Hal. gra – Halteria grandinella*, *Holo.* sp. – *Holophrya* sp., *Hol. pul – Holosticha pullaster*, *Oxyt.* sp. – *Oxytricha* sp., *Par. put – Paramecium putrinum*, *Pror.* sp. – *Prorodon* sp., *Sty. myt – Stylonychia mytilus*-complex, *Urot.* sp. – *Urotricha* sp., *Zost.* sp. – *Zosterodasys* sp.).

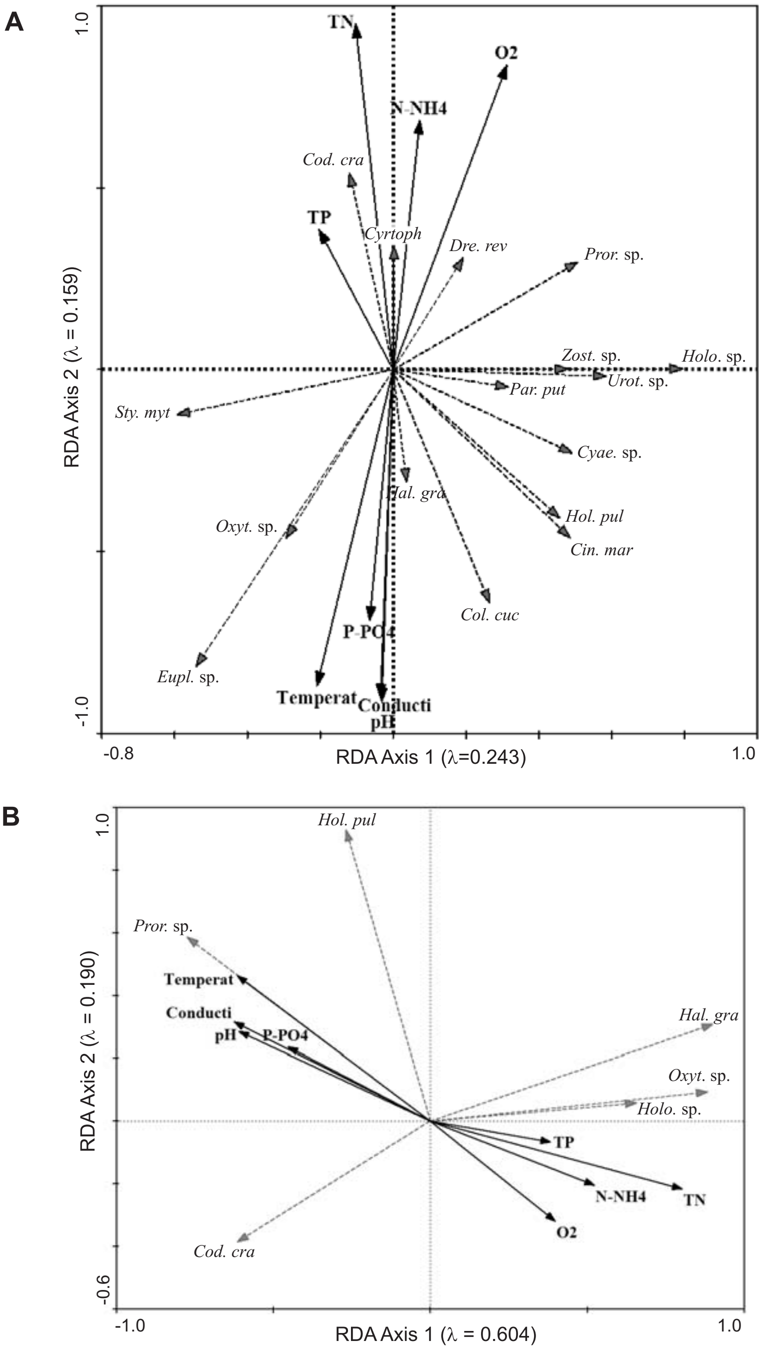

Fig. 4. Redundancy Analysis (RDA) biplots for microvertical distribution of ciliates in crioconite holes: **A**, samples collected in bottom water and environmental variables; **B**, samples collected in surface water and environmental variables. The length of arrow indicates the significance of environmental variable.

Table 1

The composition, frequency (% of samples) and abundances of ciliates found in cryoconite holes on Ecology Glacier.

Taxon	Size (µm)	Feednig groups*	Frequence (% of samples)		Mean abundance (ind.ml^{-1})	
			habitat type		habitat type	
			surface water	bottom water	surface water	bottom water
Cyrtophorida	>50	B	0	100	0	1
Cinetochilum margaritaceum (Ehrenberg, 1831)	<50	B, A	0	61	0	2
Codonella cratera (Leidy, 1877)	>50	A	45	10	6	1
Colpoda cucullus Muller, 1773	>50	O	0	100	0	2
Caenomorpha spp.	>50	B	0	100	0	3
Drepanomonas revoluta Penard, 1922	<50	B	0	30	0	1
Euplotes sp.	>50	O	0	61	0	1
Halteria grandinella (Müller, 1773)	<50	B, A	0	10	0	1
Holophrya sp.	>50	O	15	50	4	1
Holosticha pullaster (Müller, 1773)	>50	B, A	0	10	0	1
Oxytricha sp.	>50	O	10	100	2	2
Paramecium putrinum Claparede *et* Lachmann, 1859	>50	O	10	30	2	1
Prorodon sp.	>200	P	30	90	3	1
Stylonychia mytilus-complex	>200	O	0	30	0	1
Urotricha sp.	<50	B, A	0	40	0	1
Zosterodasys sp.	>200	A	0	40	0	1
Total species number: 16					5	16
Mean abundance:					17	21

* Feeding groups: A – algivores, B – bacterivores, M – mixotrophic, O – omnivores, P – predators (Foissner and Berger 1996).

Table 2

Redundancy analysis: inter-set correlation of environmental variables with axes 1 and 2 of RDA for cryoconite hole bottom and surface water.

	Bottom water		Surface water	
Variable	Environmental Axis 1	Environmental Axis 2	Environmental Axis 1	Environmental Axis 2
Temperature	-0.182	-0.709	-0.615	0.461
Conductivity	-0.030	-0.737	-0.625	0.314
pH	-0.027	-0.746	-0.609	0.284
O_2	0.270	0.684	0.398	-0.319
$N\text{-}NH_4$	0.063	0.559	0.523	-0.204
TN	-0.089	0.776	0.802	-0.216
$P\text{-}PO_4$	-0.056	-0.563	-0.453	0.235
TP	-0.176	0.314	0.384	-0.067

Table 3

Summary of RDA of ciliates and environmental variables from cryoconite hole bottom and surface water and the significance of individual variables.

Variable	Bottom water			Surface water		
	%	F	*P* value	%	F	*P* value
Temperature	24.0	0.41	0.760	25.0	1.23	0.362
Conductivity	20.0	0.24	0.834	34.0	1.57	0.190
pH	37.0	1.19	0.320	45.0	1.28	0.292
O_2	51.0	1.28	0.252	17.0	1.11	0.394
$N\text{-}NH_4$	1.0	0.22	0.740	4.0	0.04	0.102
TN	30.0	0.49	0.782	56.0	4.65	0.008
$P\text{-}PO_4$	57.0	5.63	0.006	8.0	1.54	0.340
TP	8.0	0.58	0.654	1.0	1.74	0.418

Relations between ciliates and environmental variables. — In PCA diagram the first two axes separate ciliates between surface and bottom water of cryoconite (Fig. 3A). Axis 1 (l = 0.544) and axis 2 (l = 0.218) explained 76.2% of the total variance in the ciliates data. The abundances of ciliates was most strongly correlated with the main direction of variation (Axis 1), with samples collected in bottom water (Fig. 3B). The abundance of most ciliates taxa increased toward bottom water, with exception of two ciliate species, *Halteria grandinella* and *Codonella cratera*. These two species seem to prefer surface water habitat and were rarely presented in bottom water samples (Fig. 3B). Axis 2 appeared to separate ciliate community collected in surface water from bottom water.

The direct relationships between abundance of ciliates and environmental variables were specified using redundancy analysis (RDA). The results of analysis showed differences between surface and bottom water habitats (Fig. 4). For bottom water all variables together explained 40.2% of total variance in community structure. Axis 2 showed very high correlations with most environmental variables, correlation coefficients exceeded 0.6; only $N\text{-}NH_4$, TP and $P\text{-}PO_4$ showed lower correlation values (Table 2). Temperature, conductivity, pH, O_2 and TN correlated with the abundance of ciliates in bottom water of cryoconite holes. For surface water all variables together explained 79.4% of total variance. Axis 1 showed the highest correlation with TN, conductivity, temperature and pH (Table 2). These variables are therefore associated with the abundance of ciliate species in surface water. Values of correlation coefficients between environmental variables and Axis 2 didn't exceed 0.5.

The Redundancy Analysis of individual environmental variables revealed that the proportion of ciliates data explained by each of variable and the significance varied strongly among variables and among surface and bottom water (Table 3). In the separate RDAs on bottom water and surface water samples, TN and $P\text{-}PO_4$ were significant. The highest proportion of variance in bottom water explained O_2 and $P\text{-}PO_4$ and in surface water – TN and pH.

Discussion

In total, 16 ciliate taxa were recorded in the cryoconite holes. The number of ciliate species found in the cryoconite holes during the study period was higher than those reported from glacier ecosystems by other scientists. On a glacier in the Arctic archipelago of Svalbard only four species of ciliates occurred (Säwström *et al.* 2002). The population density of ciliates in our study ranged from 14 cells ml^{-1} to 21 cells ml^{-1}. For comparison, in the cryoconite holes in a Svalbard glacier, ciliate numbers were 10 cells ml^{-1} (Säwström *et al.* 2002). However, there are no comparative data available regarding the micro-vertical diversity of these microorganisms. The increase in abundance of protozoa in the bottom water may be the result of profitable feeding conditions. The bottom water was characterized by the presence of organic and mineral materials. Such a type of environment could enhance a massive development of ciliates. The results presented show that protozoa abundance is related to nutrient concentrations. It seems that nutrients have an indirect influence on the prevalence of protozoa through the control of food abundance (mainly bacteria). Temperature is another factor apparently influencing succession of ciliates. According to Beaver and Crisman (1990) the growth and reproduction of freshwater ciliates are strongly correlated with temperature. As shown by previous research by Finlay (1980) the water temperature has additionally a significant influence on the occurrence of groups of ciliates in significantly fertile reservoirs. In the cryoconites, it was ascertained that temperature correlated with the number of ciliates.

Ciliate feeding groups consisted of bacterivores, algivores, mixotrophic, predators and omnivores. Algivorous and mixotrophic taxa prevailed in the surface water. The bacterivorous and omnivorous ciliates were more abundant in the bottom waters. This vertical micro-distribution of algivorous and bacterivorous ciliates was similar to that previously observed in shallow lakes (Jacquet *et al.* 2005). In this study, ciliates community was mainly composed of genera *Prorodon*, *Oxytricha* and *Cinetochilum*. The community composition of protozoans varied greatly with depth. In the surface water the most numerous were oligotrichs. However, the abundance of oligotrichs decreased quickly with depth. In the bottom water small-sized scuticociliates, which are known to be effective filter-feeding bacterivores, were very abundant. It has also been suggested that scuticociliates tend to concentrate at the bottom, where bacterial productivity is high. This group of ciliates presents an opportunistic bacterivorous behaviour (Foissner and Berger 1996). The species belonging to Oligotrichida have also been observed in Svalbard glacier cryoconites (Säwström *et al.* 2002). The dominance of Hypotrichida was observed in moss in Gough and Marion Islands (Foissner 1996). The domination of these orders could have resulted from wide ecological tolerance. *Oxytricha* sp. was until now reported from Europe, Israel and South Australia (Berger 1999) and thus might possibly have a cosmopolitan distribution. According to Macek *et al.* (2001), the rate of feeding on bacteria by a single Scuticociliatida individual amounts to 210 bacterial cells per hour, whereas for Oligotrichida and Colpodea individuals it ranges from 180 to 1 700 bacterial cells per hour. The relatively high density of those Ciliata suggests their crucial importance in controlling numbers of bacteria.

In conclusion, the present study showed clear vertical micro-distribution patterns of ciliates. Both the highest number of taxa, abundance and biomass of protozoans were observed in the bottom water, and the lowest values were noted in surface water. The trophic structure of protozoans changed also with depth. The surface water zone was dominated by large-sized mixotrophic and omnivorous species, while, with depth, the share of fine bacterivores increased. The results of this study suggest that the TN and P-PO_4 are more important than pH and temperature in vertical distribution of ciliates.

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