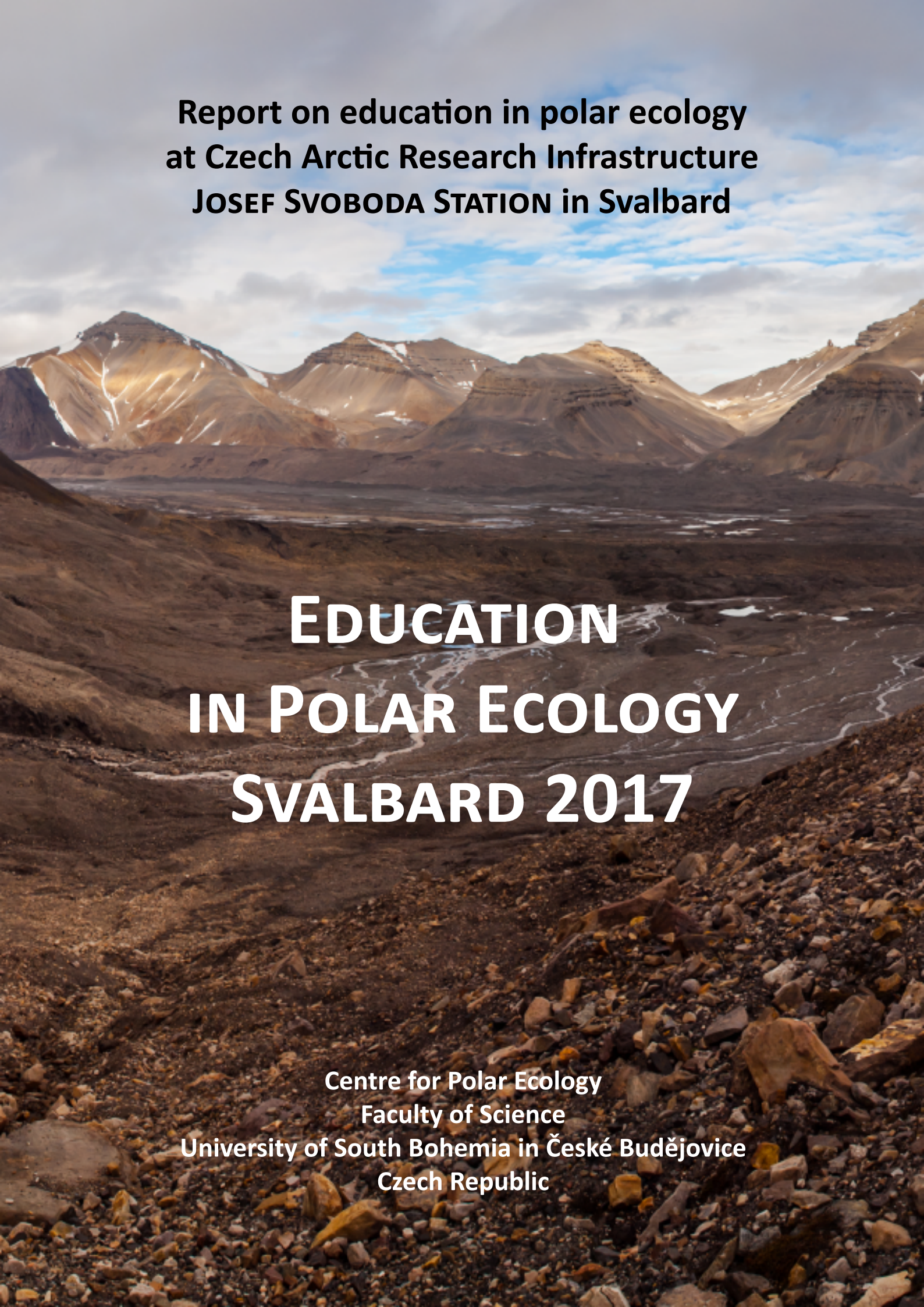
Report on education in polar ecology
at Czech Arctic Research Infrastructure
JOSEF SVOBODA STATION in Svalbard



EDUCATION IN POLAR ECOLOGY SVALBARD 2017

Centre for Polar Ecology
Faculty of Science
University of South Bohemia in České Budějovice
Czech Republic

Creating of the courses was enabled thanks to the infrastructure realized within the project of Czech Ministry of Education (MSMT) LM2015078 - CzechPolar2 - Czech Polar Research Infrastructure. The course organizers would also like to thank to the Czech Arctic Research Infrastructure "Josef Svoboda Station" (as a part of the Czech Polar Research Infrastructure, CzechPolar2) and its crew for their support.

The research reported here has been also supported by the ECOPOLARIS project No. CZ.02.1.01/0.0/0.0/16_013/0001708 provided by the Czech Ministry of Education, Youth and Sports.

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

Josef Svoboda Station
University of South Bohemia in České Budějovice
CENTRE FOR POLAR ECOLOGY

Cover photo: Martin Lulák
Editor: Jana Kvíderová
Rewieved by (in alphabetical order): Oleg Ditrich, Josef Elster & Marie Šabacká

2017

Contents

1. Introduction

In 2017, four theses focused on polar ecology were defended successfully:

Pushkareva, E.S.: Ecology and diversity of microbial phototrophs in biological soil crusts of Polar Regions. Ph.D. thesis, Faculty of Sciences, University of South Bohemia in České Budějovice, 2017.

Muchová, K.: Závislost společenstev půdních vířníků (Rotifera) na gradientu vlhkosti v polárních podmínkách [Rotifer communities on water-terrestrial gradient of central Svalbard wetlands]. MSc. Thesis, Faculty of Sciences, University of Ostrava, 2017.

Brož, M.: Střevní paraziti savců introdukovaných na Svalbard [Intestinal parasites of mammals introduced to Svalbard]. BSc. thesis, Faculty of Sciences, University of South Bohemia in České Budějovice, 2017.

Padalíková, P.: Tasemnice rejnoků *Amblyraja radiata* na Svalbardu [Tapeworms of *Amblyraja radiata* in Svalbard]. BSc. thesis, Faculty of Health and Social Sciences, University of South Bohemia in České Budějovice, 2017.

In spring, the Winter Arctic Ecology (Tab. 1.1.) was organized by the Centre for Polar Ecology, Faculty of Sciences, University of South Bohemia, together with the University Center of Svalbard (UNIS). The course took place in Longyearbyen from February 22 to March 31, 2017 (Fig. 1.1.).

Tab. 1.1. The Czech instructors and students (in alphabetical order) of the Winter Ecology Course according to their specialization. Refer to Tab. 1.2. for abbreviations explanations.

Instuctors		Students	
Josef Elster	JU+IBOT	Eva Hejduková	UK
Marie Šabacká	JU	Matouš Jimel	UK
		Anna Polášková	JU
		Petra Polická	JU

Fig. 1.1. In the field during Winter Ecology Course2017.

The 7th Polar Ecology course was organized by the Centre for Polar Ecology, Faculty of Science, University of South Bohemia in České Budějovice. The course itself consists of one month intensive the field work during the summer season and short theoretic preparation. Students were divided into two groups according to their specialization (Fig. 1.2.).

For more information, please visit polar.prf.jcu.cz.

Tab. 1.2. The instructors and students (in alphabetical order) of the Polar Ecology Course according to their specialization.

Group	Instuctors		Students	
MICRO	Josef Elster	JU+IBOT	Matouš Jimel	UK
	Jana Kvíderová	JU	Tereza Šamšulová	UK
			Claude-Eric Souquieres	JU
			Thomas Stehrer	JU
ZOO	Oleg Ditrich	JU	Oldřich Daněk	UVPS
			Margeritha Lucadello	JU + UAg
			Karel Raška	DC
			Magdalena Raška	DC
			Kamila Weissová	UK

Abbreviations:
Groups: MICRO - microbiology/phycology; ZOO - zoology/parasitology.
Affiliations: DC – Dartmouth College, Hanover (US); IBOT – Institute of Botany AS CR, Třeboň; JU – University of South Bohemia, České Budějovice; UAg – University of Algarve, Faro (PT); UK – Charles University, Prague; UVPS - University of Veterinary and Pharmaceutical Sciences Brno, Brno.

Fig. 1.2. Sampling during Polar Ecology course in summer 2017.

2. Winter Arctic Ecology course

Instructors: ***Josef Elster & Marie Šabacká***
Students: ***Eva Hejduková, Matouš Jimel, Anna Polášková & Petra Polická***

Students were given a basic training in safety procedures required in the early spring conditions, which included use of skis and their maintenance, navigation (traditional and GPS based), emergency camp setup (emergency equipment), radio communication, avoiding of avalanche danger and use of avalanche transmitters and polar bear safety measures (safe handling of firearms, use of signal flares, target shooting and camp guards).

Students of the Winter Arctic Ecology course participated at lectures, seminars and field trips at the UNIS. During the course, they worked at five different projects

- germination capability of frozen plants
- measurements of respiration of frozen plants
- evaluation of reindeer food quality
- reindeer population structure study
- viability of microalgae during winter

The Czech group focused on viability of microalgae during winter. Samples of frozenalgal mats were taken from several localities (Fig. 2.1.). This limited amount of material was subsequently used to measure cell viability and recovery in laboratory (Fig. 2.2.). The viability of cells was evaluated by fluorescence staining (DAPI, CTC and SYTOX Green, Figs. 2.3. and 2.4.) and by variable chlorophyll fluorescence measurements (Figs. 2.5.).

The results were presented at the Arctic Science Summit Week 2017 conference (Fig. 2.5.).

Fig. 2.1. Microscopic survey of glacier surface for snow algae presence. Author: Josef Elster.

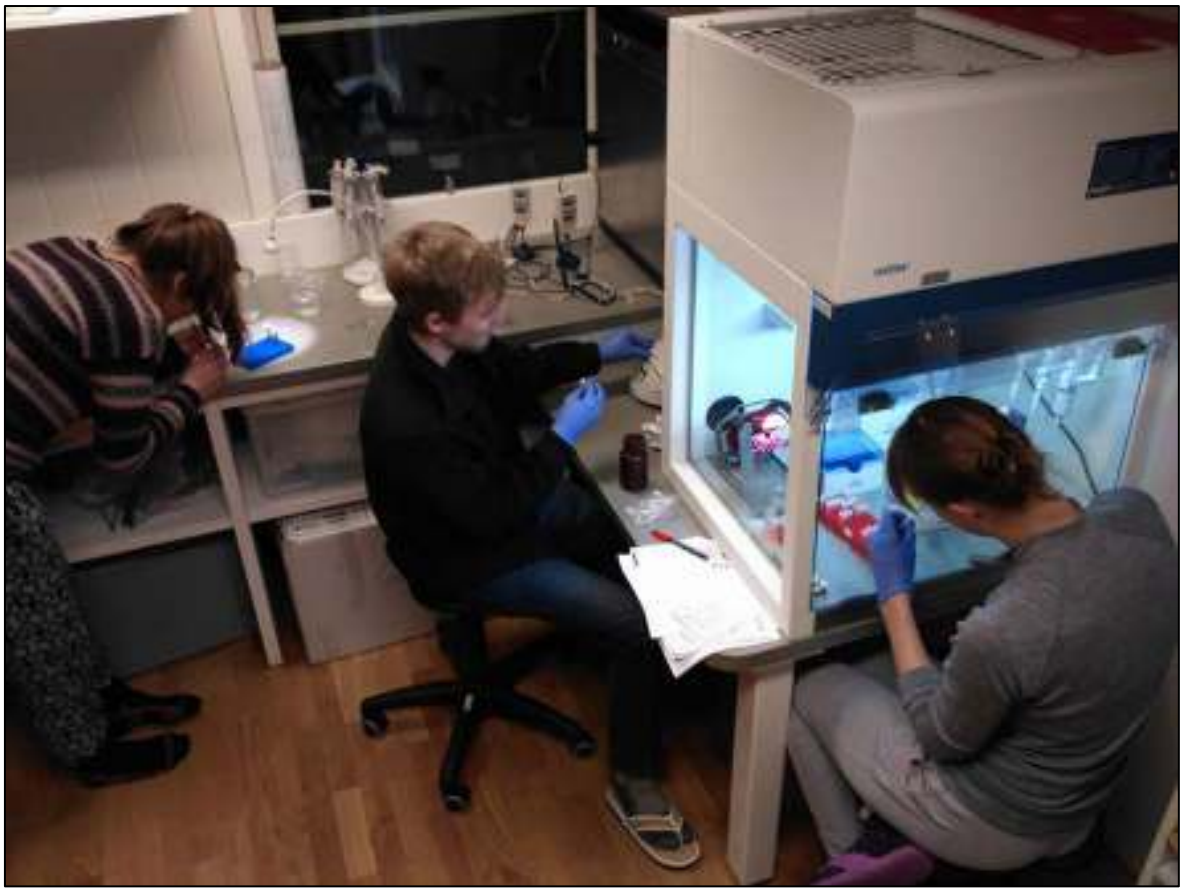


Fig. 2.2. Laboratory work during the course.

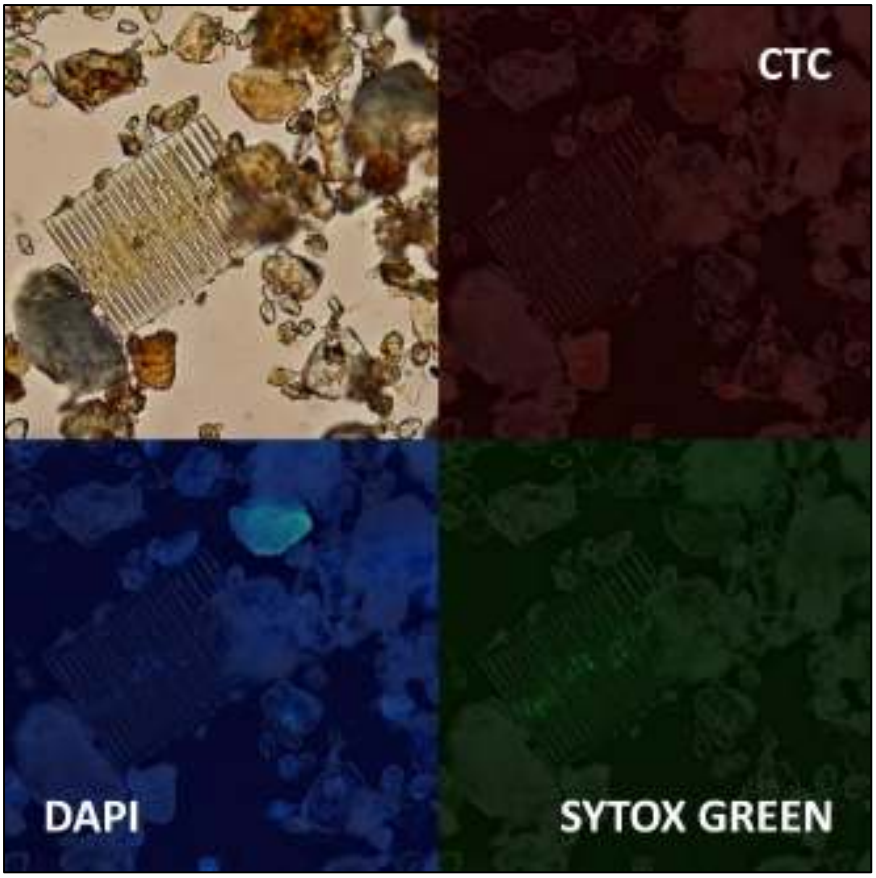


Fig. 2.3. The vitality fluorescence staining of diatom *Fragillaria* sp. DAPI for DNA , CTC for respiration and SYTOX Green for membrane integrity.

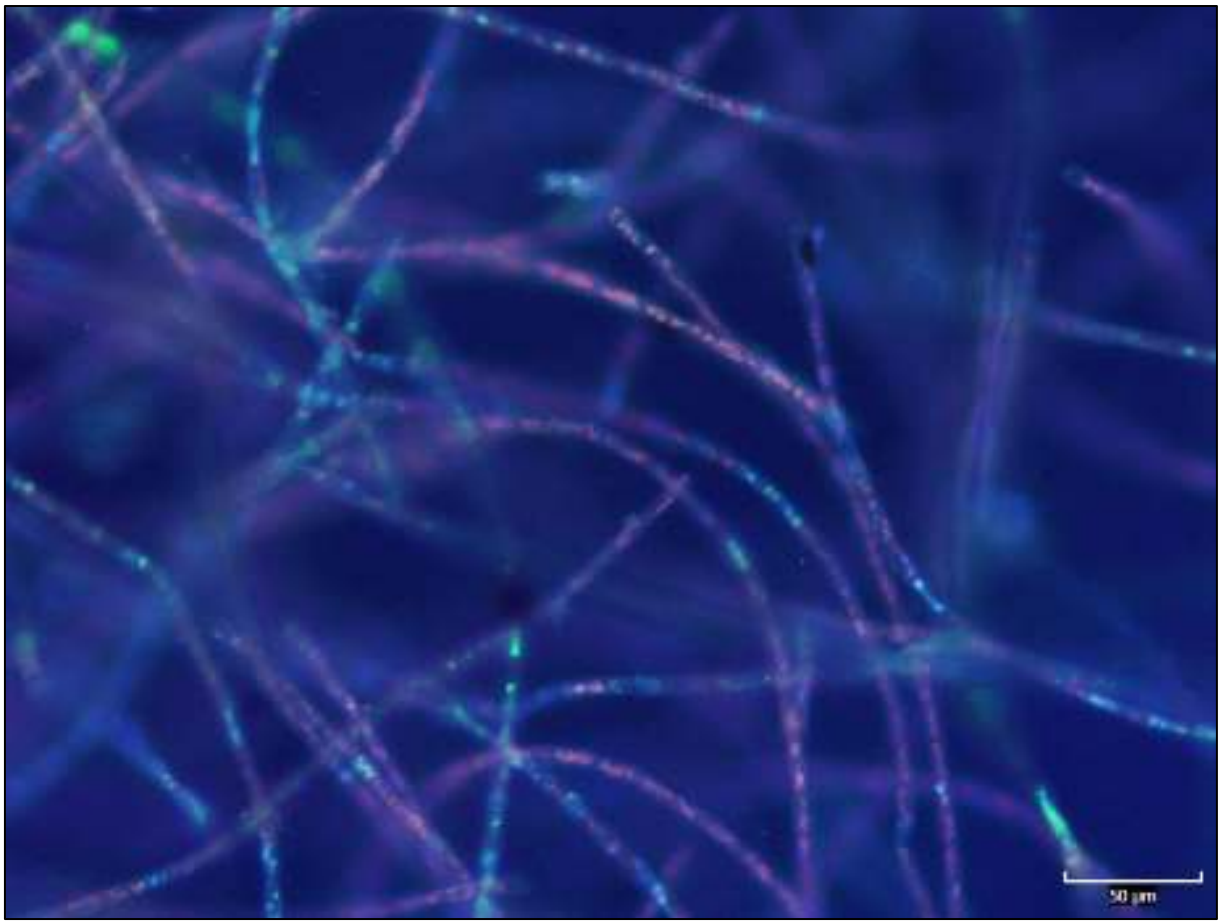


Fig. 2.3. Viability of *Tribonema* sp.Red – living cells, green – dead cells.

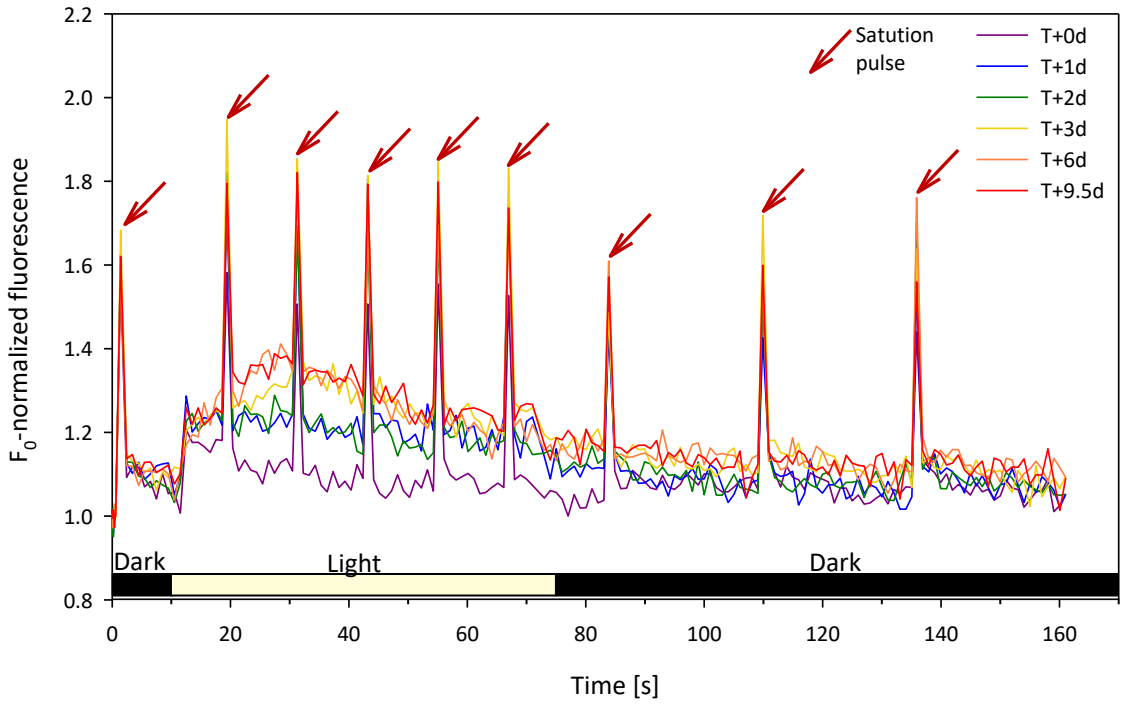


Fig. 2.4. Recovery of the photosynthetic activity in microbial mat.

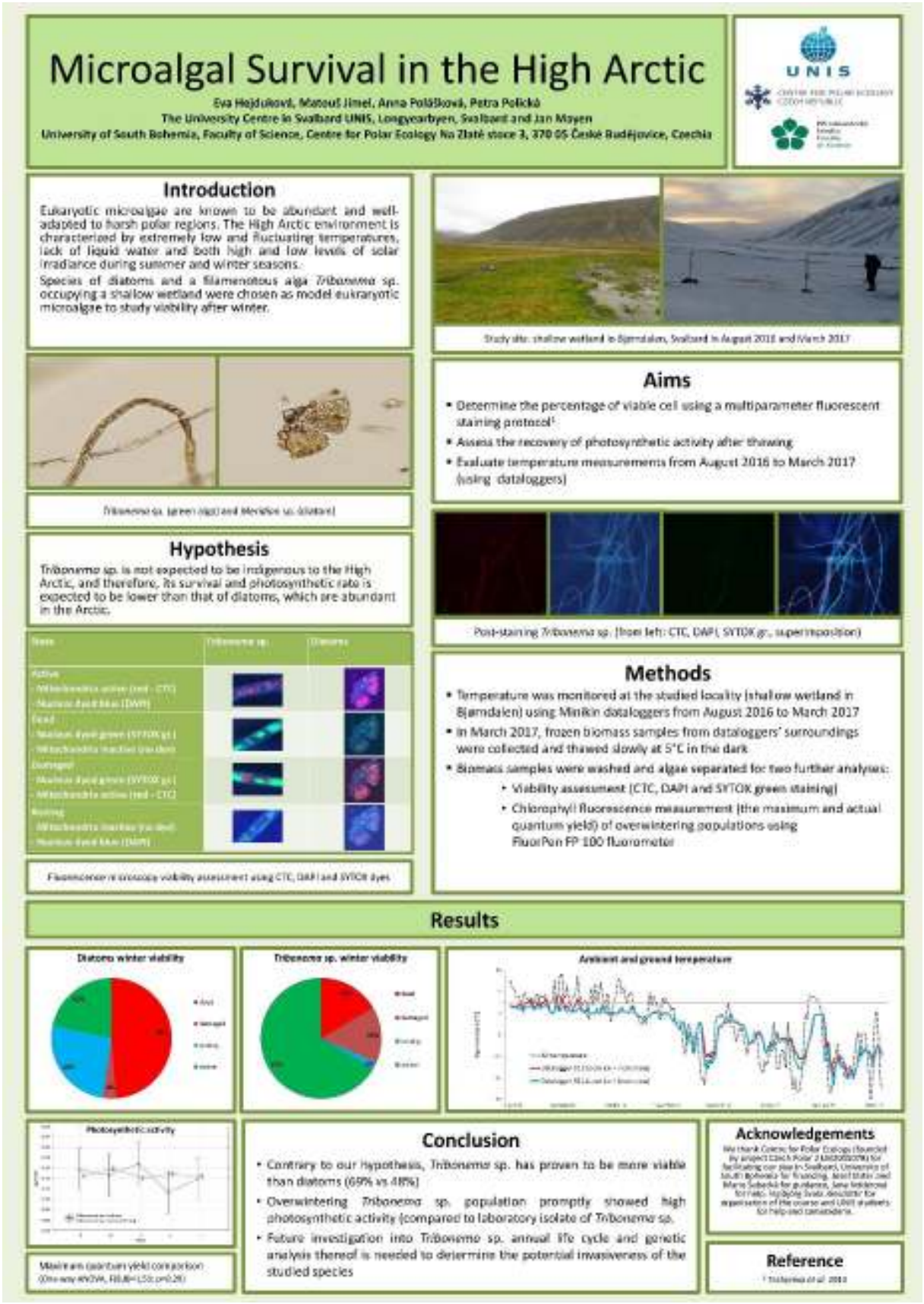


Fig. 2.5. Poster presented at the Arctic Science Summit Week 2017.

3. Polar Ecology course

Under the Faculty of Science at the University of South Bohemia (USB) in České Budějovice, The Centre of Polar Ecology (CPE) of Czech Republic is promoting the study of polar ecological sciences trough researches and educational activities. Since 2012 the CPE is honoured to represent the scientific community of Czech Republic at the International Arctic Science Committee (IASC) with the Josef Svoboda research infrastructure present in Svalbard (Norway). During August 2017 the Centre of Polar Ecology run the seasonal course of Polar Ecology. The Course of Polar Ecology it consists of two different parts, a theoretical one in which lectures about polar environments are presented, and a field work of nearly 10 days at the stations in Svalbard. During 2017 both the parts of the course were held in Svalbard under the supervision of doc. Ing. Josef Elster, CSc., head of CPE (microbiology), and doc. RNDr. Oleg Ditrich, CSc., Deputy head of CPE (zoology). The JOSEF SVOBODA STATION in Svalbard it is composed by 3 stations located in different part of Spitsbergen, the biggest island of the Svalbard's archipelago. These are: the research station in Longyearbyen (JULIUS PAYER HOUSE), the research station in Petunia Bay (NOSTOC Station) and the research motorsailer (RV CLIONE) (Fig. 3.1.).

The theoretical part of the course occurred at the Julius Payer Station. During the lectures, the origin, evolution, and ecology of polar habitats were enlightened. Arctic ecosystems were treated according to climatology, geology, geomorphology, hydrology, limnology, microbiology, phycology, botany, plant ecophysiology, zoology, oceanography and parasitology. These lectures prepared the students attending the course to work on the field consciously. During the Polar Ecology Course 2017 the class was followed by a group of 9 international student coming from Czech Republic, France, Italy and USA all with different background studies (Fig. 3.2.).

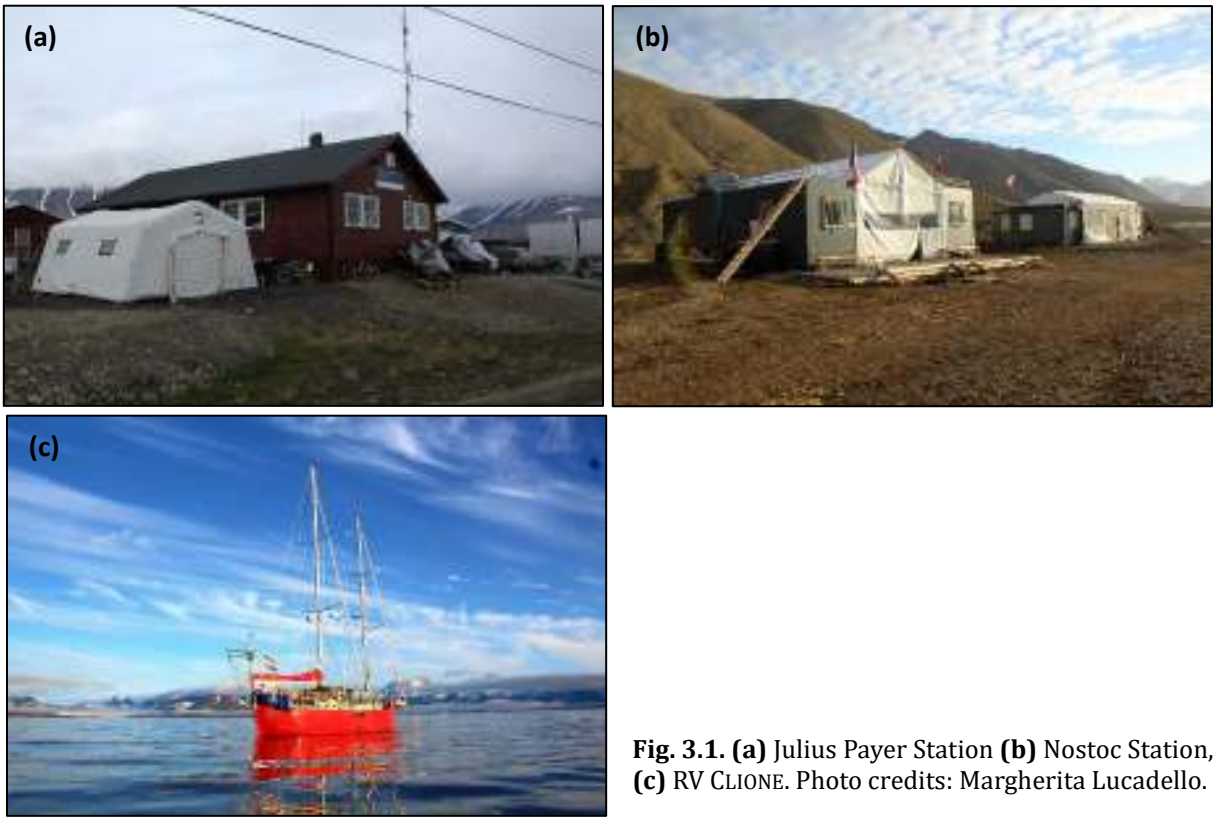


Fig. 3.1. (a) Julius Payer Station **(b)** Nostoc Station, **(c)** RV CLIONE. Photo credits: Margherita Lucadello.

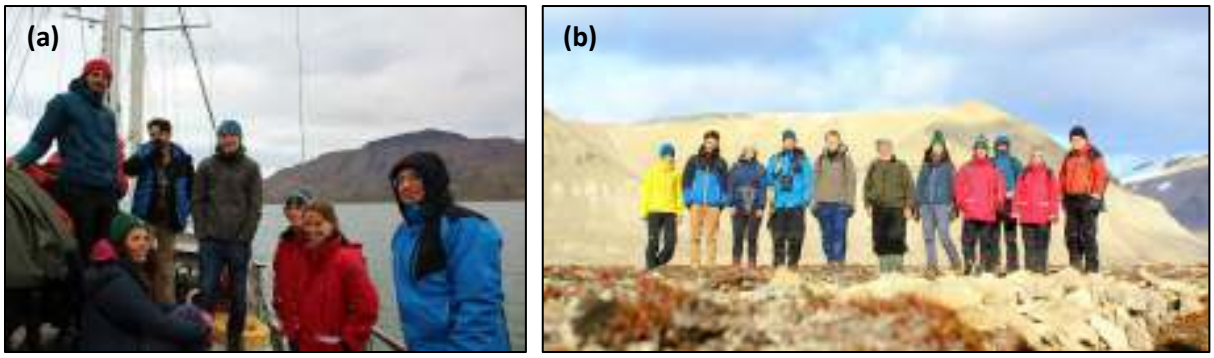


Fig. 3.2. Part of the Student attending the Polar Ecology course 2017 **(a)** on RV CLIONE and **(b)** all the students and teachers in the field. Photo credits: Margherita Lucadello.

The field trip days occurred in NOSTOC station. The station was reached by the students on board of the RV CLIONE from Longyearbyen's harbour. At the station the student followed a shooting course to being able to move safety in the area and being able to carry and use the rifles and the fleer guns to be protected against polar bear. A course explaining how to use the radios and satellite phones was also run, important especially for whom of the student were going far from the station on board of Zodiacs or on glaciers. Lastly, all the participants learnt how to properly behave on a Zodiac and how to properly wear the safety jacket to be protected by the cold water. All the courses were taught by Martin Lulak, the base manager of NOSTOC station.

The field work required from the Polar Ecology Course was different for every student (Fig. 3.3.), depending on the chosen topic:

- Thomas Stehrer: *Heavy metal and PAH contamination assessment in soil around Longyearbyen*
- Tereza Šamšulová & Matouš Jimel: *Biodiversity assessment of fresh-water ecosystems from the Polar Regions*
- Claude-Eric Souquieres: *Ecological characterization of a* Vaucheria sp. *dominated hyperborean microphytobenthos*
- Margherita Lucadello: *Metazoan fauna in cryoconite holes of Svalbard, genetical and ecological features*
- Ondřej Daněk, Karel Raška & Magdalena Raška: *Gastrointestinal nematodes of thorny skate (Amblyraj radiata)*
- Kamila Weissová: *Effect of polar day on melatonin level and clock gene expression among polar researchers*

The students presented their data at seminar at the Centre for Polar Ecology in České Budějovice on December 20, 2017.

Margherita Lucadello

Fig. 3.3. Students of the field on **(a)** river, **(b)** glacier and **(c)** lake. Photo credits: Margherita Lucadello.

3.1. Microbiology and Phycology

Instructors: ***Josef Elster & Jana Kvíderová***

Students: ***Matouš Jimel, Tereza Šamšulová, Claude-Eric Souquieres & Thomas Stehrer***

3.1.1. Heavy Metal and PAH Contamination Assessment in Soil around Longyearbyen

Thomas Stehrer, Anna Polášková

This project was realized as a cooperation of the Johannes Kepler University in Linz, Austria and the Centre for Polar Ecology. The aim was to primarily assess the contamination with certain heavy metals and EPA (Environmental Protection Agency) polycyclic aromatic hydrocarbons (PAH) around Longyearbyen. Supposed anthropogenic impact, for instance mining or industry, was a main criterion for selection of the sampling sites.

In total 15 samples comprised of 10 subsamples were collected from a depth of 5 to 10 centimetres using a stainless-steel shovel. and polyethylene plastic bags. The locations of all sites are shown in Fig. 3.1.1. The samples were sieved through a 2 mm sieve and stored in the freezer. At the Centre for polar ecology in České Budějovice preliminary parameters, namely pH, dry mass, and total organic matter were analysed. At the laboratories for analytical chemistry and chemical technology of inorganic substances in Linz the PAH and heavy metal concentrations were analysed respectively. GC-MS with a preceding microwave extraction in an acetone/hexane mixture was performed to quantitatively assess the concentration of 16 different polycyclic aromatic hydrocarbons. The second part consisted of the analysis of the metals Mn, Zn, Ni, Co, Cu, Cd and Pb with ICP-OES (inductive coupled plasma – optical emission spectroscopy). To prepare the samples for the measurement 1 g per sample was digested with hot aqua regia.

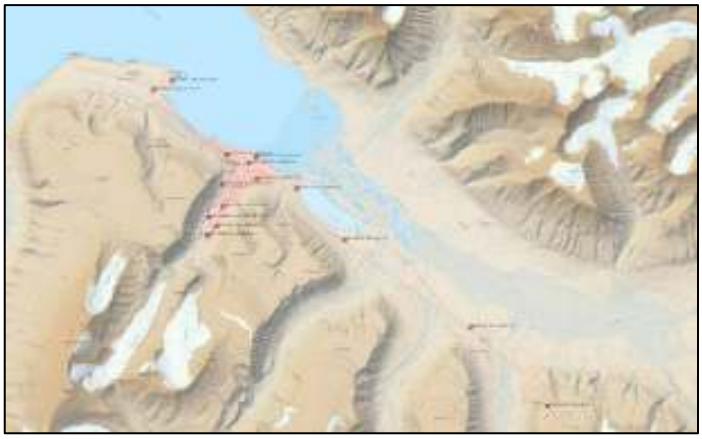


Fig. 3.1.1. Sampling sites in and around Longyearbyen. Map source: toposvalbard.npolar.no

The results of the heavy metal assessment (Table 3.1.1) were compared to two studies analysing metal concentrations around Pyramiden and Longyearbyen respectively. Compared to Krajcarova (2016)[1], comprising of samples from Pyramiden, the assessed concentrations are generally lower. Halbach (2017) analysed samples around Longyearbyen but in general further away from industry/civilization then we did and therefore on average we obtained slightly higher concentrations proposing a local anthropogenic impact (Table 3.1.2.). Comparisons to world soil average concentrations are shown in Fig. 3.1.2. The results of the analysis of the organic pollutants are summarized in Table 3.1.3.

References

[1]Krajcarova L, Novotny K, Chattova B, Elster J (2016) Elemental analysis of soil and *Salix polaris* in the town of Pyramiden and its surroundings (Svalbard). *Environ Sci Pollut Res* 23: 10124–10137

[2]Halbach K, Mikkelsen O, Berg T, Steinnes E (2017) The presence of mercury and other trace metals in surface soils in the Norwegian Artic. *Chemosphere* 188: 567-574

[3]Kabata-Pendias A (2011) Trace Elements in Soils and Plants, Fourth Edition. Taylor and Francis Group, LLCn

Table 3.1.1. Heavy metal concentrations in mg/kg of soil sample. When no value is stated the concentration was under the detection limit of 80 µg/kg.

	Mn [mg/kg]	Co [mg/kg]	Ni [mg/kg]	Cu [mg/kg]	Zn [mg/kg]	Cd [mg/kg]	Pb [mg/kg]
S 1	560.3	12.9	27.3	12.6	53.2	-	8.1
S 2	222.4	8.3	17.1	15	56	-	9.2
S 3	161.1	6.3	10.6	35.6	58.6	-	13.4
S 4	250.8	9.4	17.5	15.6	64.7	-	16.6
S 5	342	10	17.7	19	67.8	-	15.1
S 6	303.1	10.5	17.2	18.4	84.8	-	15.9
S 7	386.3	10.3	19.6	84.4	224.6	0.268	40
S 8	267.7	7.6	20.5	194.4	1014.1	12.1	32.6
S 9	213.7	9	15.1	12.9	55.8	-	10.5
S 10	211.1	7.4	8.6	7.3	39.1	-	8.3
S 11	261.2	8.6	16.4	14.1	57.1	-	10.5
S 12	166.7	8.5	16.3	14.9	57.8	-	12.6
S 13	313.5	10.4	17.1	16.6	62.8	-	13.2
S 14	219.9	8.1	18.7	23.1	66.6	-	13.8
S 15	276.2	9.7	18.1	15.6	61.8	-	15

Table 3.1.2. Comparison to Krajcarova (2016)[1] and Halbach (2017)[2]. Values in mg/kg. For the calculation of the mean values samples 1 and 8 were not considered due to their outliers in manganese respectively copper, zinc and cadmium concentrations.

	Mn	Co	Ni	Cu	Zn	Cd	Pb
Mean	256	8.9	16.2	22.5	73.7	0.02	14.9
Median (Krajcarova 2016)[1]	600	13.8	36.4	37.3	80	6.04	10.6
Mean (Halbach 2017)[2]	239	-	12	10.2	66	0.44	11.9

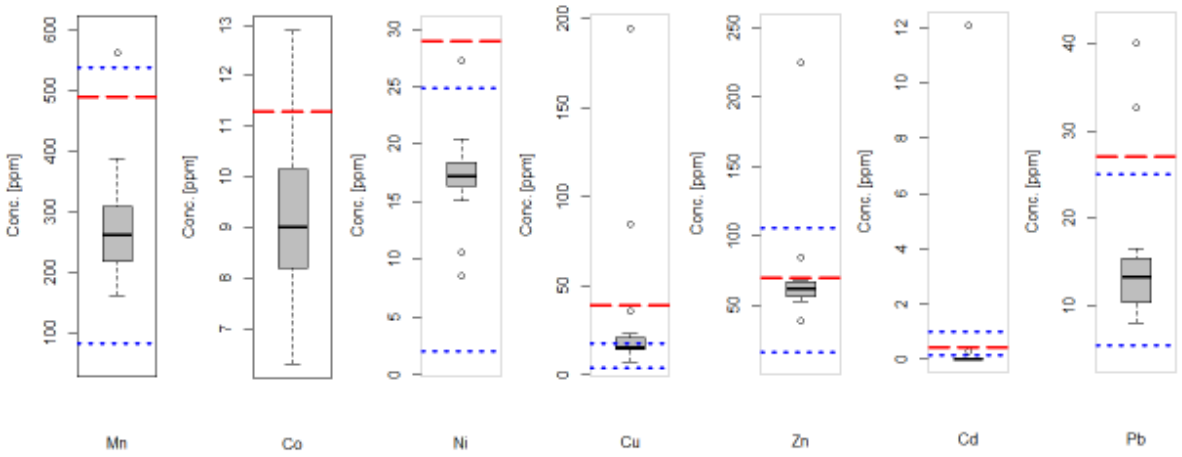


Fig. 3.1.2. Measured concentrations in ppm (mg/kg) compared to world soil average from Kabata-Pendias (2011)[3] (red dashed line) and minimum and maximum values from Halbach 2017 (blue dotted lines)

.

Table 3.1.3. Results of PAH analysis in ppb (µg/kg)

	Naphtha-lene	Acena-Phthy-lene	Acena-phthene	fluorene	Phenan-trene	Anthra-cene	fluor-anthene	pyrene	benzo (a) anthra-cene	chrysene	benzo (b + k) fluoran-thene	benzo (a) pyrene	indeno (1,2,3-c,d) pyrene	dibenzo (a,h) anthracene	Benzo (g,h,i) perylene
S 1	599.8	x	x	52.3	714.7	17.7	53.4	79.8	70.1	199.6	161.2	64.6	47.2	40.2	138.8
S 2	792.6	x	x	36.0	866.6	16.7	62.6	89.4	84.5	227.1	213.8	83.5	67.0	54.1	196.4
S 3	852.9	x	x	37.6	1022.9	29.8	122.3	141.4	98.9	254.3	215.0	50.5	60.0	44.7	145.8
S 4	2329.3	x	x	181.7	2499.4	96.3	178.3	242.3	249.5	454.3	358.2	200.2	99.8	62.9	215.4
S 5	545.6	x	x	13.0	432.0	6.1	31.7	50.9	41.7	100.9	94.5	25.8	28.6	19.9	116.5
S 6	729.3	x	x	55.1	640.0	10.7	41.2	62.8	55.4	118.7	103.6	36.6	33.2	26.6	128.4
S 7	1094.4	x	x	50.3	775.9	17.2	79.7	101.1	66.9	130.8	123.4	46.1	37.2	23.1	130.1
S 8	5141.2	x	x	469.9	4120.3	178.1	244.5	395.9	395.7	526.1	338.9	229.5	75.6	63.1	214.3
S 9	1858.5	x	x	156.5	1640.1	40.3	107.4	175.5	155.6	340.6	297.2	97.1	73.4	68.1	234.7
S 10	45.5	x	x	3.9	140.8	1.7	7.7	15.4	10.7	46.1	46.2	9.4	9.0	9.3	44.6
S 11	395.4	x	x	12.9	408.8	5.6	32.8	48.1	39.0	141.8	125.2	37.3	35.9	32.1	132.1
S 12	911.0	x	x	36.7	1438.9	26.0	221.0	219.8	146.7	388.9	358.9	124.0	144.3	66.9	258.5
S 13	401.5	x	x	13.9	470.8	6.2	36.1	52.9	41.3	160.8	150.5	43.6	35.7	32.9	121.3
S 14	988.1	x	x	50.9	1308.0	31.1	96.1	113.6	109.5	292.4	243.3	79.5	66.2	50.2	161.5
S 15	1033.4	x	x	57.2	1208.0	26.7	87.4	114.1	99.6	270.4	225.2	71.6	50.6	37.4	116.5

3.1.2. Biodiversity assessment of fresh-water ecosystems from the Polar Regions

Tereza Šamšulová & Matouš Jimel

Biodiversity in and around Billefjorden, Svalbard

The archipelago of Svalbard is located in the High Arctic, which is known for its extreme abiotic factors such as high range of temperature fluctuations, low amount or total absence of liquid water for extended periods of time and periods of both light overabundance and underabundance. The understanding of the effects of these factors is crucial for determining where life can be found and ultimately for determining the abundance of different species and genera. Our focus was, however, limited to mostly hydro-terrestrial microbial organisms and occasionally non-vascular plants.

As the distant areas that are not disturbed by frequent human activities are hard to reach, little research concerning local microbial biodiversity has been done. The facilities of the University of South Bohemia enable the research of that nature in great depth.

This research has been done as a part of the Polar Ecology Course taught by the University of South Bohemia. The lead instructor and supervisor for our microbiology specialization group was doc. Ing. Josef Elster, CSc., and its members were Thomas Stehrer (University of South Bohemia), Margherita Lucadello and Claude-Eric Souquieres (University of Algarve), Tereza Šamšulová and Matouš Jimel (Charles University).

The expedition that took place in August 2017 included a thorough evaluation of biodiversity in the surroundings of Czech research station "Nostoc" in Petuniabukta. For extended investigation of local microbial biodiversity, many trips were made around the coastal areas of Billefjorden to investigate the biodiversity of all the indigenous habitats.

42 samples in total were extracted from 24 localities (Fig. 3.1.3.) encompassing all of the habitats and kept under constant temperature and light conditions upon which they were examined using light microscopy. All unique separate cells and communities thereof had their microscopic pictures taken and were subsequently classified into their respective classes, genera and species.

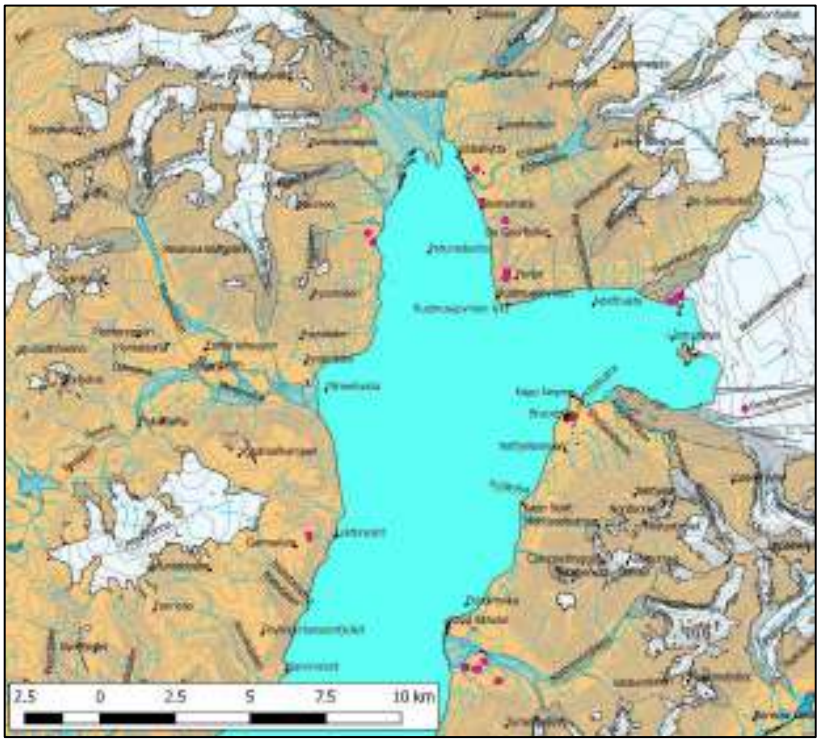


Fig. 3.1.3. The map of localities sampled in August 2017. The map was created by QGIS software and the maps were obtained from the Norwegian Polar Institute.

Since our focus was on hydro-terrestrial microbes, most of the habitats examined were either entirely water based (such as rivers, lakes and streams) or strictly water-related (wet hummock tundra, wetlands and intertidal zones). Snow and ice based habitats were examined too, although the biodiversity of such habitats tend to be, at least in comparison with the habitats with do contain liquid water, limited. Our findings later confirmed our presumption.

In addition to sample extraction, several measurements of the locality were taken. These include the water temperature, type of community, type of habitat, the GPS coordinates, pH, conductivity and altitude. This information will be necessary for improved understanding of the relationship between individual genera and physical and chemical parameters of their surroundings.

Due to the nature of the extreme abiotic factors, it is not surprising that most of the algal and cyanobacterial biodiversity is represented within communities that are attached to other surfaces and/or have its individual cells in immediate proximities – i.e. biofilms, periphytic and epiphytic communities (Fig. 3.1.4.).

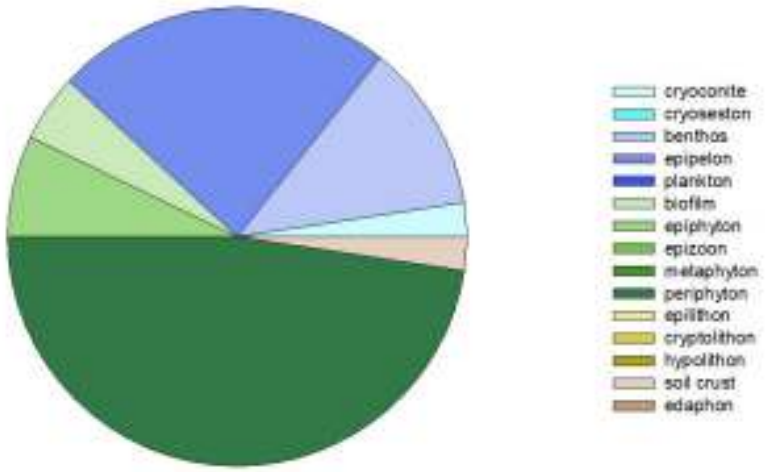


Fig. 3.1.4. The communities sampled in 2017.

Out of these communities, periphytic algae and cyanobacteria were the ones most represented in terms of biodiversity, specifically 19 out of 42 genera were found as parts of periphytic communities, and in terms of specific genera, *Leptolyngbya* sp., *Tribonema* sp., *Hydrurus* sp. and *Zygnema* sp. were most commonly found. Relating to the physical properties of the first major class group, another with similar physical properties – strict association to other surfaces – is widespread in the area. Specifically, it is the epiphytic and, to a lesser extent, benthic group, which represent 17 of the 46 genera. Other communities are largely less represented, although it should be noted that snow alga *Chlamydomonas nivalis* was discovered in cryoconites located on a nearby glacier Nordenskiöldbreen.

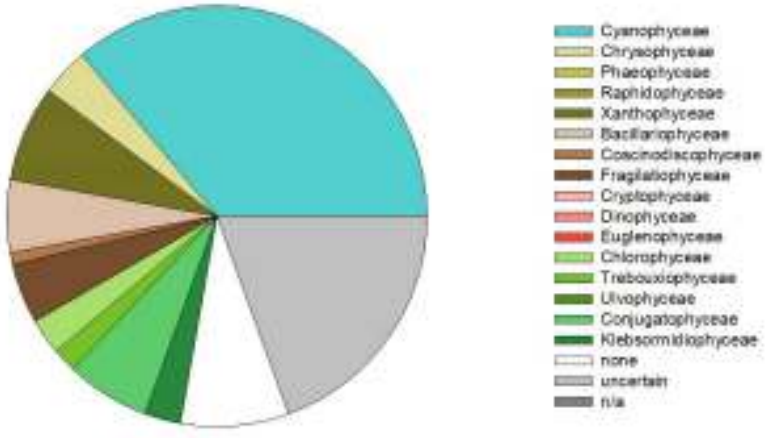


Fig. 3.1.5. The taxonomical classes observed in 2017.

The class representation itself is dominated by Cyanophyceae, which represent more than a third of the total class distribution with *Leptolyngbya* sp. being the most commonly found genus of this class. The rest of the classes found is composed of two major clades – Viridiplantae and SAR. Xanthophytic and Conjugatophytic algae along with diatoms were most dominant (Fig. 3.1.5.).

In terms of habitats, vast majority of samples that were examined were from strictly water-associated areas such as lakes, shallow pools, streams and coastal intertidal areas. Only one sample of a non-strictly water based habitat was extracted – a sample from lichenized soil crust. However, this sample contained no relevant microbial organisms. The relevance of such water depraved areas was deemed not high enough for further sample extractions (Fig. 3.1.6).

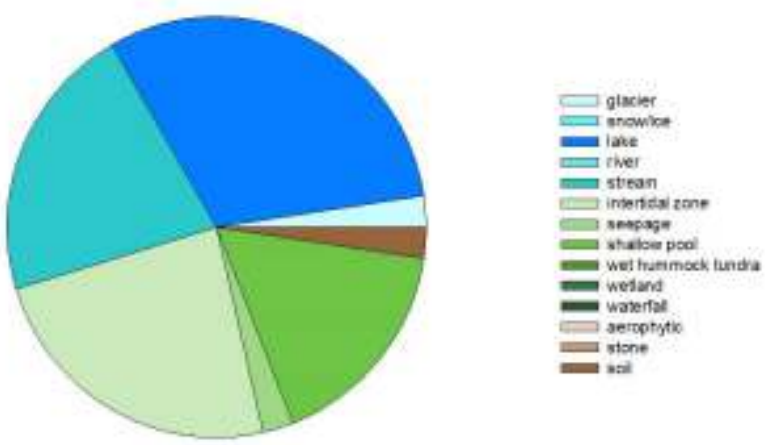


Fig. 3.1.6. The habitats sampled in 2017.

In summary, all of the 44 microbial genera and species examined were strictly water-bound and consist largely of green and SAR algae, whereas cyanobacteria form only a minority representation.

Proposals for future investigations: Our procedure had, nonetheless, certain limitations. One of these is possible imprecise classification, and more importantly, failure of classifying classes and genera of some of the samples. This shortcoming could be potentially remedied using genomic analyses as the classification was primarily based on visual characteristics. Those, however, may prove insufficient in determining the differences between two visually similar genera and even more so between two similar species.

Furthermore, it could be argued that in some cases the sample extraction might have been biased considering that we needed some of the genera encountered for other research projects. Thanks to that, some genera are most likely disproportionately represented within the total amount of samples collected. Since the naked eye visual characteristics of some algal and cyanobacterial communities are similar to those of the algae we were extracting for other projects, both of these, the ones we actively sought and those which have happened to have similar characteristics, might also be overrepresented. It should be noted, however, that the total amount of samples of genera found is not reflective of the total amount of specific classes genera found in the location of Billefjorden as a whole.

Lastly, for better understanding of current trends in local biodiversity development, the number of individual classes and their genera should be monitored, preferably during the same time of the year. In the light of changing conditions in polar areas, it would be interesting to examine the biodiversity dynamics occurring as a result of the large-scale abiotic changes.

Diversity of the freshwater green algae (Chlorophyta) in Svalbard lakes

Green algae are still a poorly known component of Arctic freshwater habitats. The main goal of this project is to contribute to our understanding of their biodiversity by comparing outputs of various approaches: 1/microscopy of field samples, 2/strain isolation and Sanger sequencing and 3/HTS of environmental DNA. This work is in progress by Tereza Šamšulová under the leadership of Linda Nedbalová and Josef Elster.

Within the Summer Polar Ecology Course (microbiology group), research on diversity of freshwater green algae was performed during August 2017. In total, 12 lakes in Billefjorden and 2 lakes in Adventfjorden were sampled (Fig. 3.1.7.). Microbial mat in the littoral zone of lakes was scraped off into 50 ml vials. During the sampling, GPS coordinates, water pH, temperature and conductivity were also recorded and photos of each site were taken. Lake water was sampled for chemical analysis of major ions and nutrients that will be performed in the Czech Republic.

Fig. 3.1.7. Ragnar lake (Billefjorden).

After returning to the station, microphotographs were taken in the field laboratory. On the day of sampling, a subsample was stored in the freezer (15 ml vial), another was fixed in LifeGuard and also stored in the freezer for planned environmental DNA isolation and a third one was fixed in formaldehyde and stored in ambient temperature. Finally, a small amount of sample was placed on agar with BBM medium and stored in ambient temperature for following isolation of unialgal strains.

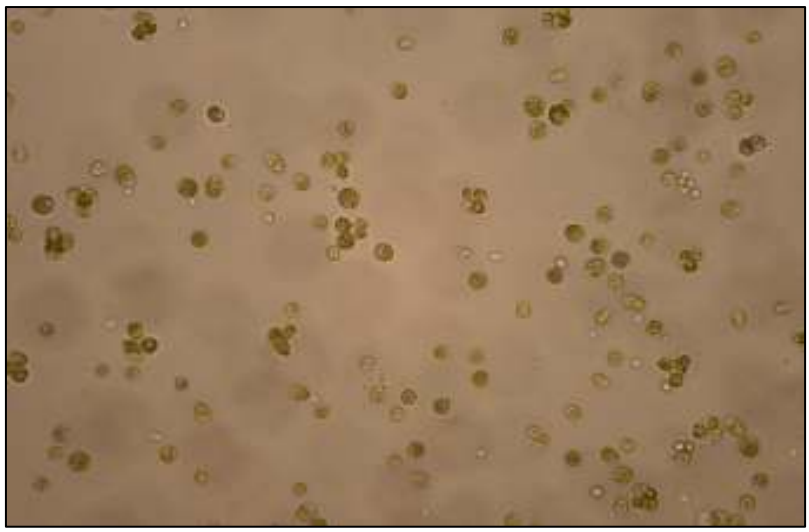


Fig. 3.1.8. A culture of green algae from a lake in Mathiesondalen (objective magnification 400x).

All samples were carefully transported to Faculty of Science in Prague and stored in the same conditions. The sample on agar was cultivated in the refrigerator (6 °C). This process of isolation consisted of transferring small amount of cultivate into the 24-well plate with BBM solution (2 ml in each well) which was repeated after 7-10 days until the sample contained only one algal species. The strain was later used for DNA isolation using DNeasy Plant MiniKit. Two molecular markers (18S rRNA and ITS-2) will be amplified for every isolate and sent to Macrogen (The Netherlands) for sequencing. Samples fixed in the LifeGuard will be used for environmental DNA isolation using DNeasy PowerLyzer PowerSoil DNA Isolation Kit and processed using high-throughput sequencing (HTS) methods.

3.1.3. Ecological characterization of a *Vaucheria* sp. dominated hyperborean microphytobenthos

Claude-Eric Souquieres, Jana Kvíderová & Josef Elster

Introduction

The Arctic coastal zone represents extreme environment characterized by frequent disturbances in the system of temporal lagoons and channels. The microbial mats play important role in substrate colonization and stabilization of the sediment. *Vaucheria* sp. (Xanthophyceae) is the main component of the microbial carpet, and seems to be adapted well to such variable environment. In 2016, we performed initial study on *Vaucheria* sp. photosynthetic activity in a microcosm which revealed that the photosynthetically active irradiance (PAR) is the most important factor controlling the diel periodicity of the photosynthetic activity and suggested low-light adaptation in *Vaucheria* sp.[1] (Kvíderová et Elster 2017).

During the summer season 2017, we focused on

- detailed characterization of the habitat
- collection of samples for molecular taxonomy analyses
- photosynthetic activity measurements *in situ* using variable chlorophyll measurements approach
- photosynthesis measurements *ex situ* using gasometrical approach

Material and methods

The locality and experimental site is situated in the Adventdalen tidal flat, near the Czech Arctic Research Infrastructure in Longyearbyen (see Kvíderová et Elster (2017)[1] for reference). The continuous climatic (PAR, air temperature) and microclimatic data in the tidal flat were recorded using Minikin dataloggers (EMS Brno, Czech Republic). Before photosynthesis measurements, temperature in the sample, irradiance and UV radiation were recorded.

The samples for habitat characterization were collected during summer 2017 (from August to September). A total of 3 transects encompassing a total of 11 sites were assayed and are represented on the map using QGIS (Fig. 3.1.9.). At each place water physicochemical properties (pH, temperature, salinity) were registered extensively using a Low Range pH/Conductivity/TDS Tester in combination with a refractometer for salinity measurements.

The photosynthetic measurements were performed in the conditions of late Arctic summer from August 12 to August 31, 2017. The variable chlorophyll fluorescence was measured using hand-held FluorPen fluorometer (Photon Systems Instruments, Czech Republic) and the CO_2 assimilation using Gas Fluorescence System GFS-3000 (Walz, Germany).

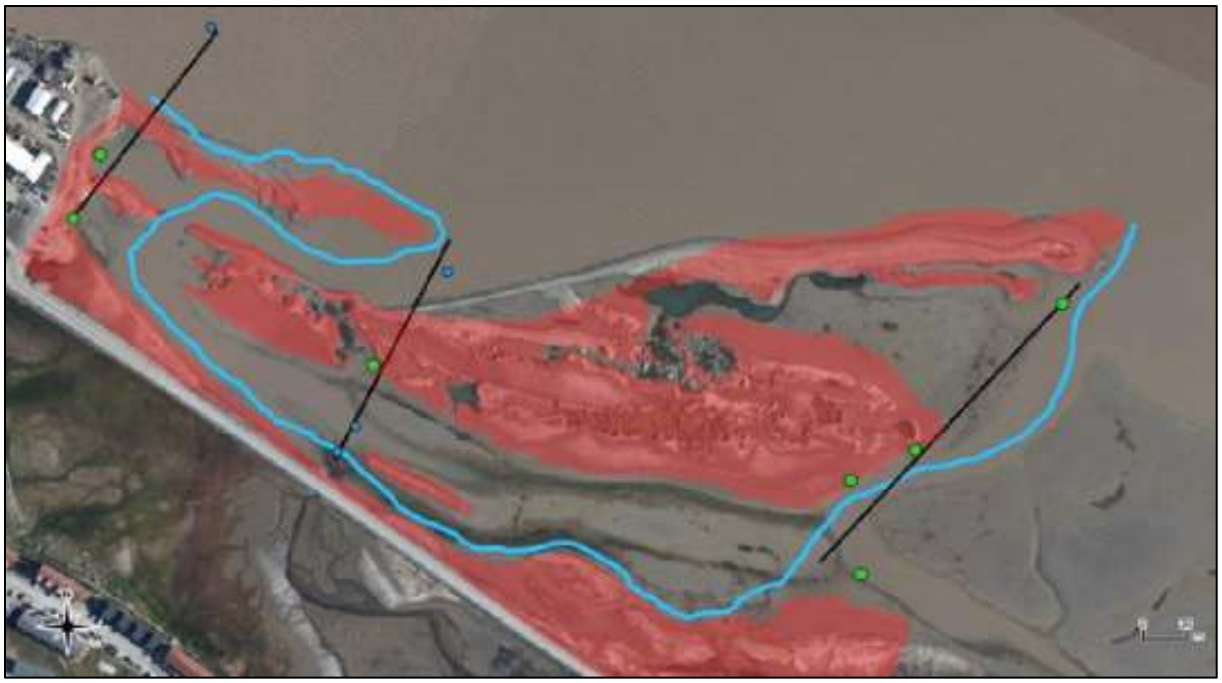


Fig. 3.1.9. Sampling area with Transects I, II and III from left to right. Sampling sites along the transect are numbered from 1 to 3/4 from down to up. The red zone delimits the high tide front. In blue the low tide channel is represented. Sampling site in green refers to the presence of *Vaucheria* communities in the close surroundings.

Results and Discussion

At present, majority of samples is being processed and data are being evaluated. Therefore, only preliminary data are presented here.

The air temperature and irradiance corresponded to late Artic summer in Svalbard[1,2]. Contrary to previous season[1] the air temperature dropped below 0 °C twice.

The water physicochemical properties (pH, temperature, salinity) were registered extensively using a Low Range pH/Conductivity/TDS Tester in combination with a refractometer for salinity measurements is drawn in Table 1.

Table 3.1.4. The physico-chemical properties of water samples.

	Mean Temperature (°C)	Mean salinity (psu)	Mean pH at Low tide	Mean Ph at High tide	depth of the permafrost active layer (cm)
TI.1	6.2	26.00	7.29	7.18	+120
TI.2	5.9	28.00	7.26	7.06	56
TI.3	5.4	27.50	7.70	7.11	+120
TII.1	4.9	16.75	6.80	6.83	50.0
TII.2	5.7	23.50	6.82	6.83	60.0
TII.3	5.8	25.25	7.33	7.10	+120
TII.4	5.7	21.25	6.69	7.41	+120
TIII.1	5.9	18.25	7.59	7.29	+120
TIII.2	6.0	17.50	7.52	7.44	+120
TIII.3	5.7	17.50	7.52	7.39	+120
TIII.4	5.6	17.25	7.34	7.20	+120

In a second place, nutrients, namely: silica, nitrogen, and phosphorus, were quantified both in the soil and water. Furthermore, the sediment was further analysed for organic carbon and granulometry.

We performed a variety of experiments as to determine the nutrient supplies of these distinct habitats within this ecosystem both in the water and the sediment. A stress was laid on a specific set of nutrients which are assumed to have a strong influence on the microphytobenthos, namely: nitrogen, phosphorus, silicate and carbon.

Sediment phosphate supplies showed low removal to no removal. Advection of phosphate seems to correlate with sites showing biomass except in TII.3 for which we assume that P-PO_4 accumulates as an effect of stagnant water in this particular small pond. Nitrogen levels are kept low upon removal by *Vaucheria* sp. in most cases. It could indicate algal adaptation to nutrient sources in the sediment rather than from the nutrient-limited water especially in this poor ecosystem. We assume that nitrogen and phosphorus stocks greatly influenced algal growth.

In situ photosynthesis measurements using variable chlorophyll fluorescence approach indicated slightly increased quantum yields at the lowest irradiances (Fig. 3.1.10). The CO_2 assimilation measurements did not show any photoinhibition at PAR values around 500 µmol m^{-2} s^{-1}.

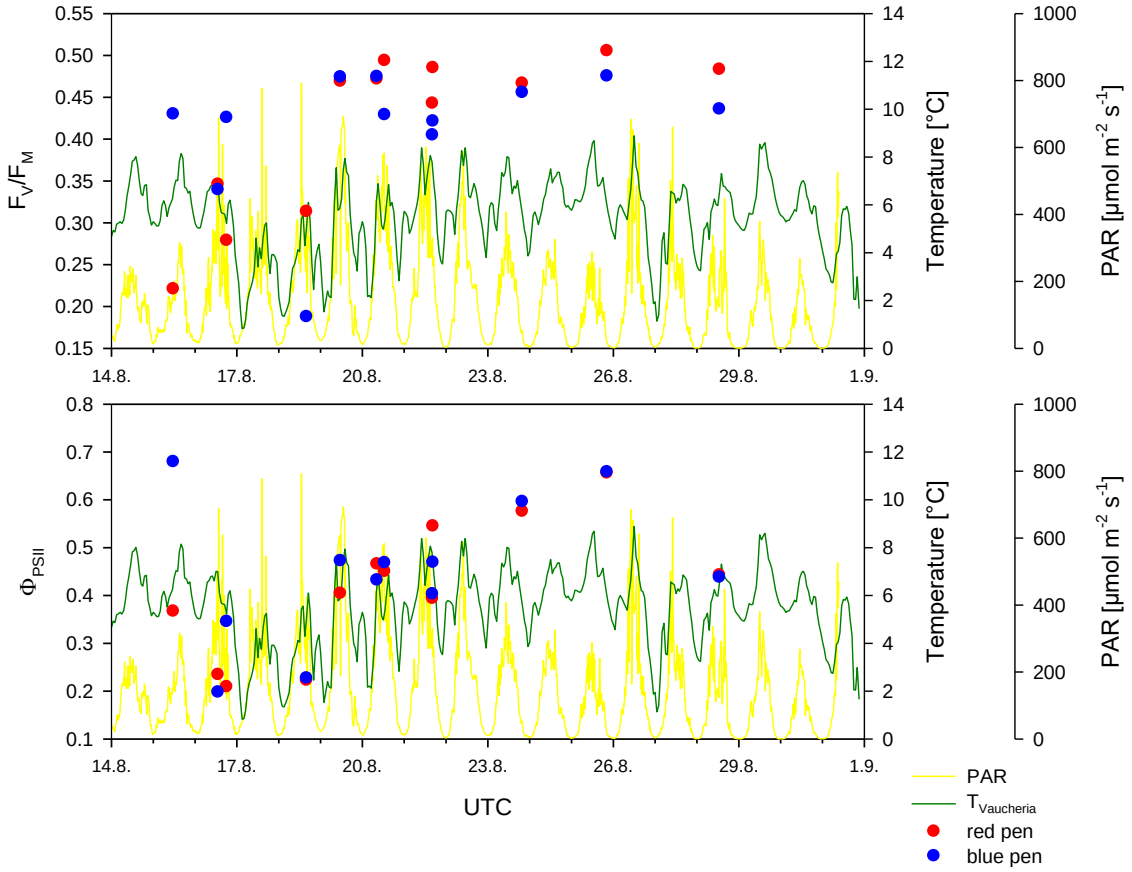


Fig. 3.1.10. The values of maximum quantum yield (F_V/F_M) and actual quantum yield (Φ_{PSII}) and courses community temperature ($T_{Vaucheria}$) and photosynthetically active radiation (PAR).

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3.2. Zoology and Parasitology

Instructors: *Oleg Ditrich*

Students: *Oldřich Daněk, Margeritha Lucadello, Karel Raška, Magdalena Raška & Kamila Weissová*

3.2.1. Metazoan fauna in cryoconite holes of Svalbard, Genetical and Ecological features

Margherita Lucadello

Introduction

This project titled is a project supervised by RNDr. Miloslav Devetter, Ph.D. The research is about the ecology and the genetic of the metazoan fauna, as tardigrades and rotifers, living in cryoconite holes of the high arctic glaciers of Svalbard. It aims to better understand the relationship between cryoconite's area, depth and number of animals that have been counted in the cryoconite's samples, in relationship with the type of glacier and the period in which they have been sampled during the season. It also aims to look at the relationship between rotifers and tardigrades, their relative abundance and correlation. Moreover, it aims to understand if the rotifers and tardigrades sampled are showing endemicity or if they show connection with rotifers and tardigrades collected along the fore field. Lastly, this study is part of an international project in which other researchers from other states are involved. The aim is to compare all the data from several different glaciers from different parts of the world, as Iceland, Greenland, Antarctic under a phylogeographic view.

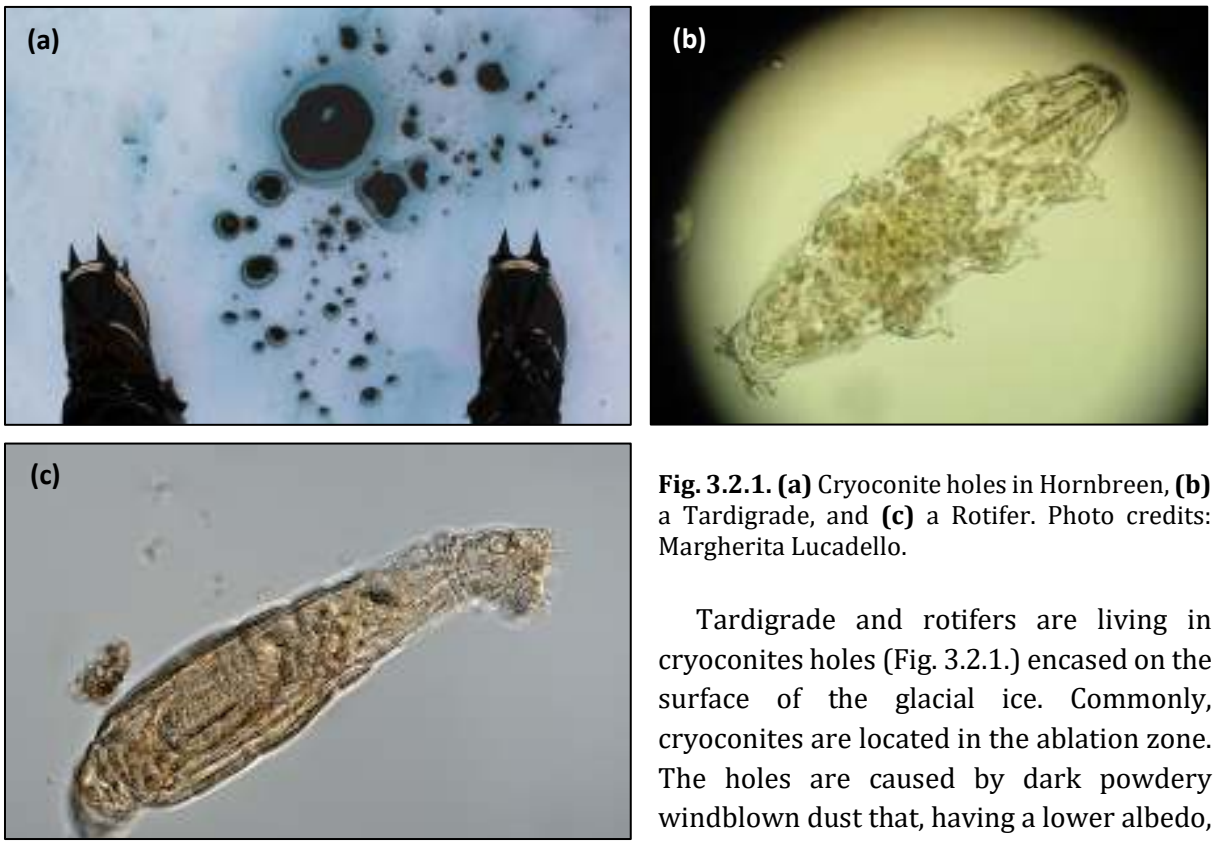


Fig. 3.2.1. (a) Cryoconite holes in Hornbreen, **(b)** a Tardigrade, and **(c)** a Rotifer. Photo credits: Margherita Lucadello.

Tardigrade and rotifers are living in cryoconites holes (Fig. 3.2.1.) encased on the surface of the glacial ice. Commonly, cryoconites are located in the ablation zone. The holes are caused by dark powdery windblown dust that, having a lower albedo, is absorbing more solar radiation than the surrounding ice. The heat is making the ice melting and the hole forming. These holes are pockets of microbial life, they are made by melted water and a thin layer of sediment on the bottom. In these sediments a hole small cold-adapted ecosystem is present, with distinct boundaries, energy

flow and nutrient cycling. It has been suggested that cryoconite holes are playing an important role on the whole glacier's ecosystem.

Materials & Methods

The research includes 7 different glaciers. The glaciers are in Spitsbergen, the bigger island of the Svalbard archipelago. The samples have been taken from 2 different parts of Spitsbergen: Billefjorden, North of Longyearbyen and Hornsund, on the South of the island. The glaciers that have been sampled are: Nordenskiöldbreen, Hørbybreen, Ebbabreen, in Billefjorden and Hornbreen, Hansbreen, Gasbreen, Torelbreen in Hornsund (Fig . 3.2.)

Fig 3.2.2. Glaciers sampled signed on topographical Svalbard's map. Map source: toposvalbard.npolar.no

To reach the sampling sites it was necessary to walk directly from the station or to use the Zodiac and walk afterword. In the case of the use of the Zodiac, life vest and waterproof bags were required. The presence of icebergs, bears or signed path of deeper water it had been taken in consideration along the trip. In Hornsund bay, two safety boxes with sleeping bag, food, medication, ammunition, a tend and a safety device against polar bears have been brought on the beach all the time. The material that have been carried on the glacier was: rifles, fleer gun, radio, satellite phone, crampons, ice axe, waterproof clothes, gloves, GPS, camera, plastic gloves, sampling ring, sampling pump, tape measure, conductivity metre, waterproof notebook, marker, bottles for sampling, whirplack bags, ziplock bags, freezer bags for fore field samples, Kopecky valecek fore field (Fig. 3.2.3.). From every cryoconite depth, width, length, GPS position, a picture, note if necessary and different samples of sediments have been taken.

Fig. 3.2.3. Sampling equipment. Photo credits: Margherita Lucadello.

During the sampling different type of samples have been taken: Samples for abundance analyses, samples of cryoconite for genetics analyses, samples of the fore field. Once in the NOSTOC station the samples for abundance have been analysed. The area sampled from the layer of sediments on the bottom of the cryoconites was equal for all the cryoconite thanks to the use of the same sample ring. From every sample, 1/3 of the sediments have been check out at the microscope looking for abundance of tardigrades and rotifers using an inverted microscopy Olympus with a magnification of 40×. It has been possible to do the abundance analyses just to the samples from the 3 glaciers in Billefjord. Any abundance analyses were done on the samples from Hornsund's glaciers, infact was not possible to use the microscope on RV CLIONE during the trip in Hornsund bay. All the rest of the samples were conserved in the bear-proof freezer of NOSTOC Station, or in RV CLIONE, then transported in Julius Payer station in Longyearbyen and then sent to Czech Republic by plane to be conserved at the Center of Polar Ecology.

Nowadays, in the lab of the Center of Polar Ecology and Biology Centrum, part of the samples from cryoconites and from the near forefield have been processed. From them the DNA was extracted using the PowerSoil® DNA Isolation Kit and conserving the DNA in the freezer at -80 °C. The DNeasy PowerSoil Kit is usually used to isolate microbial genomic DNA from soil type, both wet or dry. In this case, the metagenomic study of the genetic material recovered from cryoconite provides for use wet soil samples. The DNeasy PowerSoil Kit has also inhibitor removal technology able to interact with the soil samples. Thanks to this, the high humic acid present in the soil samples are removed, and the possibility of further problems caused by their binding with the reagents of PCR is decreased.

The same lab process and more further other protocols will be followed with all the samples sampled in Svalbard in the following days.

Results, Discussion & Conclusion

Using the data available until now it is possible to shows some results just about the 3 glaciers in Billefjorden: Nordenskiöldbreen, Hørbybreen, Ebbabreen. From these glaciers the data have been analysed respectively from 17, 26, 19 cryoconite holes. Animals have been found in 85.5 % of the cryoconites sampled (Nordenskiöldbreen: 82.40%, Hørbybreen: 92.30%, Ebbabreen: 78.95%). For the results about the rest of the samples more time is needed.

Using the cryoconite's width and length data, the area of the cryoconites have been calculated as an ellipsis. Thanks to the data analysis software RStudio, a Shapiro-Wilk normality test has been run on the 4-different set of data available, using the data of the 3 glaciers together. The variables considered were: Cryoconite area (A), Cryoconite depth (D), Number of rotifers (R) and Number of tardigrades (T).

The cryoconite area (mean ± s.d.):	591.41 ± 659.78 cm^2
The cryoconite depth (mean ± s.d.):	12.77 ± 7.26 cm^3
The number of rotifers (mean ± s.d.):	5.80 ± 8.22
The number of tardigrades (mean ± s.d.):	9.59 ± 10.50

The tests showed that all the 4 variables are not normally distributed. Consequently, a Test for association between paired samples has been used, using one of Pearson's product moment correlation coefficient, the Spearman's rho. Cryoconite Area have been paired with Number of tardigrade (A/T), Cryoconite depth have been paired with the Number of tardigrades (D/T), Cryoconite area have been paired with the Number of rotifers (A/R), Cryoconite depth have been paired with the number of Rotifers (D/R). These pairs showed no significant correlation between them, with a p-value respectively of: p-value (A/T) = 0.1628, p-value (D/T) = 0.5051, p-value (A/R) = 0.4964, p-value (D/R) = 0.5082, and rho value respectively equal to: rho (A/T) 0.1809398,

rho (D/T) 0.08697645, rho (A/R) 0.08875541, rho (D/R) 0.08633386. As well, a previous publication of 2016 about the same glaciers showed how area, depth and elevation of cryoconite holes in the Arctic do not influence tardigrades densities[1]. It was proposed that because of the persistent flushing of cryoconite sediments, the rapid melting of the glacier surface in the Arctic and inter-hole water-sediment mixing, the functioning of the cryoconite ecosystems is disrupted. It was concluded that cryoconite holes are dynamic ecosystems for micro invertebrates in the

It could show a random distribution of micro-fauna without clear ecological interactions with abiotic variables[2]. Instead, the number of tardigrades and the number of rotifers have been paired. This test showed a significant correlation between the 2 variables with a p-value (T/R) = 0.03403 and rho (T/R) = 0.2718866. Being the p-value < 0.05 it seems that there is a significant correlation between the 2 variables. But, according to the correlation factor (rho) the correlation is weak, being the value more closed to 0 than to 1. A correlation between these two variables have been founded previously in literature[3]. Tardigrade and rotifers are micro invertebrates that play a role as top consumers on glaciers. They have probably competing roles in the cryoconite food web. Most limn terrestrial and freshwater aquatic tardigrades feed on juices sucked from moss, lichens, algae, and other plants although some tardigrades are carnivorous and consume other mesofauna such as rotifers, nematodes and even other tardigrades. Rotifers instead eat particulate organic detritus, dead bacteria, algae, and protozoans[4] (Fig. 3.2.4.)

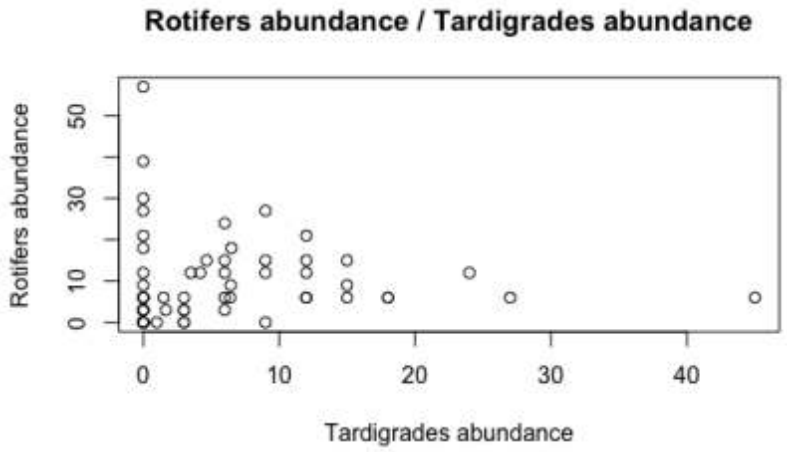


Fig 3.2.4. Correlation between abundance of invertebrates, tardigrade and rotifers, in cryoconite holes.

Furthermore, the composition of cryoconites from the different glaciers have been checked (Fig. 3.2.5.). The glacier Nordenskiöldbreen shows a more abundant presence of invertebrates composed almost equally by tardigrades and rotifers. Hørbybreen shows smaller animals abundance, with a higher presence of tardigrades. In Ebbabreen instead, the population of animals at the beginning of the season was small and composed almost totally by rotifers (Table 3.2.1.).

Table 3.2.1. The metazoan fauna composition of cryoconites from the different glaciers.

	Nordenskiöldbreen	Hørbybreen	Ebbabreen
Mean number of invertebrates	10.94	5.40	1.83
Mean number of tardigrades	10.80	7.80	0.00
Mean number of rotifers	11.10	3.00	3.67

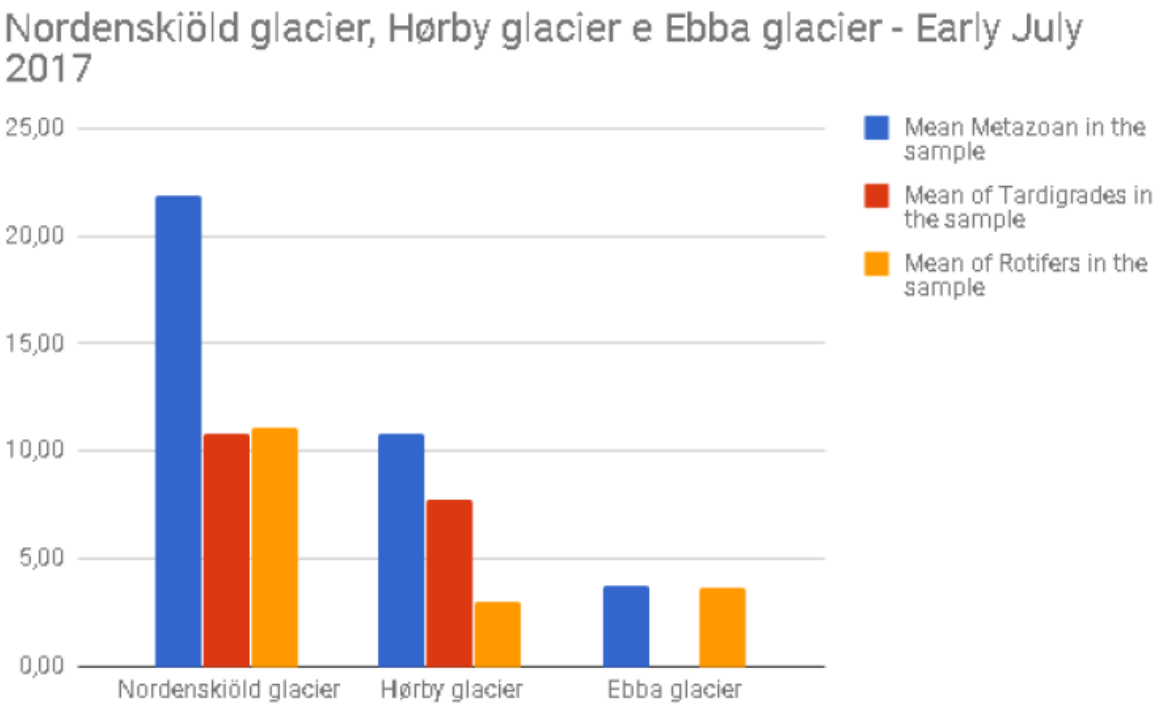


Fig. 3.2.5. Relative abundance of invertebrates in Nordenskiöldbreen, Hørbybreen, Ebbabreen in July 2017.

Besides, the cryoconites composition changed along the short summer season. Generally, the number of rotifers have been found being always more abundant compared to the number of tardigrades on all the 3 glaciers. This have been found previously on the literature. The graph shows a decrease in number of animals in the glacier Nordenskiöldbreen

Mean number of invertebrates	5.14
Mean number of tardigrades	1.71
Mean number of rotifers	8.57

An increase of abundance in the glacier Hørbybreen have been found.

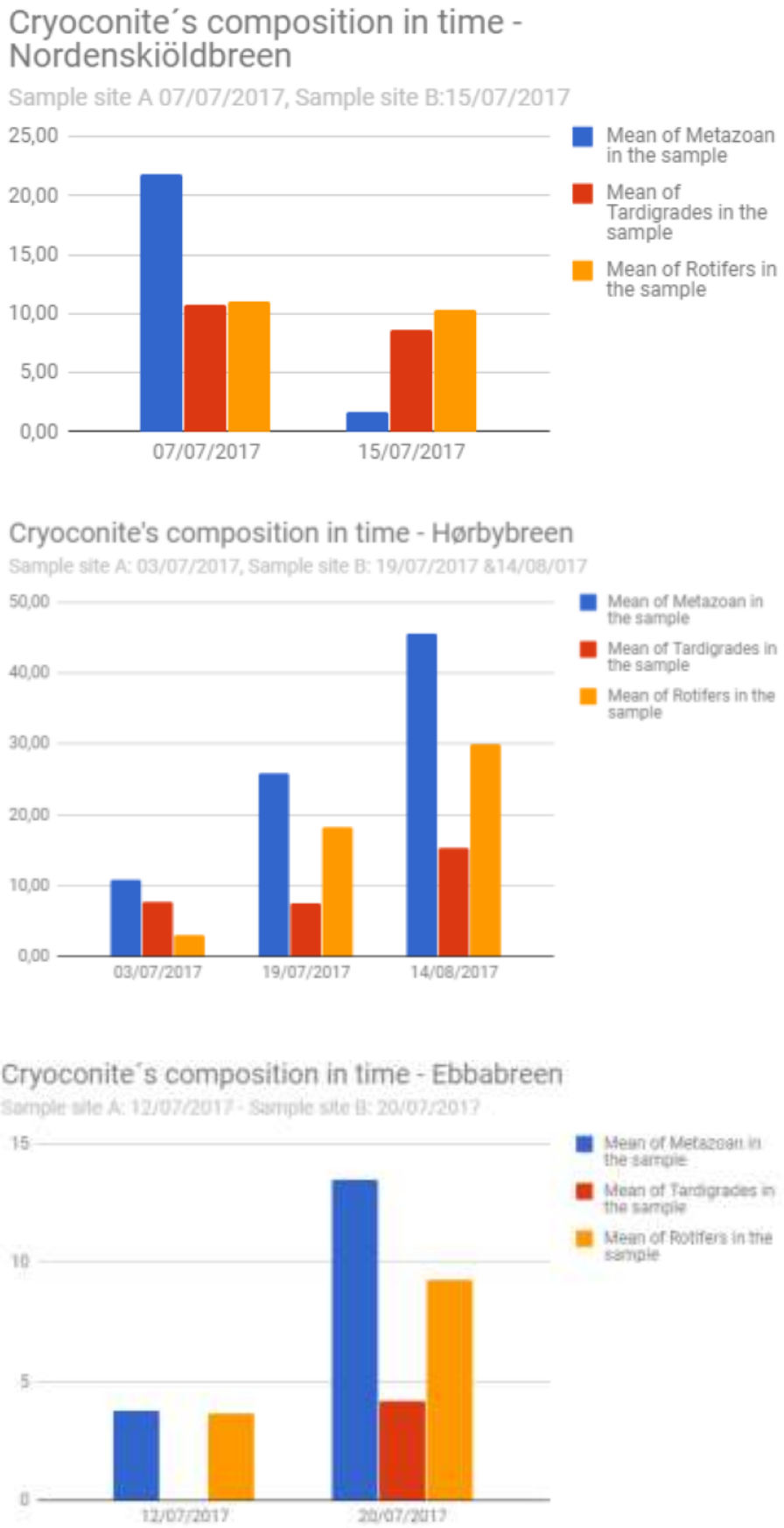
Mean number of invertebrates	11.73
Mean number of tardigrades	7.13
Mean number of rotifers	16.13

Also in the glacier Ebbabreen an increase in population number is found, together with the presence of a higher number of Tardigrades.

Mean number of invertebrates	6.74
Mean number of tardigrades	4.19
Mean number of rotifers	9.30

The standard deviation is used to quantify the amount of variation or dispersion of a set of data values. In all the cases considered was obtained a high standard deviation meaning that the data points are spread out over a wider range of values (Fig. 3.2.6.).

Many more results are expected as soon as the lab analyses are going to be completed.



Fig, 3.2.6. Cryoconite composition of invertebrates in Nordenskiöldbreen, Hørbybreen, Ebbabreen.

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3.2.2. Gastrointestinal nematodes of thorny skate (*Amblyraj radiata*)

Ondřej Daněk, Karel Raška & Magdalena Raška

Amblyraja radiata is an elasmobranch fish belonging to order Rajiformes of Rajidae family, previously known as *Raja radiata*. This skate can be found throughout Northern Atlantic, from western part around Canadian shore as well as in eastern part in Barents and Northern Sea. Thorny skate lives near seabed usually in depths ranging from 18 to 1400 m, most commonly between 27 – 439 m. Most commonly found specimens are 40 – 80 cm long but can grow up to more than 1 m. *Amblyraja radiata* preys on different kinds marine organisms ranging from decapods and other crustaceans to mollusks and fish. Preferred prey depends on body length of animal. Smaller skates around 40 cm specialize on hunting mainly amphipods (for example *Gammarus* sp.) while skates longer than 70 cm usually hunt fish.[1] This eating habits could have impact on which parasite species can be found in particular skate.

Gastrointestinal tract of elasmobranch fish is a bit different from those of other vertebrates. Stomach is divided in two parts – *pars cardiaca* and *pars pylorica*. While mucosa of *pars pylorica* is smooth there are longitudinal folds in *pars cardiaca*. *Pars pylorica* leads to intestine consisting of very short duodenum, ileum and colon. To make absorption area bigger with smaller length there is a spiral valve called *typhlosolis*. This helps to the skate a space for large liver and reproductive system.[2]

There are different types of parasites found in Rajiformes skates starting form blood protozoans and ending with “ectoparaistic” crustaceans. They can be definitive hosts as well as intermediate ones. Specifically in *Amblyraja radiata*’s gastrointestinal tract there have been found for example fluke *Otodistomum veliporum* with fish as intermediate host or tapeworms *Acantobothrium coronatum*, *Echeneibothrium dubium abyssorium*, *Pseudoanthobothrium hanseni* and *Grillota* sp.[3]

Only a few species of nematodes have been observed in Rajiformes so far. Nowadays papers mention parasites of these families: Physalopteridae, Capillariidae, Acanthocheilidae and Anisakidae. Skates serve as definitive hosts of these parasites with exception of the Anisakidae family where in this case they can be probably only intermediate hosts. *Proleptus acutus* as a representative of the Physalopteridae family was observed in Southern Atlantic near coasts of Argentina, Uruguay and Brazil. In these waters the parasite was found in stomach of *Sympterygia bonaparti* (Smallnose fanskate) but there are also records from other elasmobranch fishes across Northern hemisphere.[4] Another specimen of this family of parasites *is Heliconema psammbatidus* which was found in the spiral valve of *Sympterygia lima* (Filetail fanskate) formerly known as *Psammobatis lima* near coast of Chile.[5] Capillaridae family has only one representative found in Rajiformes skates. It is called *Piscicapillaria freemani* and was observed in the intestine of *Raja rhina* (Longnose skate), *Bathyraja interrupta* (Bering skate) formerly known as *Raja kincaidi* and

Raja stellulata (Pacific starry skate) all of them in western Canadian waters.[6] There are more parasites of family Acanthocheilidae parasiting in skates, most importantly genus *Pseudanisakis focusing* on skates as its definitive hosts. Very recently a new species, *Pseudanisakis argentinensis* was discovered in southwest Atlantic in six species of skates.[7] The type species of this genus is however *Pseudanisakis tricupola* (Fig. 3.2.7.).[8] The most studied of these families of parasites is the Anisakidae family mainly for its zoonotic potential coming from commercial fishing. Three genera were observed in skates: *Anisakis*, *Contraceacum* and *Pseudoterranova*.[9] Definitive hosts of these parasites are usually bigger predators like seals, orcas or sharks. Skates as second intermediate host (not as important as other fishes), with L3 larvae usually inside their body cavity or liver. They get infected after eating first intermediate host (for example *Gammarus* sp.) Most common and type species is *Anisakis simplex* (Fig. 3.2.8.).

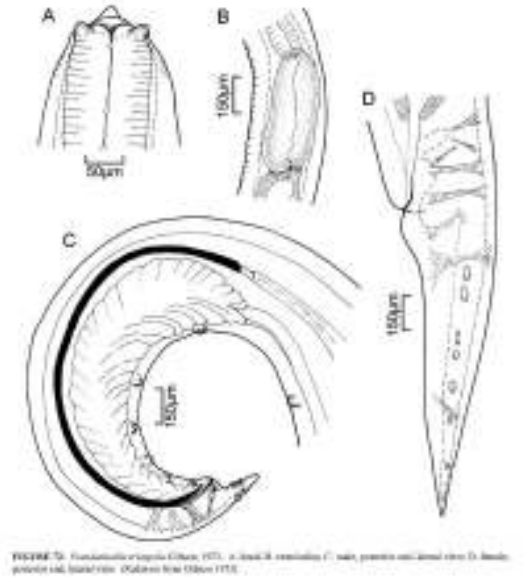

Fig. 3.2.7. *Pseudoanisakis tricopula*. Pictures used from Guide to the Parasites of Fishes of Canada Part V: Nematoda.

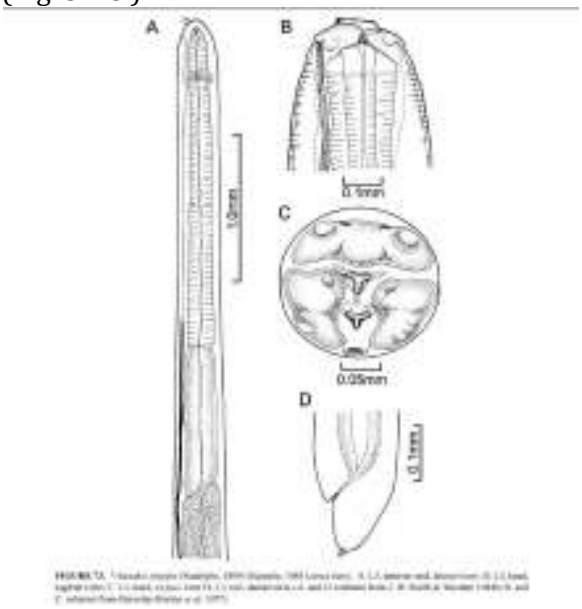

Fig. 3.2.8. *Anisakis simplex*. Pictures used from Guide to the Parasites of Fishes of Canada Part V: Nematoda.

According to literature there is only one species of nematode parasite which infect *Amblyraja radiata* as definitive host which is *Pseudanisakis tricupola*.[9] It sounds logical that this should be the only nematode observable in gastrointestinal tract of this skate. However, one can find Anisakidae species in stomach and spiral valve as well. L3 larvae of these parasites need some time to make their way inside body cavity or liver. Usually it is *Anisakis simplex* but even *Pseudoterranova dicipens* and not fully recognized specimens of *Contracaecum* sp. were found. All the above mentioned parasites can have really similar body length in range of few dozen mm. L3 larvae of *Anisakis simplex* can be 8.8-30.0 mm long and L3 larvae of *Pseudoterranova dicipens* range from 8 to 42 mm[9] while males of *Pseudanisakis tricupola* can have 5.9-60.0 mm and females 5.7-40 mm.[8] While measurements for *A. simplex* and *P. dicipens* were not conducted on skates and depend also on host size it still shows, that the ranges for parasites body length are similar. To differentiate between these parasites it is necessary to see their morphology or use PCR. Main differences are on anterior and posterior ends of these parasites as well as sexual dimorphism which cannot be observed in L3 larvae. Dorsal and two subventral labia are present on anterior end of *Pseudanisakis tricupola*. There is also missing so called boring tooth typical for Anisakidae.

Posterior part of *P. tricupola* ends with tail which is rotated in males and anal glands are missing. No tail can be observed in *A. simplex* or *P. dicipens*.[9]

If a nematode parasite is observed in gastrointestinal tract of *A. radiata* probably it is infection of *Pseudanisakis tricupola* or the skate serves as intermediate host for Anisakidae parasites. To distinguish between them morphological study needs to be performed. Not yet published date from Centre for polar ecology on Svalbard show that around 20 % of dissected *A. radiata* have nematode parasites in their gastrointestinal tract that morphologically look like *Pseudanisakis* sp. (visible labia and typical posterior end) but further determination by molecular methods is needed. Overall the theme of nematodes in skates even as common as *A. radiata* is poorly studied. The fact that there are some confusions regarding taxonomy of these parasites is not helping at all. Even thou nowadays taxonomy of *Pseudanisakis* genus was made established in 1973 by Gibson[8] use of unaccepted names can still be found throughout the literature. Recently published papers are coasts of South America and the discovery of new species *Pseudanisakis argentinensis*[7] shows that there is still a place for research to be conducted. It would be interesting to study in details parasites of skates in Svalbard waters.

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3.2.3. Effect of polar day on melatonin level and clock gene expression among polar researchers

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It´s a well know fact that during polar expeditions, researchers have to deal not only with extreme weather conditions, but also with the constant sunlight. Light as the most important synchronizing signal for our internal clock has a significant effect on our circadian rhythms. The exposure to constant light decreases the inner melatonin level and causes desynchronization of internal body rhythms.

The aim of my stay at the polar station was to study biological markers of circadian clocks and the ability of the clock to adapt to this constant light environment.

The subjects participating in the study were the other researchers staying at the polar station and working independently on their own projects. Before they left for Svalbard they agreed to participate in my study. They had to wear wrist actigraphic devices recording there activity 14 days before they got to Svalbard and then for 14 days of their stay in Svalbard. At the same time, they had to fill a sleep diary. The collection of biological samples had been done also before they

got to Svalbard and then after 14 day at the polar station. We collected two types of biological samples. One of them was saliva samples collected every 4 hours and 2 additional time points: at 9 AM and 9PM. The saliva sample has been used for measuring melatonin levels. Melatonin is a hormone secreted by the pineal gland during night time. Melatonin secretion is driven by circadian rhythms, but its production is directly inhibited by light exposure. The second biological samples were the buccal scrubs. They had been used for observing the functional state of their molecular clock. Any cell in our body expresses clock genes with defined circadian patterns. Clock genes are the key player of the circadian mechanism. The central clocks are located in the hypothalamic area of our brain, but the little clocks are present in many peripheral organs or even in the single cells of our body. In this study I used the easiest accessible cells scrubbed from the inner mouth and collected the same day as the saliva samples with 4 hour intervals. Rhythmic clock gene patterns are supposed to provide information about the effect of the polar day on the functional state of the clock (Fig. 3.2.9.).

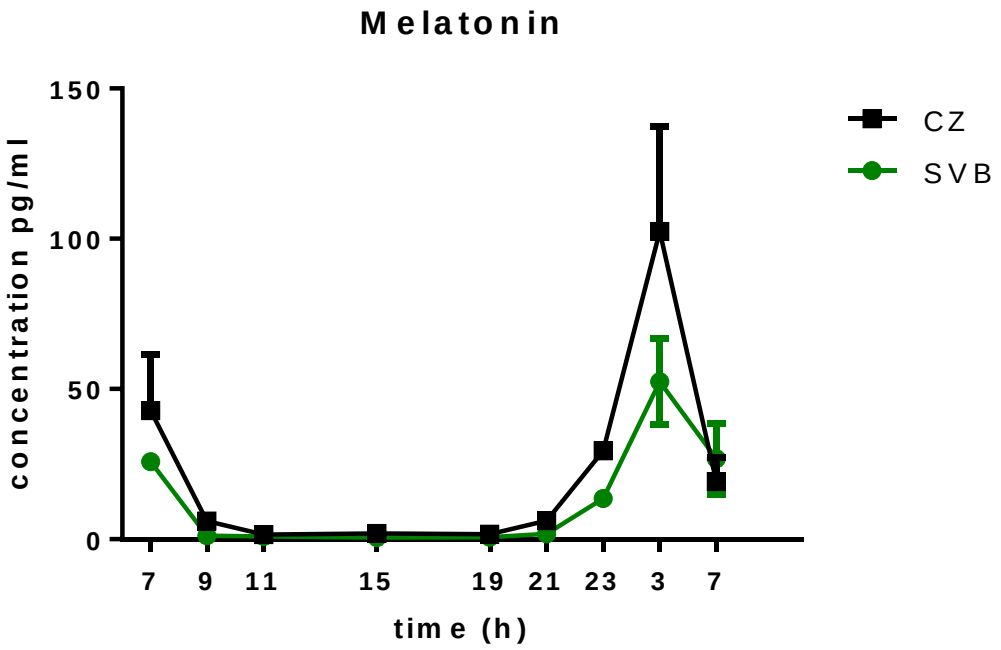

Fig. 3.2.9. The example of the daily melatonin profile of one of the subjects. The black line represents melatonin secretion in the Czech Republic (before the stay in Svalbard) and the green line represents melatonin secretion after 14 days of stay in Svalbard.