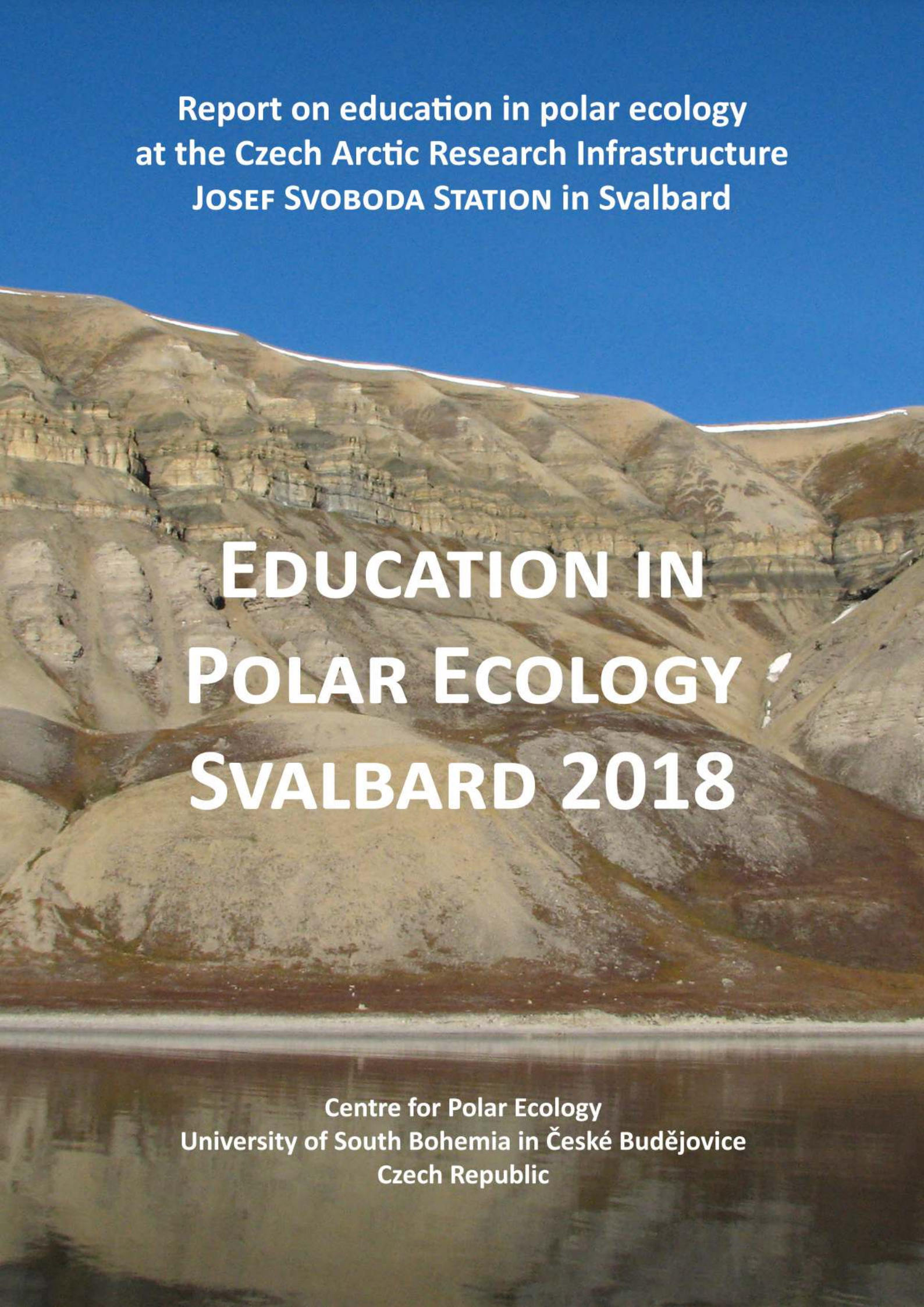
Report on education in polar ecology
at the Czech Arctic Research Infrastructure
JOSEF SVOBODA STATION in Svalbard



EDUCATION IN POLAR ECOLOGY SVALBARD 2018

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

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Faculty of Science

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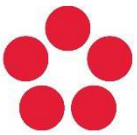
University of South Bohemia in České Budějovice



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

Cover photo: Jana Kvíderová
Editor: Jana Kvíderová

2018

Table of content

1. Introduction

The 8th 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 week of intensive theoretic preparation in respective fields of interest, and of approximately 14 days of field work at the Czech research station in Svalbard. Eleven students were selected (Tab. 1.1.).

In 2018, The theoretical part of the course took place in CPE facilities in České Budějovice during spring semester (21/05 – 25/05 2018). For the field work during the summer season in Svalbard, students were divided into three groups according to their specialization. The groups performed their field work in Svalbard on 13/08-27/08 2018, (microbiology/phycology) and on 20/08-03/09 2018 (botany/plant physiology + zoology/parasitology).

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

Tab. 1.1. The instructors and students (in alphabetical order) of the Polar Ecology Course according to their specialization.

Group	Instuctors		Students	
MICRO	Josef Elster	JU+IBOT	Dominika Činčarová	JU+MBU
	Jana Kvíderová	JU	Martina Flegrová	JU+ENTU
	Marie Šabacká	JU	Vít Nahlík	JU+MBU
			Luca Sanchez	JU
			Deborah Walter	JU
BOTA	Tomáš Hájek	JU+IBOT	Hana Dvořáková	JU
	Petr Macek	JU	Lada Klimešová	JU
			František Trkal	CZU
			Stanislava Wolfová	JU
ZOO	Miloslav Devetter	IPB+JU	Daniel Bartoň	JU
	Oleg Ditrich	JU	Vendula Branišová	JU
	Tereza Hromádková	JU	Dominik Horký	JU
	Václav Pavel	UPOL+JU	Jana Marešová	JU+ENTU
			David Novotný	JU
			Veronika Žánová	JU

Abbreviations:
Groups: BOTA - botany/plant physiology; MICRO - microbiology/phycology; ZOO - zoology/parasitology.
Affiliations: CZU – Czech University of Life Sciences, Prague;ENTU – Institute of Entomology, Biology Centre CAS, České Budějovice; IBOT – Institute of Botany CAS, Třeboň; ISB – Institute of Soil Biology, Biology Centre CAS, České Budějovice; JU – University of South Bohemia, České Budějovice; MBU – Institute of Microbiology CAS, Třeboň; UPOL – Palacký University, Olomouc.

2. Polar Ecology Course

2.1. Microbiology/Phycology

Instructors: ***Josef Elster, Jana Kvíderová & Marie Šabacká***
Students: ***Dominika Činčarová, Martina Flegrová, Vít Nahlík, Luca Sanchez & Deborah Walter***

The long-term aim of the microbiology/phycology group is to characterize the microbial diversity of algae and cyanobacteria in various freshwater and aero-terrestrial biotopes (streams, pools and lakes, seepages, soil surface, wet rocks, snow, snow cryoconites). We focus not only on taxonomical diversity, but also on diversity in ecology and physiology. The sampling sites are shown in Fig. 2.1.1.

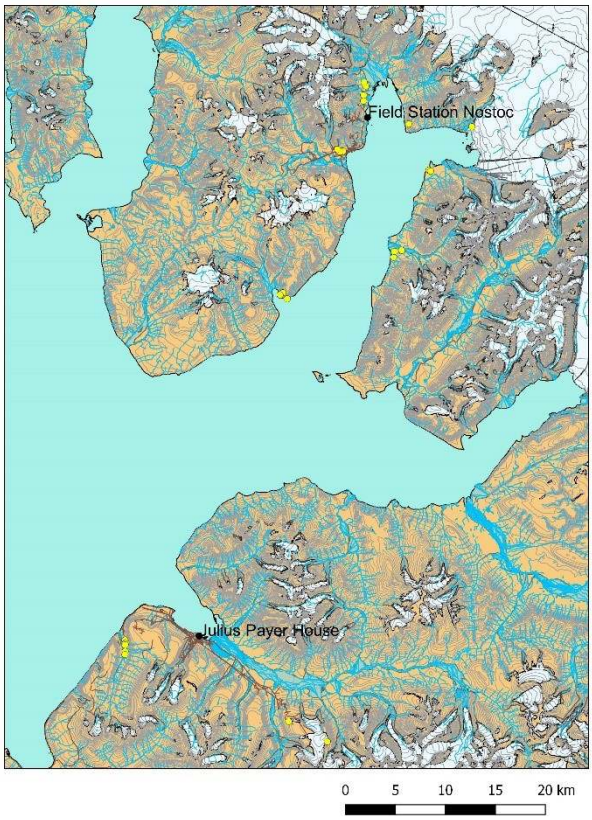


Fig. 2.1.1. Map of sampling sites of the microbiology/phycology group in 2018. QGIS, map source: Norwegian Polar Institute (2014). Kartdata Svalbard 1:100 000 (S100 Kartdata) / Map Data [Data set]. Norwegian Polar Institute. https://doi.org/10.21334/npolar.2014.645336c7)

2.1.1. Algae & Cyanobacteria in Svalbard - a small overview

Deborah Walter

In 2018, we took 39 different samples at 11 sites in different habitats around Svalbard. The habitats reached from bird cliff to hummock tundra to glacier ice and cryoconites (Fig. 2.1.2.). For each sample the exact GPS position, the altitude, the time and the date were recorded and temperatures, water conductivity and pH were measured when possible. The microscope was used later in the field laboratory to observe the found organisms. The observed species were recorded and we saved some pictures of important algae and cyanobacteria to the computer. The observed classes of algae are summarized in Fig. 2.1.2.

Most found species belonged to periphyton. Second most of our samples were growing on stones, so called epilithon and the third most samples were from soil crust. *Nostoc* sp. for example was very abundant in wetland and soil crust samples and under the microscope we could clearly distinguish heterocytes from other cells. *Tribonema* sp. was also present in some wetland samples and some good examples from the H-piece were found. The H-piece is the shape

of unfinished cells at the end of the filaments. Large biomass of *Prasiola* sp. could be observed in filamentous and sheet form and also surrounded by a mucilaginous layer in Bjørndalen and Pyramiden town. The cyanobacteria *Rivularia* sp., which grows on the upper side of stones as mucilagious colonies was present in a shallow lake in Brucebyen. Opposite the bay in the Fortet stream amongst other species also *Hydrurus foetidus* sp. and *Schitzothrix* sp. were found. In some samples we could identify Zygnema sp. with thick cell walls, as preparation for the winter. We found also some species such as *Ancylonema nordenskioeldii*, *Cylindrocystis brebissonii*, *Leptolyngbya* sp. and *Phormidesmis* sp. in cryoconites and the snow algae *Chlamydomonas* cf. *nivalis* was present in a sample of red snow taken on Foxfonna.

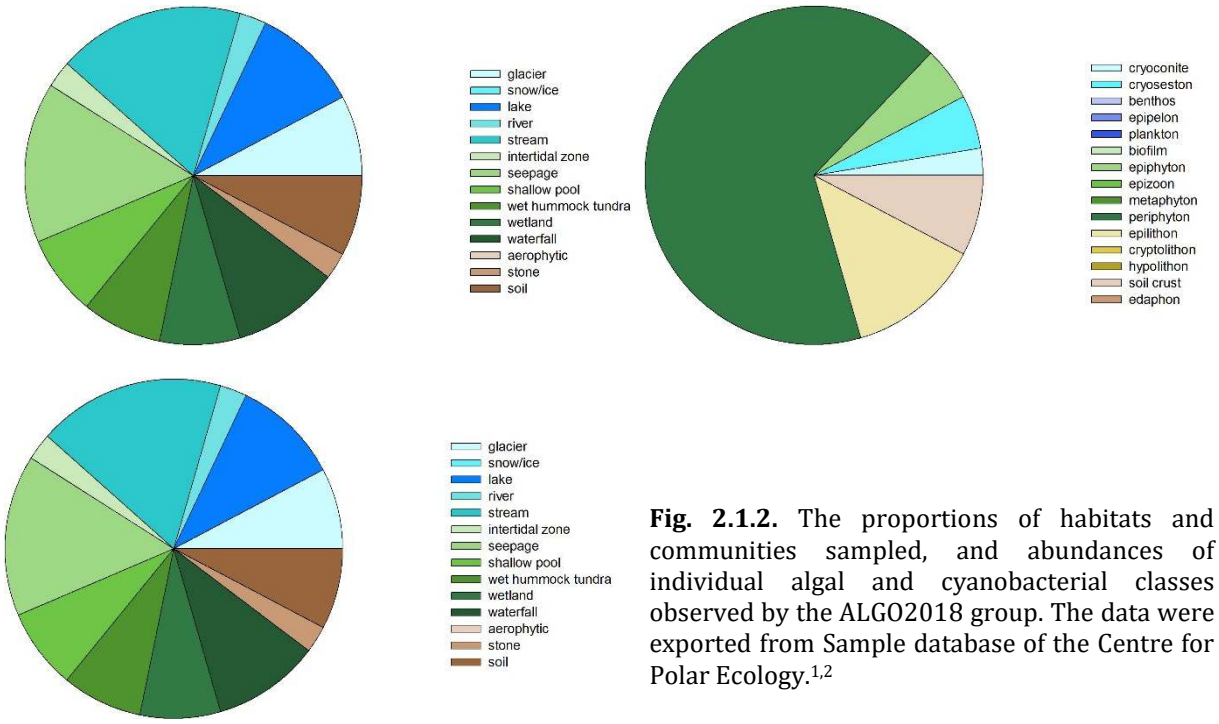


Fig. 2.1.2. The proportions of habitats and communities sampled, and abundances of individual algal and cyanobacterial classes observed by the ALGO2018 group. The data were exported from Sample database of the Centre for Polar Ecology.[1,2]

For the first time during our observations, we recorded green algae *Stichococcus pelagicus* and *Geminella* sp. (Fig. 2.1.3.), and desmids *Staurastrum* sp. and *Hyalotheca* sp. (Fig. 2.1.4.)

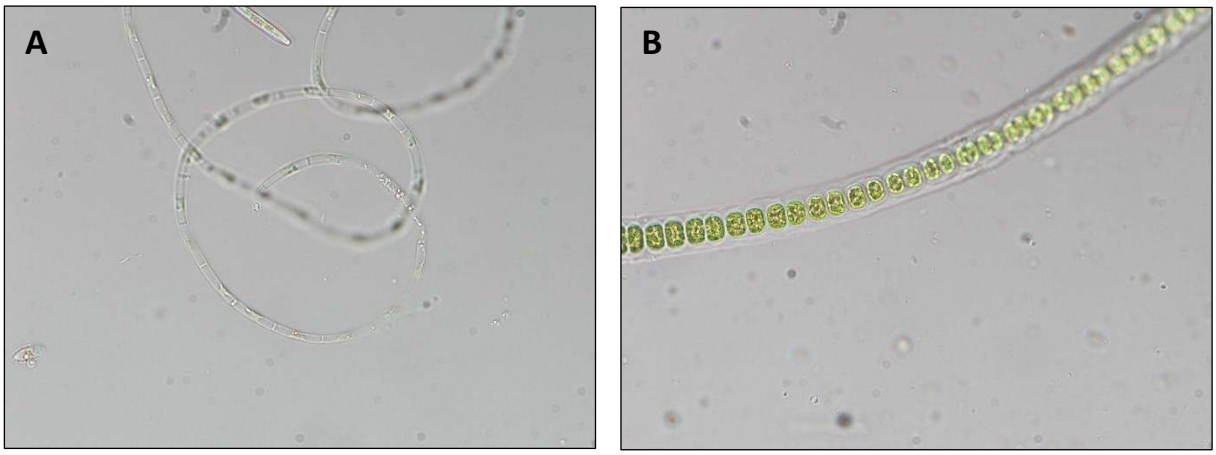


Fig. 2.1.3. Newly recorded taxons of green algae in our observations. **(A)** *Stichococcus pelagicus*, **(B)** *Geminella* sp.

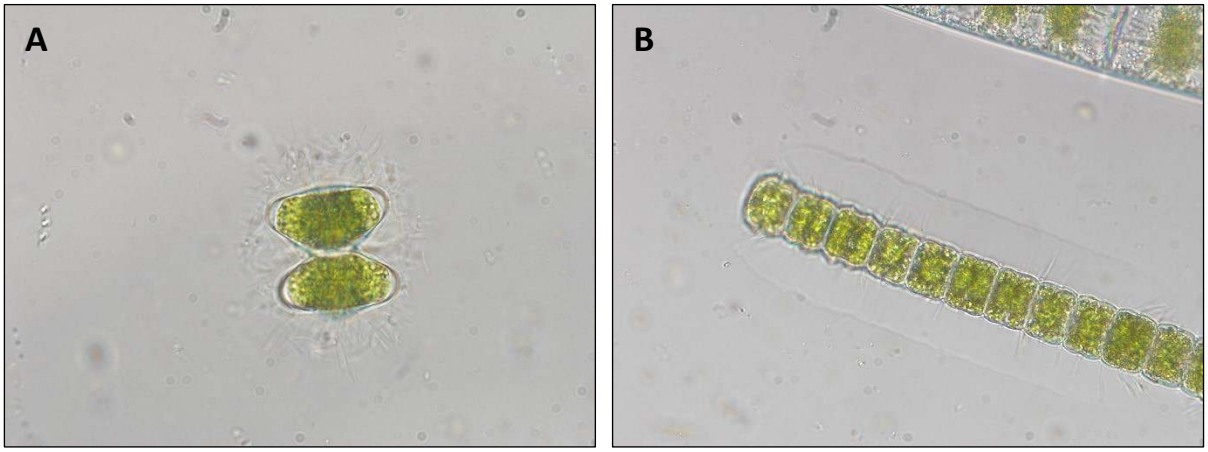


Fig. 2.1.4. Newly recorded taxons of desmids in our observations. **(A)** *Staurastrum* sp., **(B)** *Hyalotheca* sp.

References

[1]Kvíderová, J. (2014): Sample database of the Centre for Polar Ecology - Database design and data management. Czech Polar Reports, 4, 140-148.

[2]Kvíderová, J., Elster, J., Iliev, I. (2015): Exploitation of databases in polar research-Data evaluation and outputs. Czech Polar Reports, 5, 143-159.

2.1.2. Searching for valuable fatty acids in Svalbard microbial communities

Dominika Činčarová

Fatty acids are from the chemical point of view hydrocarbon chains ended with carboxyl group on the one site and methyl group on the second end of the chain. They are on the base of double bonds presence in the chain divided into saturated and unsaturated fatty acids. Unsaturated fatty acids have one double bond at least and if there are more than two double bonds we call them polyunsaturated fatty acids or briefly, PUFAs. Regarding to cell function unsaturated fatty acids have main role in fluidity of the cell membrane[1].

Mammals are not able synthetize linoleic acid (LA) and α- linolenic acid (ALA) which are precursors for other valuable fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). There are number of studies which found out connection between intake of these two acids (EPA and DHA) and beneficial properties on human health. Especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) show protective or even healing influence on many diseases such as for instance, heart disease or inflammation and their positive effect in treatment of rheumatoid arthritis was proven as well[2,3]. Although human body is able to create these two mentioned acids, conversion efficiency is very low[4].Due to this fact, their direct income from external source is very important.

Even though, the main PUFAs source is considered marine fish oil, there are many risks of its consumption (the presence of harmful contaminants, heavy metals, mercury)[5]. Another example of potential source is microalgae and valuable fatty acids obtained from them have even better purification potential than PUFAs achieved from fish[6].

I collected samples containing microbial communities from different Svalbard areas (Table 2.1.1.) which I used for lipid extraction and subsequent specifying of fatty acids composition.

Tab. 2.1. 1. List of the Svalbard areas of collected samples with expected microorganisms.

Graph label	Locality	Microorganisms
1	Skansbukta (rock bird cliff)	*Pinnularia* sp. *Ulothrix* sp.
2	Skansbukta (anhydride rock)	Uncertain marine alga
3	Unknown locality (upper soil crust)	unspecified yet
4	Pyramiden (metal eaves on the ground)	*Haematococcus pluvialis* *Nostoc* sp. *Oscillatoria* sp. *Phormidium* sp.
5	Foxfonna (shallow pool)	*Cylindrocystis brebissonii* *Leptolygbya* sp. *Phormidium* sp.
6	Foxfonna (snow)	*Chlamydomonas* cf. *nivalis*
7	Bjørndalen (seepage)	*Closterium* sp. *Cymbella* sp. Diatoms *Chroococcus* sp. *Phormidesmis* sp. *Zygnema* sp. *Hyalotheca* sp. *Woronichinia* sp.
8	Bjørndalen (flowing stream)	*Prasiola* sp. *Hydrurus foetidus* *Meridion circulare*
9	Mathiesondalen (hummock tundra seepage)	*Aphanothece* sp. *Chroococcus* sp. *Leptolyngbya* sp. *Phormidium* sp. *Pseudanabaena* sp. *Zygnema* sp.
10	Mathiesondalen (empty lake)	Diatoms *Klebsormidium* sp. *Navicula* sp.
11	Mathiesondalen (lake)	*Klebsormidium* sp.

Lipid extraction was done as follows: Lyofilizated samples were transferred into the 25ml Erlenmayer flasks and sonicated on the ice more than two hour in the mixture of distilled water, dichloromethane (DCM) and methanol in final ratio 2:2:2. After the extraction, samples were centrifuged on 800 rcf for 10 minutes. Three layers of the extract were created and bottom (DCM) layer with lipids presence was withdrawn. Lipids extracts were transesterificated where methylation is going on the border of hexane and methanolic HCl. Extracts prepared like that were used for Gas Chromatography (GC) analysis. Obtained data from GC were processed and following graph (Fig. 2.1.5.) was created.

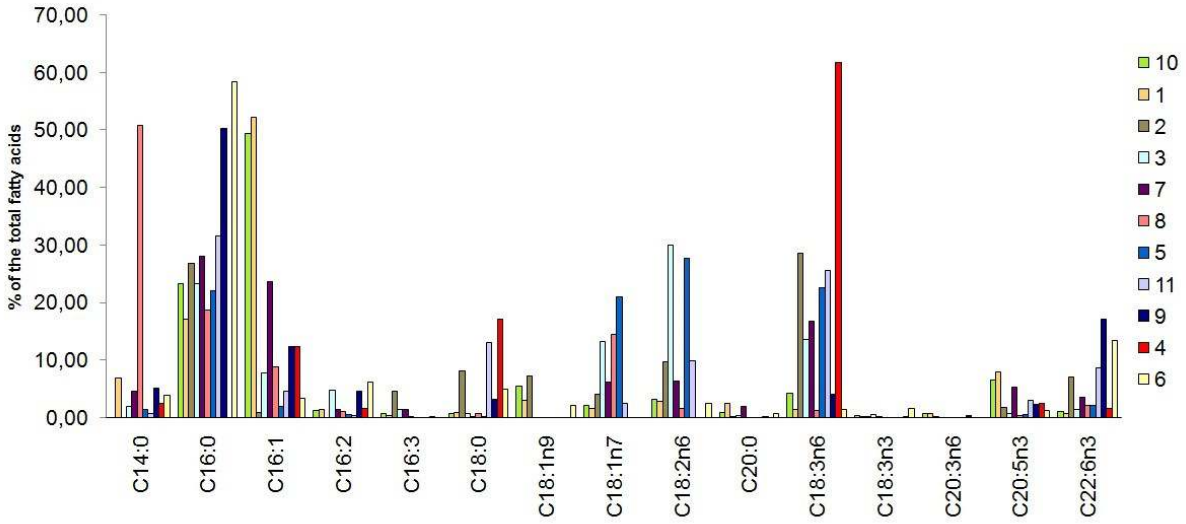


Fig. 2.1.5. Fatty acids (%) in different samples collected from Svalbard areas (no. 1-11).

Base on the graph we can conclude that samples from different Svalbard areas are differ in the fatty acids composition from each other.

In some of them was detected significant content of valuable unsaturated fatty acids. From this point of view, we can consider findings in sample no. 4 (Pyramiden – metal eaves) interesting, where amount of γ-linolenic acid (C18:3n6) achieved over 60% of its total fatty acids. Also, samples no. 9 (Mathiensondalen – hummock tundra) and no.6 (Foxfonna – snow) are noticeable because of the presence of the docosahexaenoic acid (C22:6n3). In both cases was its content more than 10% of its total fatty acids.

Others determined fatty acids are not so attractive for biotechnological industry.

However, there is not known how the results would look like after isolation of each microorganisms and determination of their fatty acids separately.

References

[1]Hagve, T. A. (1988): Effects of unsaturated fatty-acids on cell-membrane functions. Scandinavian Journal of Clinical & Laboratory Investigation, 48 (5), 381-388.

[2]Allayee, H., Roth, N. Hodis, H.N. (2009): Polyunsaturated fatty acids and cardiovascular disease: implications for nutrigenetics. Journal of Nutrigenetics and Nutrigenomics, 2 (3), 140-148.

[3]Calder, P. C. (2006): n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. The American journal of Clinical Nutrition, 83 (6 Suppl): 1505S-1519S.

[4]Qiu, X. (2003): Biosynthesis of docosahexaenoic acid (DHA, 22:6-4, 7,10,13,16,19): two distinct pathways. Prostaglandins, Leukotrienes and Essential Fatty Acids, 68 (2), 181-186.

[5]Sidhu, K. S. (2003): Health benefits and potential risks related to consumption of fish or fish oil. Regulatory Toxicology and Pharmacology 38 (3), 336-344.

[6]Pulz, O. and W. Gross (2004): Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology, 65 s(6), 635-648.

2.1.3. Use of wild samples from Svalbard in cell cycle research

Vít Náhlík

Algae and cyanobacteria are a suitable model organism for the study of cell cycles of algae and for biotechnological purposes. Many algae have high metal storage capacities and there is considerable potential for their use in recycling and bioremediation.[1] These model organisms are used in research concerning the recycling of lanthanides (REEs) and other rare metals.

Lanthanides are widely used in electronics and fertilizer production. Recycling of lanthanides is highly desirable, as the main exporter of this "industrial gold" is China, who recently began to use this natural resource as a tool to dictate political and economic conditions all the while reducing exports and continuing to use it within its own industry.[2,3]

In the polar regions, cyanobacteria and algae are primary producers.[4] In these areas, the cyanobacteria and algae are very well adapted to stress conditions such as low temperature, desiccation, freezing, and high salinity.[5] Some types of snow algae such as *Chlamydomonas* spp. and *Chloromonas* spp. are able to adapt to strong radiation by producing secondary carotenoids - astaxanthin etc. These algae species are interesting due to their potential use in biotechnology.[6]

Fig. 2.1.6. Cultivation of samples in nutrient media.

Each sample was taken placed in a 50ml Falcon tube, the GPS position registered, and the pH and temperature of the site measured. Samples were stored at 4-8 °C. Variation and types of cyanobacteria and algae were determined using light microscopy. Upon return to the Czech Republic, samples were seeded into BBM[7] and BG 11[8] nutrient broths. Subsequently, the samples were cleaned and innoculated into BBM and BG11 broths, supplemented with 1.5% agar and antibiotics (Ampicillin and Carbendazy) for better growth. Samples were cultured in under daylight for two weeks (Fig. 2.1.6.).

Samples from the creek from the mining town Pyramiden (Fig. 2.1.7.) showed interesting compositions of heavy metals in soil and water, subsequently samples were sent to the laboratory team of Mgr. Marian Rucký, Ph.D., from the State Institute of Health in Prague for ICP-MS analysis to obtain a more detailed profile of the heavy and rare metals present.

After two weeks of cultivation, surviving samples were selected, i.e. *Hydrurus foetidus* and *Nostoc* sp. These samples were frozen in liquid nitrogen to be used in further research.

Fig. 2.1.7. Coal mine in the ghost town Pyramiden.

References

[1]Mehta, S.K., Gaur, J.P. (2005): Use of algae for removing heavy metal ions from wastewater: Progress and prospects. Critical Reviews in Biotechnology, 25,113-152.

[2]Schwabe, A., Meyer, U., Grün, M., Voigt, K.D., Flachowsky, G., Dänicke, S. (2012): Effect of rare earth elements (REE) supplementation to diets on the carry-over into different organs and tissues of fattening bulls. Livestock Science, 143: 5–14.

[3]Tse, P.-K. (2011): China's rare earth industry, U.S. Geological Survey Open-File Report 2011–1042, pp.11.

[4]Zakhia, F., Jungblut, A.-D., Taton, A., Vincent, W.F., Wilmotte, A. (2008): Cyanobacteria in cold ecosystems. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds.) Psychrophiles: from biodiversity to biotechnology. Springer-Verlag, Berlin Heidelberg, pp 121-135.

[5]Elster, J., Benson, E.E. (2004): Life in the polar terrestrial environment with a focus on algae and cyanobacteria. In: Fuller BJ, Lane N, Benson EE (eds.) Life in the frozen state. CRC Press, Boca Raton, pp 111-150.

[6]Műller, T., Bleiß, W., Martin, C-D., Rogaschewski, S., Fuhr, G. (1998): Snow algae from northwest Svalbard: their identification, distribution, pigment and nutrient content. Polar Biology 20, 14-32

[7]Bischoff, H.W., & Bold, H.C. (1963): Phycological Studies. IV. Some soil algae from Enchanted Rock and related algal species. – Univ. Texas Publ., 6318: 1-95.
[8]Stainer, R.,Y., Kunisawa, R., Mandel, M., & Cohen-Bazire, G. (1971): Purification and properties of unicellular blue-green algae (Order Chroococcales). Bacteriological Reviews, 35: 171-205.

2.1.4. Entomopathogenic nematodes on Svalbard

Martina Flégrová

Nematodes are considered to be a very diverse group of animals; the number of species is currently estimated to more than one million species. Even though only a small fraction of this amount has been described until now, it is obvious that nematodes occur in various types of habitats, including tundra and polar areas.[1] Due to numerous adaptations and survival strategies, they can tackle even challenging life conditions like desiccation or freezing[2], and many groups of nematodes are believed to develop these skills independently.[3] Entomopathogeny might be one of those strategies; however, from the evolutionary point of view it is considered to be an impasse.[4,5] Entomopathogenic nematodes actively search the soil to find their host - insects larvae.[6] The infection is always lethal in this case – this is the main difference between classical parasitism and entomopathogeny.

Since there are other kinds of nematodes present on Svalbard, we decided to look for the entomopathogenic nematodes as well. The conditions on Svalbard are extremely tough; on the other hand, there are other living organisms on Svalbard including 230+ species of insects, in which some of their larvae live in the soil (e.g. flies, beetles etc.;[7]). This is essential assumption for the entomopathogenic nematodes to occur (correspondingly with the active layer of soil in summer months). In order to examine possible presence of entomopathogenic nematodes on Svalbard, 12 samples of soil were collected in different locations. We focused on habitats with vegetation, since it could possibly be grazed by herbivorous insect larvae and thus attract nematodes.

Each sample was carefully wrapped in a plastic bag and kept partially open to insure the air flow; furthermore we recorded its vegetation cover composition, GPS position and approximate temperature in situ. The samples were stored in 4-8 °C for about one week and examined in the laboratories of Entomological Institute (Academy of Sciences, Czech Republic) right after the field course. In the laboratory, we placed one or two individuals of the honeycomb moth, *Galleria mellonella*, in specialized cages depending on the weight of the sample. The cages were inserted directly into the soil and incubated them in approx. 16°C in the thermobox for a week (Fig. 2.1.8.).

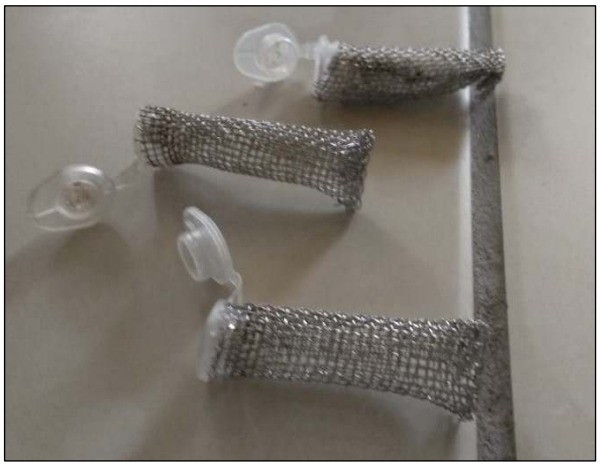


Fig. 2.1.8. Specialized cages for *Galleria mellonella*.

The honeycomb moth normally lives in bee hives, therefore it is an ideal „naive“ host for virulence testing in entomopathogenic nematodes, since it has no adaptive immunity. It has been successfully used in many studies, so it would be suitable for comparison of our results in wider context.[8,9]

After the seven days of incubation the mortality rate check was performed in *Galleria* larvae.

Since the mortality level was very low (only one larva out of 16 was found dead), we repeated the incubation for one more week and after the evaluation we found seven cadavers in total. In four of them, the cause of death was probably fungus, since the cadavers displayed signs of fungal infection. Remaining three cadavers (namely from samples collected in hammock tundra in Petuniabukta, slope vegetation in Skansbukta and tundra vegetation under Svenbreen) showed typical marks of entomopathogenic infection. Those cadavers were dissected under the microscope to uncover possible presence of entomopathogenic nematodes. Unfortunately, no nematodes were found during and after the dissection, so we conclude that there are no entomopatoghenic nematodes present in our samples. Since the sample collection was focused on inspecting different habitats and vegetation cover types, we hypothetize that there might be no entomopathogenic nematodes on Svalbard (Fig. 2.1.9.).

Fig. 2.1.9. Microbiology group: tea break in the field.

References

[1]Sommer, R., Streit, A. (2011): Comparative genetics and genomics of Nematodes: genome structure, development and lifestyle. Annual Review of Genetics, 45 (1), 20.

[2]Perry R., (1999): Desiccation survival of parasitic nematodes. Parasitology, 119, 109-130.

[3]Blaxter, M., Koutsovoulos, G. (2014): The evolution of parasitism in Nematoda. Parasitology, 142, 26-39.

[4]Dillmann, A., Chaston, J.M., Adams, B.J., Ciche, T.A., Goodrich-Blair, H., Stock, S.P., Sternberg, P.W. (2012): An entomopathogenic Nematode by any other name. PLoS Pathogens, 8 (3), e1002527.

[5]Sudhaus, W. (1993): The nematode genera *Heterorhabditis* and *Steinernema*, both entomopatogenic by means of symbiotic bacteria, are not sister taxa. Verhandlungen der Deutschen Zoologischen Gesseschaft, 86, 146.

[6]Nermuť, J., Půža, V., Mráček, Z. (2012): Entomopathogenic and molluscoparasitic nematodes – the invisible soil killers. Živa, 1/2012, 10.

[7]Svalbardinsects.net (2018). The insects of Svalbard. Downloaded 26.10.2018 from http://svalbardinsects.net

[8]Noosidum, A., Hodson, A., Lewis, E., Chandrapatya A. (2010): Characterization of new entomopathogenic Nematodes from Thailand: foraging behavior and virulence to the Greater Wax Moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). Journal of Nematology, 42 (4), 281-291.

[9]Půža, V., Mráček, Z. (2009): Mixed infection of *Galleria mellonella* with two entomopathogenic nematode (Nematoda: Rhabditida) species: *Steinernema affine* benefits from the presence of *Steinernema kraussei*. Journal of Invertebrate Pathology, 102 (1), 40-43.

2.1.5. Metazoan fauna in cryoconite holes of Svalbard. Genetics and ecological features

Luca Sanchez

Between July and August 2018 I was part of the project titled ''Metazoan fauna in cryoconite holes of Svalbard, Genetical and Ecological features'' supervised by RNDr. Miloslav Devetter, Ph.D.

The project concerns the genetic and ecological study of metazoic fauna, such as Rotifers and Tardigrades, microscopic animals in our case present in the sediment presents in cryoconite holes of the Svalbard glaciers (Figs. 2.1.10. and 2.1.11.).

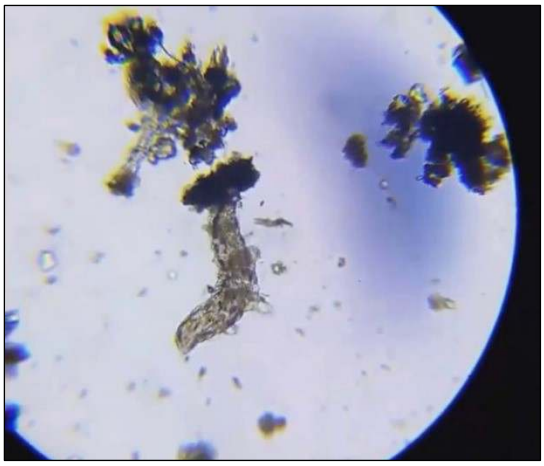


Fig. 2.1.10. Sediment sample.

Fig. 2.1.11. Rotifer.

My task was to continue a part of the work of the student Margherita Lucadello who went to the same place during the summer of 2017.

During my stay in the Nostoc station we sampled, collected data (degrees and direction of the slope, depth, width, length) and marked with spray paint the cryoconite to be able to recognize them avoiding to sample them more than once or to be able to sample again in the following days. The total number of cryoconite from which we have taken samples are approximately 60 from 3 different glaciers, Mimerdalen, Nordenskioldbreen and Horbyebreen through the use of materials like sampling ring, sampling pump, tape measure, waterproof notebook, marker pen, bottles for sampling, ziplock bags, spray can. The cryoconite have been sampled using standard measures thanks to the use of the same sampling ring.

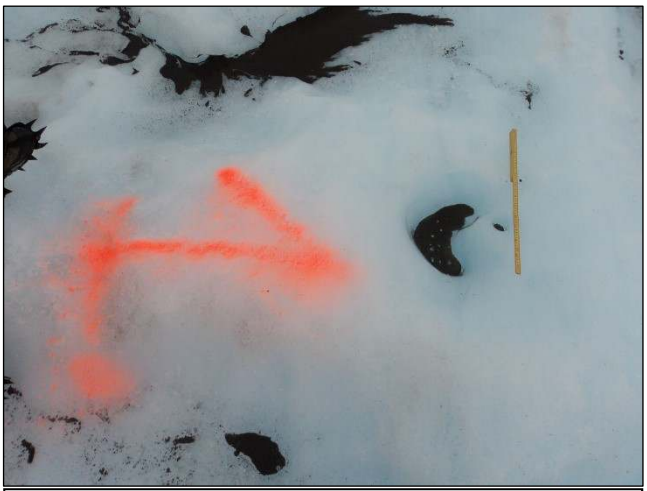


Fig. 2.1.12. Experimental cryoconite.

Tab. 2.1.2. The results of the calculation for Sed= 0.5ml / Water= 6ml.

Site	Rotifers	Tardigrades
Ling 06	Ind	12
N Frank 10	Ind	15
Goose Bukta 10	Ind	43
Dunne 3	Ind	0
Dunne 5	Ind	0
Dunne 6	Ind	1
Dunne 7	Ind	0
Dunne 10	?	0
Nord 1	17	43
Nord 2	50	29
Nord 3	27	11
Nord 4	52	21
Nord 5	6	8
Nord 6	12	59
Nord 7	9	34

Fig. 2.1.13. Cryoconite.

Once in the Nostoc station I counted the number of Tardigrade and Rotifers present in the samples through the use of an optical microscope and a petri dish to place the sediments. For each sample I took 0.5ml of sediment and added 6ml of distilled water, then observed under an optical microscope with a 40x magnification (Tab. 2.1.2.). The remaining samples were stored in the bear-proof freezer of the NOSTOC Field Station for future analysis.

2.2. Botany/Plant Physiology

Instructors: ***Tomáš Hájek & Petr Macek***
Students: ***Hana Dvořáková, Lada Klimešová, František Trkal & Stanislava Wolfová***

2.2.1. Tree-ring analysis of *Empetrum hermaphroditum* in Svalbard

František Trkal

Studies of tundra shrubs have become integral part of Arctic activities. The range of their applications varies significantly in fields such as population ecology, carbon storage, permafrost thaw, or environmental reconstructions. However, ecology as such has been chronically understudied for many shrub tundra species.

Under the extreme growth conditions in high arctic, dwarf shrubs are the only woody life form that is able to withstand the harsh environment. In comparison with trees, additional steps of analysis are required to extract the common growth patterns of dwarf shrubs. Extremely small ring-widths and numerous anomalies in growth formations make the dendrochronological analysis of dwarf shrubs difficult and time consuming. Nevertheless, dwarf shrubs attract research interest due to the large number of new species for dendroecological analysis in areas without tree cover.

For purpose of this work dwarf shrub *Empetrum hermaphroditum* was chosen as the most suitable species. Selection criteria were the expression of clearly visible, countable and measurable annual growth rings and occurrence both in Arctic and Krkonoše mountains. Populations of both areas will be compared in the future.

The collection of individuals for this work was carried out in the Colesbukta (78.1091750N, 15.0400622E) in Svalbard. In august 2018, 40 complete individuals including the woody root and branch system were collected in the field. The physiognomic structure of each individual was documented by a digital photo and the position of the soil surface was marked with a tape. All samples were collected from south facing slope, 50 – 150 meters a.s.l. Samples were all preserved in zip-lock bags with 40% alcohol.

For more reliable data was every individual cut in more parts (serial sectioning). In order to explore oldest part of an individual, first cross section was taken from stem base, close to root collar. However, the main root was often replaced by adventitious root, so that root, stem and branches were often difficult to distinguish. Distance from one cross section to another was always measured and write down.

Microtome sections of 12 – 15 μm thickness were prepared from the whole diameter of selected segments using a sledge microtome. After staining with safranin and astrablue, sample was rid of water using 96% ethanol and stuck in Canada balsam.

Ring widths were measured along two radii using a microscope and the software TSAPWin. The examination of complete cross sections allowed a careful determination of the measured radii with minimum number of discontinuous rings or scars.

Till now 49 radii were measured (Tab. 2.2.1.). The biggest number of measured tree rings in one radius was 68. The lowest number was 5. Mean annual increment of all individuals was 0.08 mm per year with standard deviation 0.02 mm. Because of measuring the distances between cross sections, we can calculate mean annual primary growth between cross sections. Mean primary growth of all individuals was 1.24 cm/yr with standard deviation of 0.88 cm/yr.

Tab. 2.2.1. Summary of existing data

Sample	Usable cross sections	Max number of tree rings radius	Mean annual increment (mm)	Mean primary growth (cm/yr)
E1	1	27	0.09	-
E2	5	68	0.07	0.6
E23	5	23	0.12	3.0
E31	2	32	0.07	0.8
E37	3	33	0.08	0.4
E39	5	42	0.07	1.4
E40	2	22	0.07	1.6

This paper introduces approach for dwarf shrub dendrochronology. We can assume that examined individuals start to grow between years 1945 and 2000. It's not possible to reliably determine exact start of growth of an individual because samples have not been cross-dated yet. Till now it was done several first steps, however, there is a lot of work ahead before we will have some solid data. At first whole collection of Svalbard samples needs to be measured. For correct dating of tree rings, we need to correct measured data with cross dating software (PAST4 for this purpose). Properly dated tree rings will be used for comparison of growth of *Empetrum hermaphroditum* with climatic conditions in Svalbard and to try to estimate main limiting factors for growth of this species. Data from this locality will be compared with data of *E. hermaphroditum* from Krkonoše mountains as a representative of tundra ecosystem in Czech Republic.

References

Bär, A., Bräuning, A., Löffler, J. (2006): Dendroecology of dwarf shrubs in the high mountains of Norway–A methodological approach. Dendrochronologia, 24 (1), 17-27.

Bär, A., Pape, R., Bräuning, A., Löffler, J. (2008): Growth-ring variations of dwarf shrubs reflect regional climate signals in alpine environments rather than topoclimatic differences. Journal of Biogeography, 35 (4), 625-636.

Buchwal, A., Rachlewicz, G., Fonti, P., Cherubini, P., Gärtner, H. (2013): Temperature modulates intra-plant growth of *Salix polaris* from a high Arctic site (Svalbard). Polar Biology, 36 (9), 1305-1318.

Trkal, F., LEHEJČEK, J. (2017): Primary growth of *Juniperus communis* in southwest Greenland. Czech Polar Reports, 7 (2), 331-333.

2.2.2. Climate impact on growth rate of *Silene acaulis*

Stanislava Wolfová

Silene acaulis (Fig. 2.1.1.) is a cushion-forming gynodioecious plant and the most widespread alpine cushion plant in the Northern Hemisphere.[1] It generally grows on wind exposed ridges, rocky slopes, and open alpine grasslands between 1700 and 2400m in elevation. *Silene acaulis* can survive extreme temperatures from -80 to 60°[2], and the dense, dome-shaped structure has been shown to moderate temperature, reduce wind, increase moisture, and increase soil nutrients.[2,3]

Silene is well known as a nurse plant because it ameliorates microclimatic conditions by yielding warmer temperatures, creating more stable moisture conditions and improving soil quality within its compact canopy.[2,4]

Cushion plants are specifically adapted to the alpine climate because their low stature and compact form make them heat traps that decouple their internal climate from the outside.[2]

Fig. 2.2.1. *Silene acaulis* in the cage area.

With increasing habitat loss due to climate change, cushion plants can thus be a critical first step in assessing the responsiveness of community to change.[5] Cushion morphological changes are associated with mitigating effects on microclimate, indicating that cushions effectively act as a heat-trap.[5]

Alpine ecosystems are important globally with high levels of endemic and rare species. Given that they will be highly impacted by climate change.[4]

Small open-top chambers (OTC; (Fig. 2.2.2.) are used widely in ecosystem warming experiments. The efficacy of the open-top as an analogue of climatic warming is examined.[6] They are used to passively increase temperature (as a small greenhouse) in tundra at Svalbard. They do not require technological maintenance. The open top design allows free air

Fig. 2.2.2. 50 experimental areas in Pyramiden.

exchange and minimizes undesirable chamber effects and access of herbivores. OTCs modify the microenvironment, in order to interpret plant response.

Recently, there has been considerable interest in temperature relations of species in response to predicted global temperature rise.[7] The devices are used to manipulate temperature in order to forecast responses of species to climate change. This is particularly relevant in the Arctic Tundra, where the temperature change is seen.

The data presented here were collected in July and August between the seasons 2014-2018. In season 2018 the increase was measured for twice as an increase during the season. From 2014 until 2015, OTCs and cages have not yet been installed. This baseline data serves as the initial state of the objects.

The data were collected at the beginning of June and at the end of August. It was measured at the two sites. The first study site is located in Pyramiden on Svalbard (78°38'N 16°11'E) and the second one is located near the Josef Svoboda station - AWS (78°42'N 16°26'E). In both of the spots, there were 50 experimental areas of which it was 15 OTCs, 20 controls and 15 cages. *Silene* was monitored in each plot. The radical increment during the season was average 4.2 mm in Pyramiden and 8.3 mm in AWS.

Statistics program was used to evaluate the data (ANOVA). Individual measurements from the years are interdependent. This must be matched by the variance analysis model used to evaluate such data - ANOVA Repeated Measures.

It was started by verifying the assumption of variance homogeneity (Bartlett). The probability of the first type error is too high to reject the zero hypothesis of match variance. This means that the assumption of homogeneity variations was not contested.

Tab. 2.2.2. AWS. ANOVA Repeated Measures results.

Repeated measures Analysis of Variance (AWS)					
Effect	SS	Degr. Of Freedom	MS	F	p
Plot	101.735	2	50.868	1.2377	0.299957
Error	1808.367	44	41.099		

In the case of both spots we have not rejected a zero hypothesis (H0 = surface type has no effect on the increase of the *Silene acaulis* cushion). Pyramiden (p=0,2999; df = 2; F =1,2377; Tab. 2.2.2.) and AWS (p =0,0737; df = 2; F =2,7609; Tab. 2.2.3).

Tab. 2.2.3. Pyramiden. ANOVA Repeated Measures results.

Repeated measures Analysis of Variance (Pyramiden)					
Effect	SS	Degr. Of Freedom	MS	F	p
Plot	125.678	2	62.839	2.7609	0.073729
Error	1046.98	46	22.76		

Regarding to that there have not been proven the ANOVA testing conclusively, it makes no sense to do Multiple Comparison (Tukey Test) if there is a significant difference between test groups.

In the graph, we can see that there is little difference in the Pyramiden area. It can also be seen that the greatest increase is in the *Silene* measured in OTCs.

It is based on long-term observation. It is a permanent experiment. The fact that ANOVA results did not show that the surface type had effect on the growth of the *Silene acaulis* cushion could be changed in the future. Data is known only from observing a couple of seasons in a low-dynamic tundra (Figs. 2.2.3. and 2.2.4.).

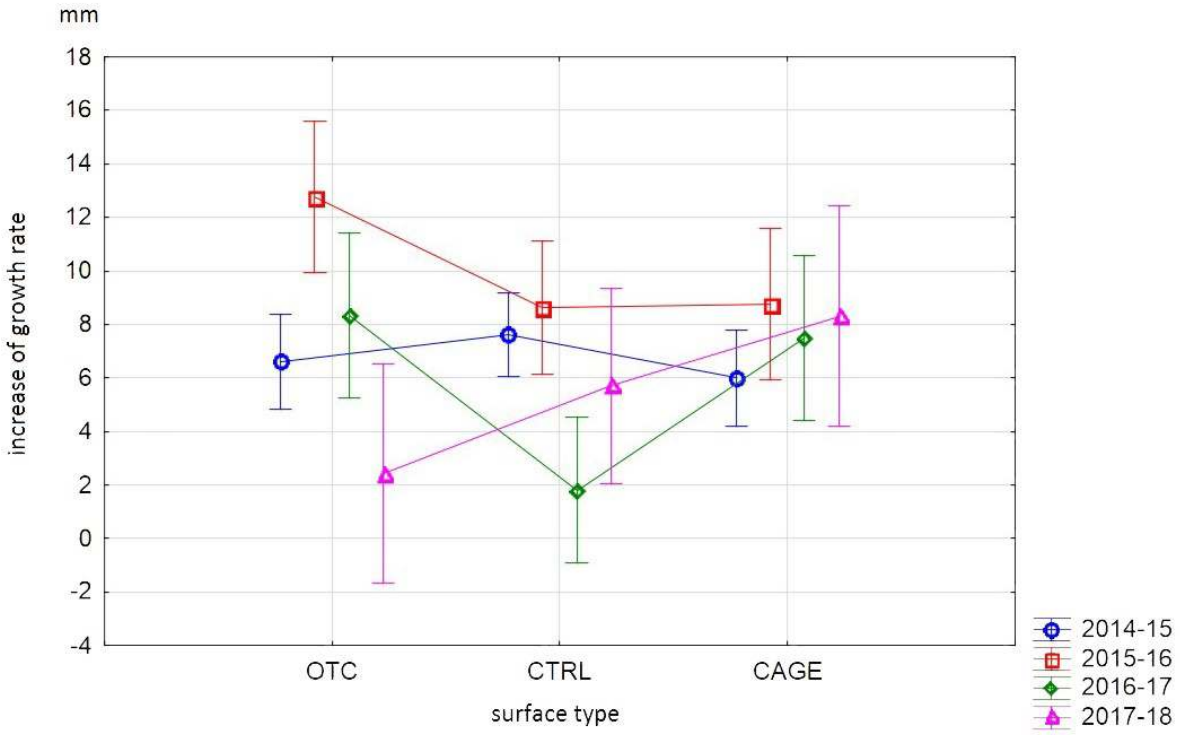


Fig. 2.2.3. The yearly increase depending on the surface type at the AWS.

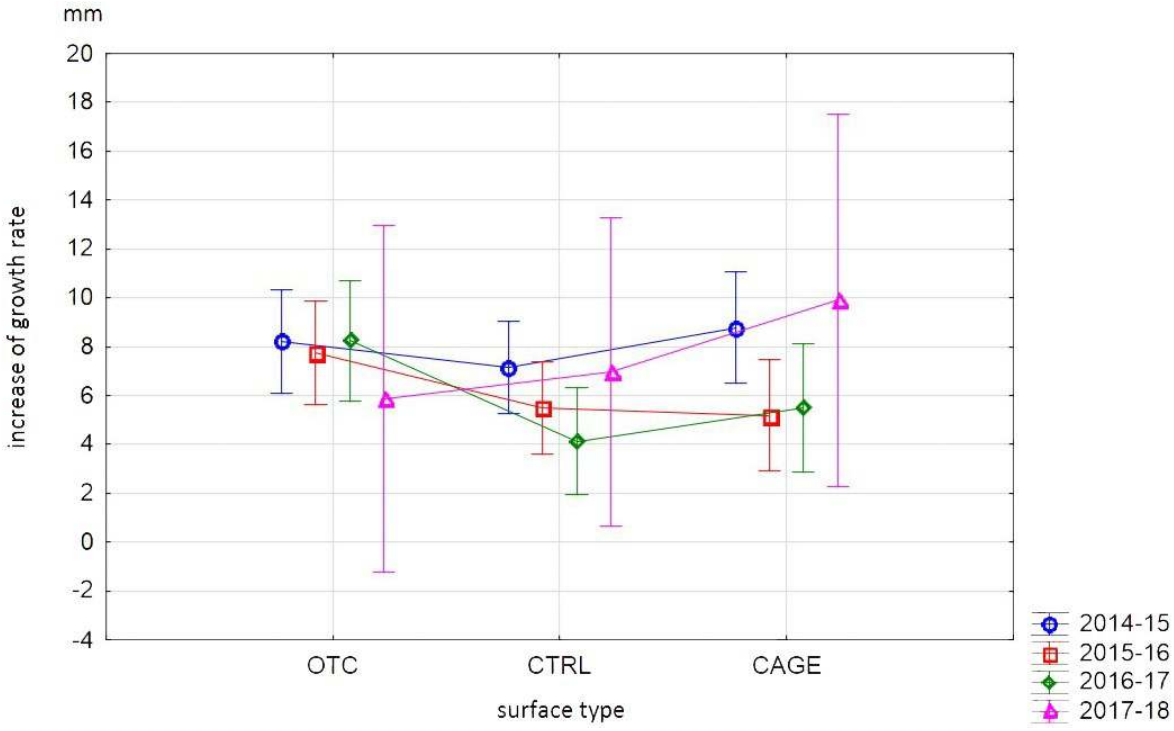


Fig. 2.2.4. The yearly increase depending on the surface type at Pyramiden.

References

[1]Körner, C. (2003): Alpine Plant Life. Functional Plant Ecology of High Mountain Ecosystems, 2nd edn. Springer-Verlag, Berlin, Germany.

[2]Larcher, W., Kainmuller, C., Wagner, J. (2010): Survival types of high mountain plants under extreme temperatures. Flora 205, 3–18.

[3]Körner, C., Demoraes, J. (1979): Water potential and diffusion resistance in alpine cushion plants on clear summer days. Oecologia Plantarum, 14, 109–120.

[4]Molenda, O., Reid, A., Lortie, C.J. (2012): The alpine cushion plant *Silene acaulis* as foundation species: a bug's-eye view

to facilitation and microclimate. PLoS ONE, 7, e37223.
[5]Bonanomi, G., Stinca, A., Chirico, G.B., Ciaschetti, G., Saracino, A. & Incerti, G. (2016): Cushion plant morphology controls biogenic capability and facilitation effects of Silene acaulis along an elevation gradient. Functional Ecology, 30, 1216–1226.
[6]Hollister, R.D., Webber, P.J. (2000): Biotic validation of small open-top chambers in tundra ecosystem. Global Change Biology, 6, 835-842.
[7]Henry, G.,H.R., Molau, U. (1997): Tundra plants and climate change: The International Tundra Experiment (ITEX). Global Change Biology, 3, 19.

2.2.3. Influence of OTCs on reproductive traits of plants living inside and outside cushions

Hana Dvořáková

The data were collected in the two experimental localities in Pyramiden and in Petuniabukta, where open top chambers (OTCs) were installed 3 years ago to simulate a climatic change (slight temperature increase) and observe its effects on the local plant species' functional traits and interaction. My project aimed to compare reproductive effort of the most abundant species at two levels; 1) by comparing differences in reproductive traits between plants living inside OTC and in control plots, 2) by looking for differences between plants living inside cushions of *Silene acaulis* and solitarily. In the first case, there were expected differences due to increased temperature inside OTCs. In the second case, the potential variability could be related to the nursing effect of the cushion plants interactions that was proved to enhance soil resources[1,2] and mitigate climatic extremes[3] in Alpine ecosystems.

The most pronounced effects of OTCs were observed on reproductive height and seed mass of *Bistorta vivipara* and *Silene acaulis*, the two most abundant species (Fig. 1 and 2). The effects of cushion plant interactions did not turn out significantly in any species. However, we can observe some positive effects of both cushions and OTCs on the germination success of seeds of various species (Fig. 3).

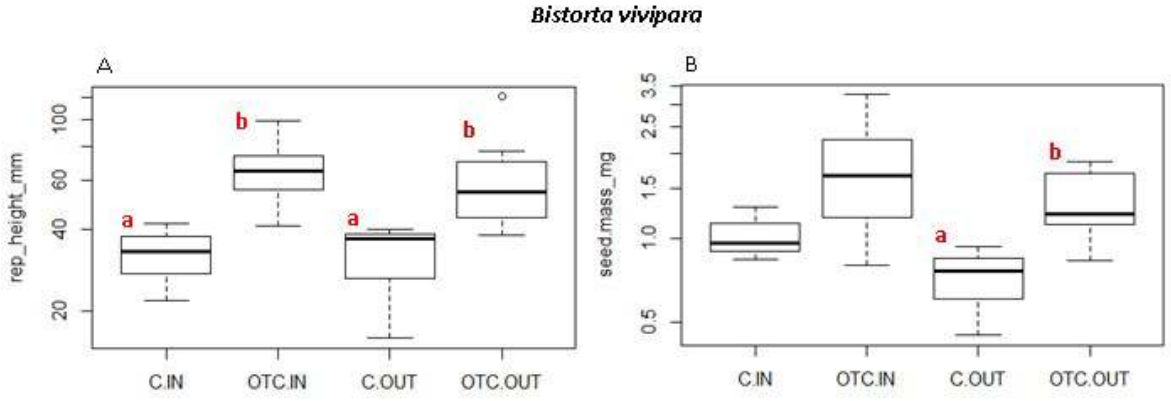


Fig. 2.2.5. Positive effect of living inside OTC on **(A)** reproductive height and **(B)** seed mass of *Bistorta vivipara*. Abbreviations: C - control plot, OTC - open top chamber, IN - inside cushion, OUT - outside cushion.

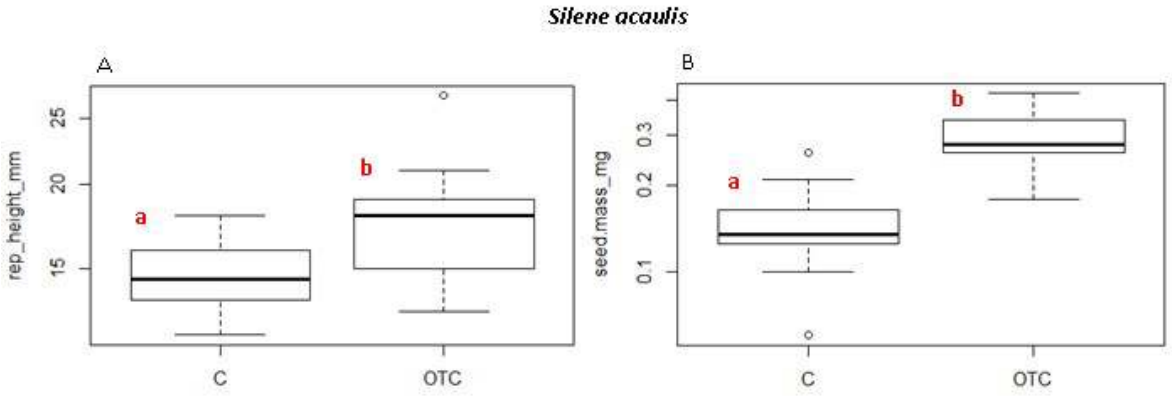


Fig. 2.2.6. Positive effect of living inside OTC on **(A)** reproductive height and **(B)** seed mass of cushion species *Silene acaulis*. Abbreviations: C - control plot, OTC - open top chamber.

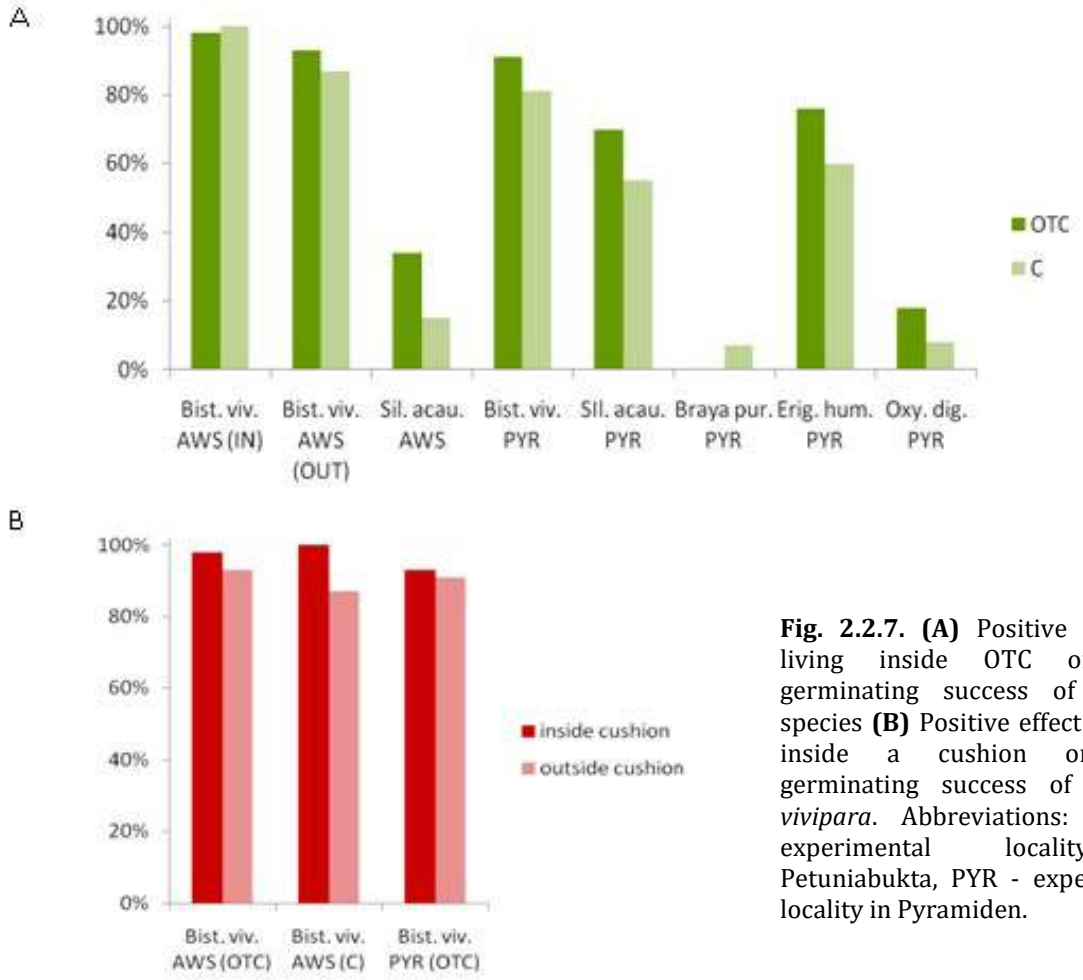


Fig. 2.2.7. (A) Positive effect of living inside OTC on seed germinating success of various species **(B)** Positive effect of living inside a cushion on seed germinating success of *Bistorta vivipara*. Abbreviations: AWS – experimental locality in Petuniabukta, PYR - experimental locality in Pyramiden.

References

[1]Arroyo, M. T. K., Cavieres, L.A., Peñaloza, A., Arroyo-Kalin, M.A. (2003): Positive associations between the cushion plant *Azorella monantha* (Apiaceae) and alpine plant species in the Chilean Patagonian Andes. Plant Ecology, 169 (1), 121-129.

[2]Schöb, C., Armas, C., Guler, M., Prieto, I., Pugnaire, F. I. (2013): Variability in functional traits mediates plant interactions along stress gradients. Journal of Ecology, 101 (3), 753-762.

[3]Cavieres, L.A., Badano, E. I., Sierra-Almeida, A., & Molina-Montenegro, M. A. (2007): Microclimatic modifications of cushion plants and their consequences for seedling survival of native and non-native herbaceous species in the

high Andes of central Chile. Arctic, Antarctic, and Alpine Research, 39 (2), 229-236.

2.2.4. Research of arbuscular mycorrhiza by selected plant species of snowbeds

Lada Klimešová

Mycorrhiza is a concept of a (mostly) symbiotic association between a fungus and a root system of some plants. It is possible to distinguish between two types of root colonization, firstly intracellular and secondly extracellular. Arbuscular mycorrhiza (AM) is the most common type of intracellular association between a fungus and plants[1], which occurs by 80% of all plants.[2]

Research of AM is especially important in grasslands[3] but it plays an important role also in colder regions such are arctic regions.[4] The occurrence of AM was also confirmed by several species in Svalbard[5], they have observed mycorrhizal structures by: *Alopecurus ovatus*, *Deschampsia alpina*, *Festuca rubra* ssp. *richardsonii*, putative viviparous hybrids of *Poa arctica* and *Poa pratensis*, *Poa arctica* ssp. *arctica*, *Trisetum spicatum*, *Coptidium spitsbergense*, *Ranunculus nivalis*, *Ranunculus pygmaeus*, *Ranunculus sulphureus* and *Taraxacum arcticum*. On the other hand there are also studies from Spitzbergen which have not been successful in findings of AM.[6]

I have assumed that AM could occur later during succession therefore can be problematic to spot it in yearly successional stages of plant communities.[6] I have selected for my study three snowbeds plant species: *Oxyria digyna* (AM recorded in Myko-database, by Inga Hiiesalu), *Rannunculus pygmaeus* (AM recorded from Svalbard[5]) and *Silene acaulis* (AM is not recorded).

WI have collected three individuals of each specie at three to five localities which were preferably chosen together for at least two species. First of all I have excavated several roots for selected plants and kept them dried by silica gel till final evaluation. Lately I have evaluated the plant roots in a laboratory.

The roots were hydrated by placing them for one hour in water and then soaked in solution of KOH for about 10 hours. After the first treatment which lighten root structures is possible to color them by boiling for three minutes in solution of ink and vinegar. Eventually, I have observed the structures of each root samples and recorded the roots for any signs of AM.

I have not found any AM in the collected samples which could be caused by small sampling number. Moreover, the abundance of mycorrhiza is smaller than in warmer regions.

References

[1]Gryndler, M., Baláž, M., Hršelová, H., Jansa, J., Vosátka, M. (2004): Mykorhizní symbióza: O soužití hub s kořeny rostlin. Academia, Praha.

[2]Smith, S.E., Read, D.J. (2008): Mycorrhizal symbiosis, 3rd edn. Academic Press, San Diego, California.

[3]Hartnett, D.C., Wilson, G.W.T. (2002): The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. Plant and Soil, 244, 319–331.

[4]Pietikäinen, A., Kytöviita, M.-M., Husband, R., Young, J.P.W. (2007): Diversity and persistence of arbuscular mycorrhizas in a low-Arctic meadow habitat. New Phytologist, 176, 691–698.

[5]Newsham, K.K., Eidesen, P.B., Davey, M.L., Axelsen, J., Courtecuisse, E., Flintrop, C., Johansson, A.G., Kiepert, M., Larsen, S.E., Lorberau, K.E., Maurset, M., McQuilkin, J., Misiak, M., Pop, A., Thompson, S., Read, D. J. (2017): Arbuscular mycorrhizas are present on Spitsbergen. Mycorrhiza, 27, 725–731.

[6]Těšitel, J., Těšitelová, T., Bernardová, A., Janková Drdová, E., Lučanová, M. Klimešová, J. (2014): Demographic population structure and fungal associations of plants colonizing High Arctic glacier forelands, Petuniabukta, Svalbard. Polar Research, 33: 20797.

2.3. Zoology/Parasitology

Instructors: ***Miloslav Devetter, Oleg Ditrich & Václav Pavel***
Students: ***Hana Dvořáková, Lada Klimešová, František Trkal & Stanislava Wolfová***

2.3.1. Invertebrate predation of freshwater zooplankton

Daniel Bartoň

Aim of the project was to evaluate ichthyofauna of Petuniabukta. In previous years all fish were caught for parasitology purposes and no proper knowledge of benthic ichthyofauna of the bay where Czech polar station is located was recorded. Reviewing protocols from the previous years we found out that only season 2009 can provide strong data of gillnet fish catch so to compare ichthyofauna of Petuniabukta we decided to duplicate methodology to see if we could record some change in fish diversity. In recent years there are many studies of polar ward migration of fish fauna including fish as a response to the global warming. [1,2]

According to data from parasitology research from season 2009 the fish were caught using benthic gillnets (Fig. 2.3.1.). Unfortunately, no depth was recorded so we put nets into random depths but in similar spots as remembered from the comparing season. The nets used for the study were European Standard EN 14757 gillnet with mesh sizes that range from 5 to 55 mm (knot-to-knot) for the standard monitoring of fish which are commonly used and very effective for catching fish smaller than approximately 30 cm of standard length.[3] Nets were set for approximately for 12 hours every time from August the 1st to August 20.

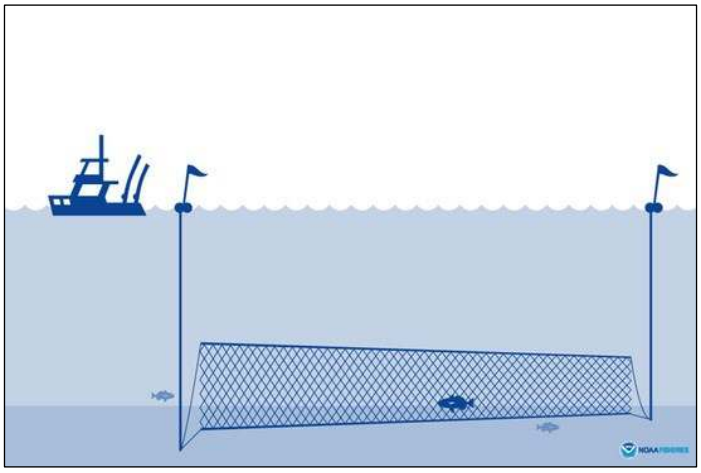


Fig. 2.3.1. Benthic gillnet set on the bottom (Fishing Gear: Gillnets | NOAA Fisheries, 2018[4]) Retrieved from https://www.fisheries.noaa.gov/national/bycatch/fishing-gear-gillnets .

In total, we caught 9 fish species which is similar to 2009 but capelin was missing that year in the catch (Tab. 2.3.1.). What was also interesting that in the year 2009 only 5 snakeblennies were caught but this season it was the second most abundant species (23.43 %). All the fish were caught near the bottom and close to shore from depth of 2 meters to 25 meters. Both of the sculpins and Snakeblenny are strictly benthic bottom living fish, rest of the species can use both benthic and pelagic habitat[5] but in this case were caught in benthic nets. In recent years poleward expansion of Atlantic cod to areas of Svalbard where Polar cod were more dominant was reported.[6] Much higher abundancy of polar cod in the benthic area could support theory that other cods in pelagic areas are forcing smaller native Polar cod to hideouts near shore by predation and diet competition (Fig. 2.3.2.).

Tab. 2.3.1. Total catch and relative abundancies of fishes. Note: number of nets is different from 2009 (12 nets) to 2018 (17 nets).

Species	2009		2018	
	Total number of catch	Relative abundancy (%)	total number of catch	relative abundancy (%)
American plaice *Hippoglossoides platessoides*	3	1.90	9	2.57
Arctic staghorn sculpin *Gymnocanthus tricuspis*	34	21.52	46	13.14
Atlantic cod *Gadus morhua*	1	0.63	8	2.29
Atlantic herring *Clupea harengus*	12	7.59	16	4.57
Atlantic salmon *Salmo salar*	1	0.63	1	0.29
Capelin *Mallotus villosus*	0	0.00	13	3.71
Polar cod *Boreogadus saida*	14	8.86	55	15.71
Shorthorn sculpin *Myoxocephalus scorpius*	88	55.70	120	34.29
Snakeblenny *Lumpenus lampretaeformis*	5	3.16	82	23.43
total	**158**		**350**	

Fig. 2.3.2. Polar cod *Boreogadus saida* caught by benthic gillnets.

References

[1]Beaugrand, G. (2009): Decadal changes in climate and ecosystems in the North Atlantic Ocean and adjacent seas. Deep-Sea Research Part II: Topical Studies in Oceanography, 56 (8-10), 656-673.

[2]Gregory, B., Christophe, L. and Martin, E. (2009): Rapid biogeographical plankton shifts in the North Atlantic Ocean. Global Change Biology, 15 (7), 1790-1803..

[3]Šmejkal, M., Ricard, D., Prchalová, M., Říha, M., Muška, M., Blabolil, P., Čech, M., Vašek, M., Jůza, T., Herreras, A.M., Encina, L. (2015): Biomass and abundance biases in European standard gillnet sampling. PLoS ONE, 10(3), e0122437.

[4]Fishing Gear: Gillnets | NOAA Fisheries (no date). Available at: https://www.fisheries.noaa.gov/national/bycatch/fishing-gear-gillnets (Accessed: 1 November 2018).

[5]Helfman, G.S., Collette, B.B., Facey, D.E., Bowen, B.W. (2009): The diversity of fishes: biology, evolution, and ecology, Atlantic. John Wiley & Sons, Oxford.

[6]Renaud, P.E., Berge, J., Varpe, Ø., Lønne, O.J., Nahrgang, J., Ottesen, C., Hallanger, I. (2012): Is the poleward expansion by Atlantic cod and haddock threatening native polar cod, *Boreogadus saida*? Polar Biology, 35(3), 401-412.

2.3.2. Invertebrate predation of freshwater zooplankton

Dominik Horký

Two morphs of *Daphnia middendorffiana* have been observed throughout the high Arctic. These two morphs differ in pigmentation of their carapace (Fig. 2.3.3.). The pigmented morph seems to have an advantage in shallow ponds where it needs protection from sunlight.[1] The nonpigmented morph on the other hand is less likely to be eaten by a visual-feeding predator. The situation, however, can differ in areas with no visual-feeding predator. The pigmentation might function as protection against both the sunlight and the only present predator *Lepidurus arcticus* which is blind.

The preliminary data from our 2015 qualitative sampling suggest that *Daphnia* populations of deep and shallow lakes (nonpigmented and pigmented morphs respectively) differ due to the influence of *Lepidurus arcticus*. *Lepidurus* is benthic and cannot swim too far from the bottom. For that reason, the population of *Daphnia m*. in the deep lake behaves as if it had no predator at all and it does not have to have any protection. Whereas the population in the shallow lake encounters *Lepidurus* continuously.

Fig 2.3.3. Two morphs of *Daphnia middendorffiana*, magnification 200×.

Our hypothesis states that a population of a shallow lake might have evolved to have a protection from its only predator, in this case *Lepidurus arcticus*. *Lepidurus* is not a visual-feeding predator due to its blindness, thus its choice of pray should not be influenced by the pigmentation.[2]

In August 2018 we carried out two experiments testing our hypothesis. One experiment was designed to compare the predation of each morph separately when the predator was only offered one morph at a time. The second experiment tested the *Lepidurus*' preference when presented with equal number of individuals of both morphs at the same time.

The first experiment compared the predation when the predator is offered only one morph. In this experiment there were four columns with five repetitions (see Fig. 2.3.4.). The first and second columns (NC and NT) had 20 individuals of the nonpigmented morph and the third and fourth columns (PC and PT) had 20 individuals of the pigmented morph put in each of the beaker. The first and third columns served as control groups (NC and PC) with no predator. The second and fourth columns (NT and PT) had one individual of *Lepidurus arcticus* put in each of the beaker. To prevent any shock for the Daphnia each morph was placed in filtered water coming from their lake of origin.

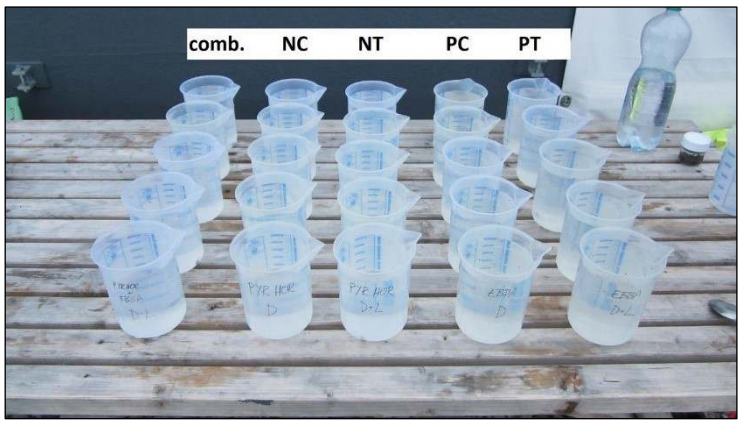


Fig. 2.3.4. Design of experiment.

The second experiment compared the predation when the predator had an equal possibility to choose either of the two morphs. This experiment had only one column with five repetitions (see Fig. 2 – comb). Each of the beakers had 10 individuals of each morph put in them along with one individual of *Lepidurus* arc. One *Lepidurus*, however, underwent ecdysis during our experiment so its sample had to be removed from the analysis. In this experiment the water was a filtered 50:50 mix of both lakes.

Both experiments took place on August 24, 2018, from 0:00 to 16:00 in Petuniabukta on Svalbard on a cloudy day, meaning there was more or less constant dimmed daylight throughout the whole period of 16 hours and the temperature stayed constant on 7°C ± 0.5°C. For both experiments 1-liter beakers were used. The experiments were stopped when one of the predators consumed all of the *Daphnia* individuals in its beaker which took 16 hours. The remaining content was then concentrated and fixed by formaldehyde.

The fixed samples were later transported to the Czech Republic for further analysis. Here they were counted and measured under an optical microscope. The data from the first experiment were then analyzed using two-way ANOVA, the data from the second experiment were analyzed using the paired samples t-test. The data comparing the size of each morph's carapace were obtained by measuring 30 randomly selected individuals of each morph followed by using the independent samples t-test.

Tab. 2.3.2. The results of the two-way ANOVA. Red-marked differences are significant at p < 0.05.

Effect	Univariate Tests of Significance				
	SS	df	MS	F	p
colour	0.450	1	0.450	0.0186	0.893235
Lepidurus	252.050	1	252.050	10.4153	0.005267
colour**Lepidurus*	6.050	1	6.050	0.2500	0.623882
Error	387.200	16	24.200		

The analysis of the first experiment did not confirm our hypothesis as the p-value for the *Lepidurus**lake interaction was too high (p = 0.62; Tab. 2.3.2.). This could have also been caused by a human error; in some of the samples of both the control and the testing groups more than 20 individuals were found. This could be the result of insufficient equipment used for setting up the experiment as at that time only a common tea-spoon was available. The human error, nevertheless, would be the same for all samples.

The t-test of the second experiment disproved the null-hypothesis which stated that there was no difference between the predation of each morph (p = 0.03). This could be interpreted as confirming our hypothesis (Tab. 2.3.3.).

Tab. 2.3.3. The results of the paired samples t-test. Red-marked differences are significant at p < 0.05.

Variable	T-test for Dependent Samples									
	Mean	Std.Dv.	N	Diff.	Std.Dv.	t	df	p	Confidence	Confidence
N	1.50	1.00								
P	4.50	1.91	4	-3.00	1.63	-3.674	3	0.035	-5.60	-0.40

The t-test used for comparing the sizes of each morph's carapaces has shown that there is no significant difference between the size of both morphs. This eliminated size as one of the factors for *Lepidurus* choosing its pray in our experiments (Fig. 2.3.5., Tab. 2.3.4.).

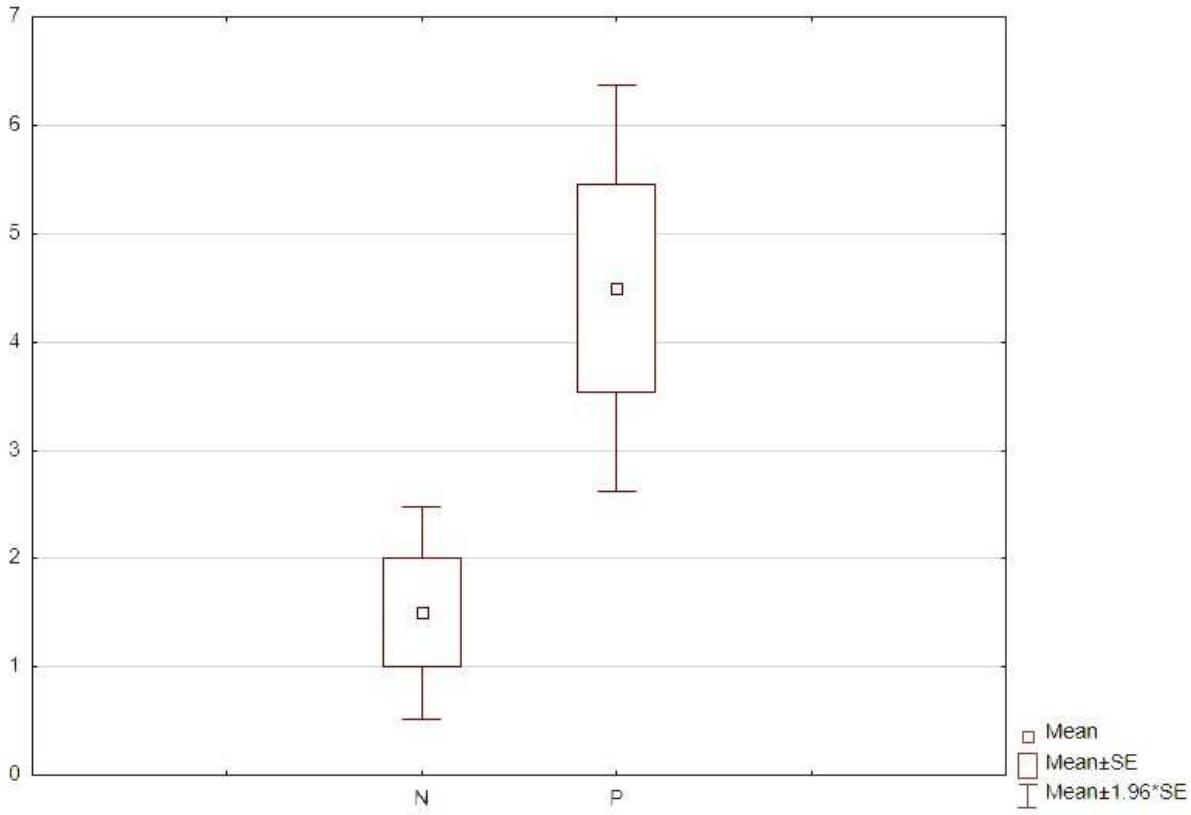


Fig. 2.3.5. Box & Whisker plot of the paired samples t-test.

Tab. 2.3.4. The results of the independent samples t-test.

Variable	T-tests; Grouping: colour										
	Mean N	Mean P	t-value	df	p	Valid N N	Valid N P	Std.Dv. N	Std.Dv. P	F-ratio	p
size	128.4	126.5	0.550	58	0.585	30	30	11.8	14.4	1.491	0.288

It can be therefore concluded that *Lepidurus arcticus* used in our experiments consumed both pigmented and nonpigmented morphs of *Daphnia middendorffiana* at the same rate when having no other option. When given the choice between these two morphs, *Lepidurus* prefers the nonpigmented one. This might suggest that the pigmented individuals have not evolved to protect themselves from the predator. They might be, however, harder to consume as their carapace might be hardened by the pigmentation. Our future plans involve observing ultrathin sections of both morphs under an electron microscope and comparing the thickness of their carapaces.

References

[1]Luecke, C., O'Brien, W. J. (1983): Photoprotective pigments in a pond morph of *Daphnia middendorffiana*. Arctic, 36 (4), 365–368.

[2]Tollrian, R. (1995): Predator-induced morphological defenses : costs, life history shifts, and maternal effects in *Daphnia Pulex*, 76 (6), 1691–1705.

2.3.3. Macrofauna associated with the kelp forests in Adventfjorden (Svalbard)

Jana Marešová

Kelp forests support extremely productive benthic ecosystems, creating hot spots of invertebrate diversity in temperate and boreal coastal waters. They are often regarded as cold water analogs of tropical coral reefs.[1,2] Kelps act as ecosystem engineers[3] by altering water motion, sedimentation and light penetration. Thus, they provide habitats (physical substrate) and energy (fixed carbon) to myriad of fauna and fora.[2]

There are several studies concerning macrofauna inhabiting kelp forests on Svalbard, however they are restricted to Kongsfjorden (north)[4-7] and/or Hornsund (south)[6,8-10], leaving the central coast unexplored. Conveniently, both Czech polar stations are located in this area. Therefore, we attempted to find out more about fauna associated with macroalgae in Adventfjorden and Billefjorden.

To gather material, we constructed device (Fig. 1A), inspired by triangular biological dredge (Fig. 1B), which supposed to collect algae from the seabed. Moreover, we sewn special casting net (Fig. 1C), to catch mobile fauna associated to seaweed growing near the shore. Unfortunately, after several experiments (collecting with and without float and/or fishing lead), we were not able to fish out any good samples and collecting by scuba diver was not possible due to bad weather conditions.

Fig. 2.3.6. Collecting tools: **(A)** homemade triangular dredge, **(B)** professional triangular dredge sold by KC Denmark A/S company, **(C)** homemade casting net.

Despite the failure of our experimental collecting methods, we obtained one sample of benthos and several kelp individuals captured to the classical fishing nets. All living specimens gathered in Adventfjorden were photographed using a binocular microscope and digital camera (Fig. 2). Majority of the fauna was concentrated on kelp holdfasts. We found 34 species from 8 different phyla (Annelida, Arthropoda, Bryozoa, Cnidaria, Echinodermata, Mollusca, Nematoda, Nemertini). Annelida, Mollusca, Arthropoda and Cnidaria yielded the highest numbers of species (9, 6, 6 and

5, respectively), however, Bryozoa and Cnidaria were the most abundant.

Fig. 2.3.7. Overview of species collected in Adventfjorden.

References

[1]Steneck, R.S., Graham, M.H., Bourque, B.J., Corbett, D., Erlandson, J.M., Estes, J.A., Tegner, M.J. (2002): Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental Conservation, 29, 436–459.

[2]Graham, M.H., Kinlan, B.P., Druehl, L.D., Garske, L.E., Banks, S. (2007): Deep-water kelp refugia as potential hot spots of tropical marine diversity and productivity. Proceedings of the National Academy of Sciences USA, 104, 16576–16580.

[3]Jones, C.G., Lawton, J.H., Shachak, M. (1994): Organisms as ecosystem engineers. Oikos, 69, 373–386.

[4]Carlsen, B.P., Johnsen, G., Berge, J., Kuklinski, P. (2007): Biodiversity patterns of macro-epifauna on diVerent lamina parts of *Laminaria digitata* and *Saccharina latissima* collected during spring and summer 2004 in Kongsfjorden, Svalbard. Polar Biology, 30, 939–943.

[5]Hop, H., Kovaltchouk, N.A., Wiencke, C. (2016): Distribution of macroalgae in Kongsfjorden, Svalbard. Polar Biology, 39, 2037–2051.

[6]Legeżyńska, J., Włodarska-Kowalczuk, M., Gluchowska, M., Ormańczyk, M., Kędra, M., Węsławski, J.M. (2017): The malacostracan fauna of two Arctic fjords (west Spitsbergen): the diversity and distribution patterns of its pelagic and benthic components. Oceanologia, 59, 54 –564.

[7]Lippert, H., Iken, K., Rachor, E., Wiencke, C. (2001): Macrofauna associated with macroalgae in the Kongsfjord (Spitsbergen). Polar Biology, 24, 512–522.

[8]Kedra, M., Pabis, K., Gromisz, S., Weslawski, J.M. (2013): Distribution patterns of polychaete fauna in an Arctic fjord (Hornsund, Spitsbergen). Polar Biology 36, 1463–1472.

[9]Ronowicz, M., Legeżyńska, J., Kuliński, P., Włodarska-Kowalczuk, M. (2013): Kelp forest as a habitat for mobile epifauna: case study of Caprella septentrionalis Kröyer, 1838 (Amphipoda, Caprellidae) in an Arctic glacial fjord. Polar Research, 32, 21037.

[10]Włodarska-Kowalczuk, M., Kukliński, P., Ronowicz, M., Legeżyńska, J., Gromisz, S. (2009): Assessing species richness of macrofauna associated with macroalgae in Arctic kelp forests (Hornsund, Svalbard). Polar Biology, 32, 897–905.

2.3.4. Trematoda of the family Hemiuridae on Svalbard

Vendula Branišová

The main aim of the project was to collect samples of the trematodes of family Hemiuridae from infected fish and Chaetognaths in two localities of Svalbard. The first locality was near the capital of Longyearbyen, the other near Nostoc Field Station in Petuniabukta (Fig. 2.3.8.).

Fig. 2.3.8. Map with labeled locations.

These flukes are elongated, cylindrical worms with a nonspinous tegument (Fig. 2.3.9.). They are sometimes called appendiculate flukes, and they vary length from a few to 15 mm. A characteristic feature of the body of some is its division into an anterior soma and a posterior ecsoma. These two parts may be telescoped together, the escoma being withdrawn into the soma. The dividing line between the thick-walled posteriori part is often clearly evident as a cosnstriction.

Fig. 2.3.9. Hemiuridae sp.

Often the vitellaria consists of a few large bodies rather than many scattered particles. The flukes usually inhabit the gut, stomach, goldbladder, esophagus, or pharynx of marine fish, and they worldwide in their distribution.[1]

Fishes were trapped in nets and traps. Nets were laid at different depths for different fish species. For example, for fishing of the fishes of the family Cottidae, the nets were primarily laid at a smaller depth of about 10 m. For fishing of the fishes of the family Gadidae, nets were laid down to a depth of about 30 m. I was also interested in Chaetognathes. They are part of the zooplankton and they were caught by nets or only by picking up water in a bucket (fig. 2.3.10.). Also the gastropods of the Naticidae family, precisely the species of *Euspira pallida* were investigated.

Fig. 2.3.10. Catching Chaetognaths by the net.

Caught Chaetognaths were optically examined (fig. 2.3.11.). Because of their transparent body, we can clearly see if they are infected. None of the investigated individuals showed signs of infection.

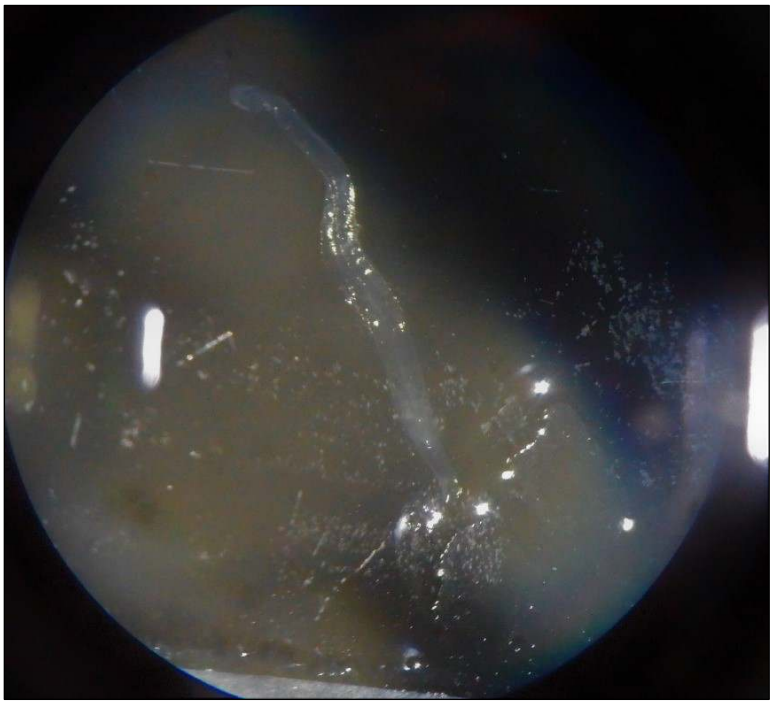


Fig. 2.3.11. Chaetognatha sp.

Caught fishes were autopsy (fig.5.). Although trematodes are found primarily in the stomach, I was interested in the entire digestive tract, as they may also be found in the intestine of an individual (fig.6.) In total, it was examined 274 individuals dedicated to the 13 species sorted into the 10 families (for example- Gadidae, Cottidae, Rajidae..). Hemiurid trematodes were found in 38 specimens from family Gadidae (*Gadus morhua* and *Boreogadus saida*).

Fig. 2.3.12. The autopsy of *Melanogrammus aeglefinus.*

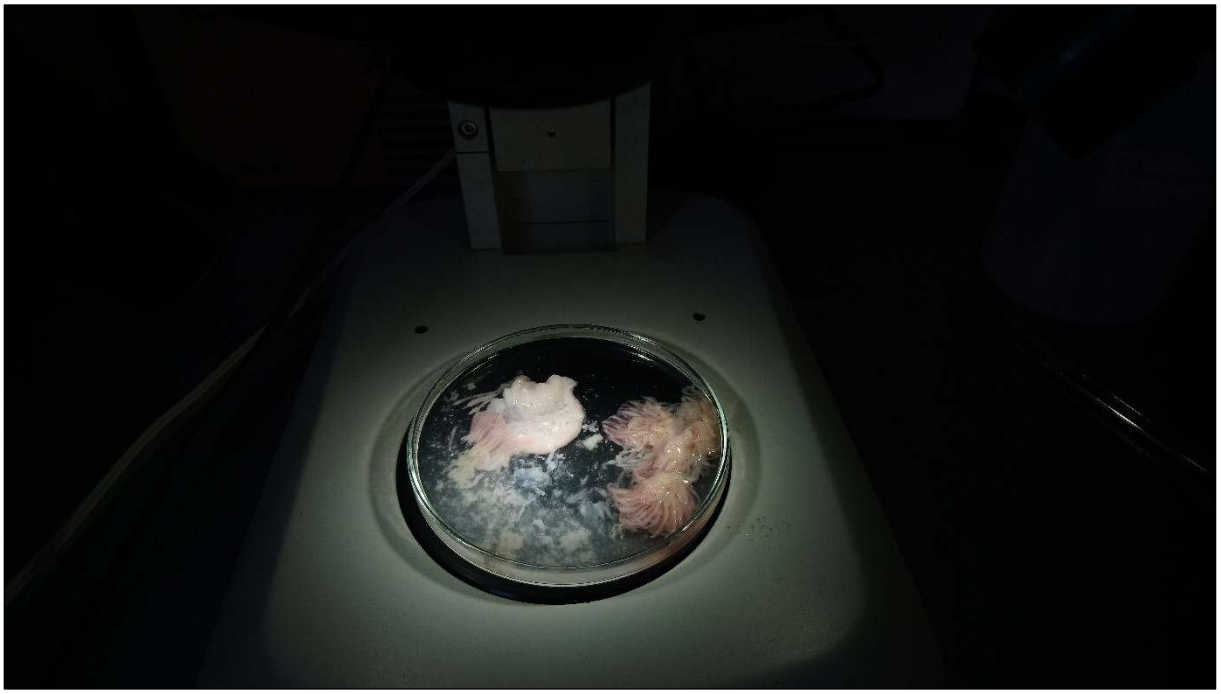


Fig. 2.3.13. The stomach of *Melanogrammus aeglefinus.*

Samples gained from this project will be used in my bachelor thesis. They will be morphologically characterized and phylogenetically assessed.

References
[1]Noble, E.R., Noble, G.A. (1971): Parasitology: the biology of animal parasites. 3rd edn. Lea & Febiger, Philadelphia.

2.3.5. Parasitic trematodes (Opecoelidae) of marine organisms of Svalbard

Daniel Novotný

Svalbard is an archipelago located between 74°- 81° of the north latitude and 10°- 34° of the east longitude. 60% of the land is covered by the glaciers, which also made lot of bays named fjords. In the fjords, the conditions are more stable than the open sea and they are more suitable for studying the marine life surrounding the archipelago. My main research was done during 20. 8. 2018- 3. 9. 2018 in Billefjorden in its part named Petunia bay and also in the waters surrounding the Longyearbyen (LYR) which is capital city of Svalbard.[1]

The fjords around Svalbard are inhabited with many species of organisms which some of them have high commercial value like fishes from order Gadiformes or Clupeiformes and many other species. Usually the diversity of marine organisms is much higher in arctic regions than the terrestrial ones and lot of them even contain the large variety of parasites. My study is focused on helminth parasites (especially trematodes of family Opecoelidae Oazaki, 1925). These trematodes are usually found in guts of marine fishes, mainly two species of sculpins - *Myoxoccephalus scorpius* Linnaeus, 1758 and *Gymnacanthus tricuspis* J. C. H. Reinhardt, 1830 where we can find adult (sexual) stages of them and in gastropods of genus *Buccinium linnaeus*, 1758. The gastropods are first intermediate hosts of most of trematodes. It means they contain asexual stages of trematodes (sporocysts, rediae, cercaria). The second intermediate host is usually crustacean and in the Svalbard littoral zone it is amphipod of genus *Gammarus setosus* Dementivea, 1931. The amphipods were not dissected due to low prevalence of infection (lower than 1%). From previous studies of Otáhal[2] there was recorded only one genus of Opecoelidae trematodes- *Podocotyle* Dujardin, 1845 which was described as species *Podocotyle atomon* Rudolphi, 1802.[2,3]

Main aim of this study is to collect the samples of Opecoelidae trematodes for molecular and morphological analysis.

The samples were collected in the Petuniabukta which is part of Billefjorden that is located in the center of Svalbard and second location were shallow waters around Longyaerbyen. The gastropods were usually caught by sein net used for fishing or by box traps used for catching crustaceans. The depth, where the traps were usually deployed was around 30- 40 m in Longyearbyen and around 10- 20 m in Petuniabukta. The fishing nets were deployed around 30- 40 m in Longyearbyen and 9- 15 m in Petuniabukta. After collecting the gastropods and fishes, most of them were dissected and samples were fixed in ethanol or hot formaldehyde.

The samples fixed in hot formaldehyde will be used for morphological measurements and histological staining. The stain usually used for helminths is carmine, which has bright red colour. The samples fixed in ethanol will be used for molecular analysis of 28S rDNA and ITS1 rDNA.[4,5]

During my course in Svalbard (20. 8. 2018- 3. 9. 2018) there were dissected 28 specimens of both final host species (*M. scorpius*, *G. tricuspis*). These fishes were only caught in Petunia bay in shallow waters (9- 20 m) (Tab. 2.3.5.).

The specimens fixated in hot formalin were stained and measured (Fig. 2.3.14.) and molecular analysis is still not done.

Tab. 2.3.5. List of dissected final host species of *Podocotyle* sp.

Host species	Sex	Number of samples	Prevalence (positive: negative)
Myoxocephalus scorpius	M	5	4:1
	F	8	6:2
Gymnacanthus tricuspis	M	8	3:5
	F	7	5:2

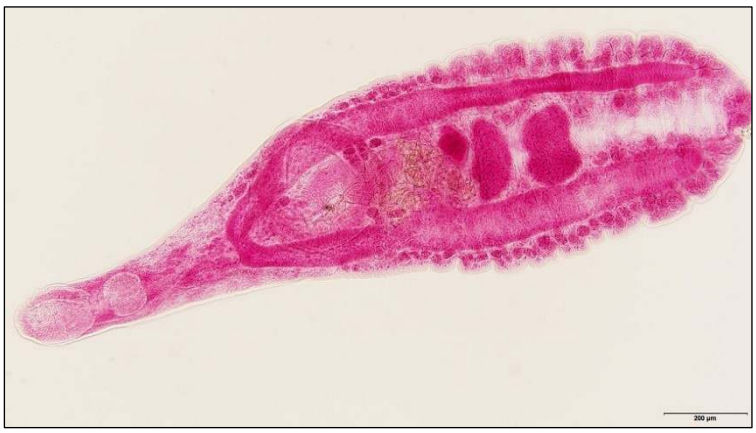


Fig. 2.3.14. Stained *Podocotyle* sp. (zoom 100x).

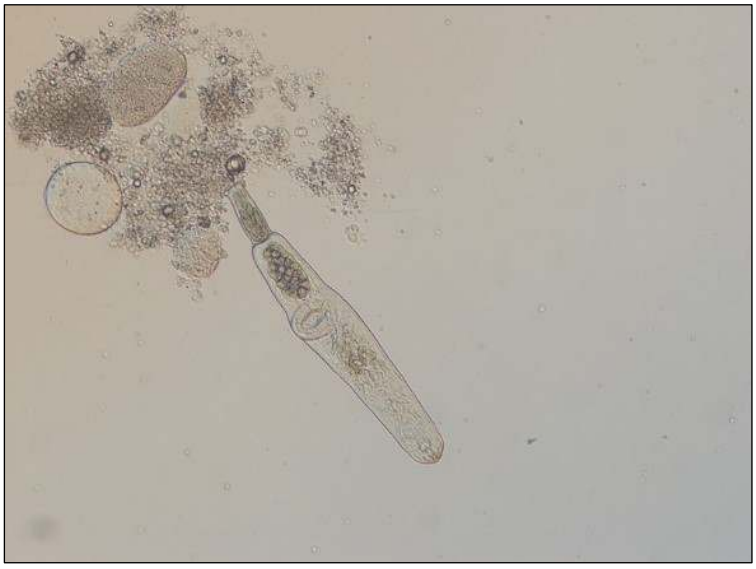


Fig. 2.3.15. Cercaria of Opecoelidae trematode isolated from *Buccinium undatum*.

In Longyearbyen, there were also dissected intermediate hosts – gastropods of genus *Buccinium* Linnaeus, 1758 (3 species: *B. undatum* Linnaeus, 1758, *B. polare* J.E. Gray, 1839, *B. glaciale* Linnaeus, 1761). There were dissected 37 specimens, but only one (*B. polare*) was positive with asexual stages of *Podocotyle* sp. For instance in Petunia, there were dissected only 7 specimens (*B. undatum*), but 3 were positive (Fig. 2.3.15). In specimens from LYR there were also found asexual stages of other trematode families for example Heterophyidae Leiper, 1909.

Also, during the course there were caught many other species of marine organisms with their parasites (Tab. 2) which can be used for later studies. There were collected samples of parasites (ethanol, hot formalin), chime (potassium dichromate) and fin/tissue samples (ethanol).

Tab. 2.3.6. List of other caught marine organisms with their parasites. Abbreviation: LYR – Longyearbyen.

Higher classification group	Species	Number. of dissected species	Locality	Parasites
Rajiformes (skates)	*Amblyraja radiata*	11	LYR	*Rajochoncotyle* *Acanthocotyle* Copepoda *Pseudanisakis* *Pseudantobothirum*
Teleostei fishes	*Cyclopterus lumpus*	1	Petunia	---
Teleostei fishes	*Gadus morhua*	18	Petunia	Copepoda Hemiuridae *Anisakis*
Teleostei fishes	*Hippoglosoides platessoides*	20	Petunia LYR	*Anisakis* *Aporocotyle* Copepoda
Teleostei fishes	*Melanogrammus aeglefinus*	8	LYR	*Anisakis* Hemiuridae
Teleostei fishes	*Sebastes mentella*	1	LYR	*Anisakis* Cestoda?
Bivalvia	*Mya trunkata*	5	Petunia	*Malacobdella grossa* (larvae) *Gymnophallis*
Gastropoda	*Coleus kroyeri*	1	LYR	---

Reference

[1]The editors of Encyclopedia Britannica, Svalbard [online], Encyclopedia Britannica [cit. 25. 10. 2018], Available from: https://www.britannica.com/place/Svalbard.

[2]Otáhal, O., Ditrich, O. (2015): Trematodes of family Opecoelidae in central part of Svalbard. Faculty of science, University of South Bohemia in České Budějovice, České Budějovice.

[3]Rohde, K. (2005): Marine parasitology. CSIRO Publishing, Collingwood.

[4]Bray, R.A., Cribb, T.H., Littlewood, D.T.J., Waeschenbach, A. (2016): The molecular phylogeny of the digenean family Opecoelidae Ozaki, 1925 and the value of morphological characters, with the erection of a new subfamily. Folia Parasitologica, 63, 1-13.

[5]Jousson, O., Bartoli, P., Zaninetti, L., Pawlowski, J. (1998): Use of the ITS rDNA for elucidation of some life-cycles of Mesometridae. International Journal for Parasitology, 28, 1403-1411.

2.3.6. Intestinal parasites of Svalbard wild birds

Veronika Žánová

Most birds occur in Svalbard only during summer months due to the nesting and breeding. After summer they migrate south and their wintering grounds vary from one species to another. Only Svalbard rock ptarmigan (*Lagopus muta hyperborea*) is an exception, it stays in Svalbard for the whole season. The variety of migration and flight paths may raise some questions, f.e. if there is some correlation between wintering ground and the occurrence of the specific parasite.[1]

The main goal was to collect samples for the project focusing on the prevalence and diversity of avian intestinal parasites in Svalbard and on migratory differences affecting the occurrence of concrete parasites. Especially the representatives of the phylum Apicomplexa (subclass Coccidia and Cryptosporidea) and Microspora. These parasites have the huge medical and economic importance because they cause coccidiosis,[2] cryptosporidiosis[3] and microsporidiosis diseases.[4] Field research have provided not only the opportunity to collect faecal samples but also to observe

an interactions of potential hosts with their natural environment. To understand these behaviors could be helpful to figure out the possible transmission of parasites.

Fresh faeces specimens were collected from the identified birds during the fieldwork. Most of samples were taken in the Longyearbyen area and its surrounding. Some samples were collected in Billefjorden (namely from Pyramiden and an island called Retrettøya near to the Nordskiøldbreen) and in Wijdefjorden (Sørbreen) (Fig. 2.3.16.).

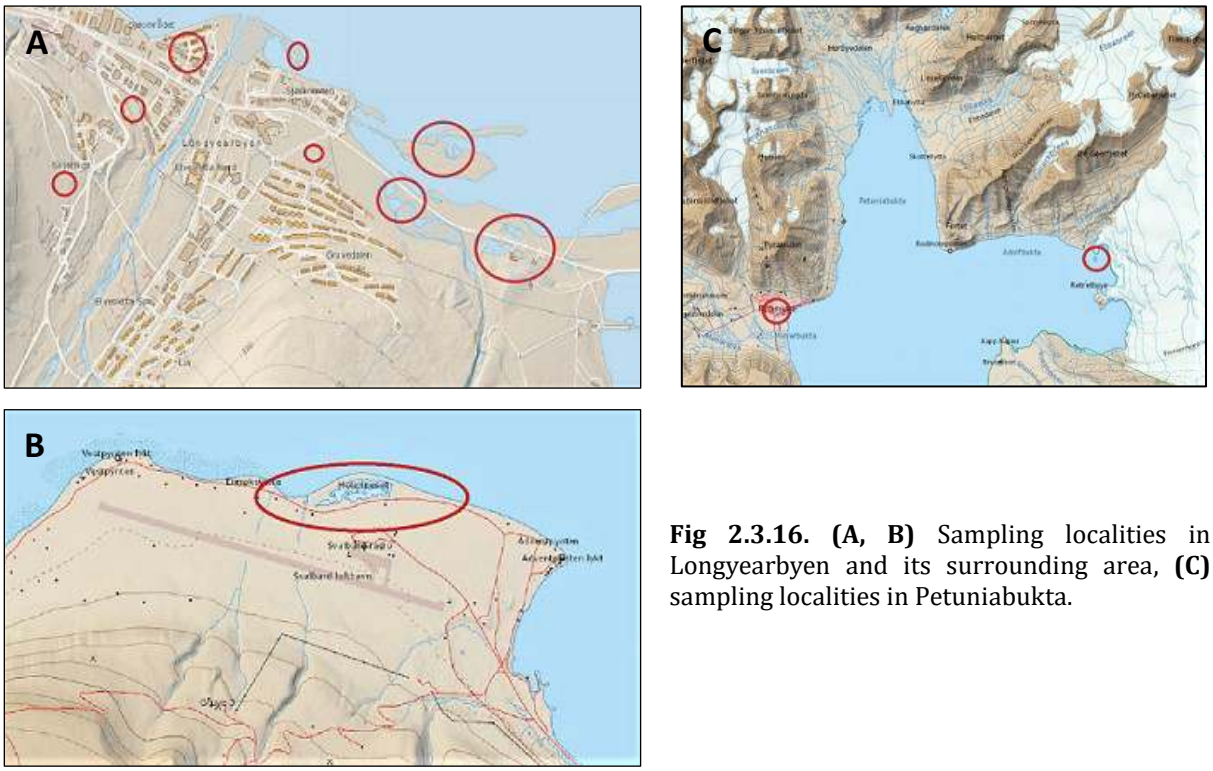


Fig 2.3.16. (A, B) Sampling localities in Longyearbyen and its surrounding area, **(C)** sampling localities in Petuniabukta.

In total 168 samples from different species were collected : *Branta leucopsis* (28 samples), *Anser brachyrhynchus* (6 samples), *Somateria mollissima* (17 samples), *Charadrius hiaticula* (3 samples), Calidris maritima (17 samples), *Phalaropes fulicarius* (2 samples), *Stercocarius parasiticus* (1 sample), *Larus hyperboreus* (4 samples), *Rissa tridactyla* (5 samples), *Plectrophenax nivalis* (7 samples), *Sterna paradisaea* (60 samples), *Fratencula arctica* (1 sample), *Lagopus muta hyperborea* (1 sample). (Fig. 2.3.17.).

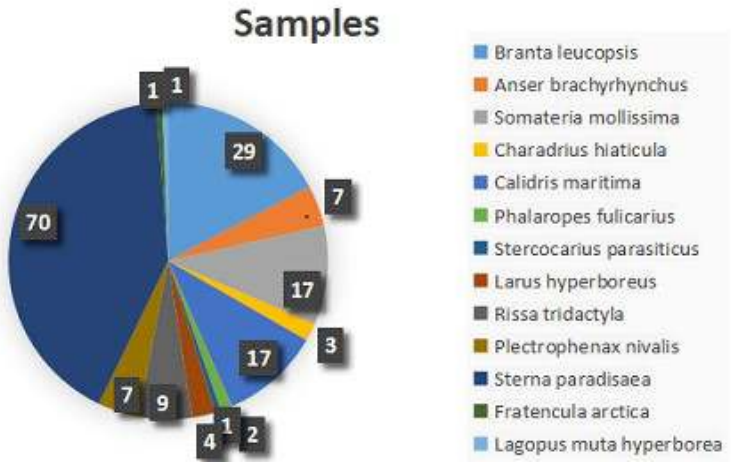


Fig 2.3.17. The proportions of the samples divided according to the bird species.

Eight of the all samples are cloacal swabs of arctic terns (Fig. 2.3.18.). To collect these specimens was made possible thanks to the project "Migration route of Arctic terns (*Sterna paradisaea*) from the northernmost breeding colonies in Svalbard.". The rest is fresh faeces samples. Part of the samples are stored in 4% solution of potassium dichromate ($K_2Cr_2O_7$), which should help potential oocyst to sporulate and part of the samples are native. All samples are stored in refrigerator.

Fig 2.3.18. Taking a cloacal swab from the Arctic tern.

In the Czech Republic there will be used specific methods to prove presence of parasites in collected samples. Samples will be examined with the Sheather´s flotation method and then light microscopy will be used to find oocysts of coccidia. For smaller cryptosporidium and microsporidium is better to colour the thin layer of scattered and fixed feces before microscopy. Positive samples will be examined with molecular methods (DNA isolation, PCR and sequencing). Furthermore, the gained data will be used for phylogeny.

Up to now 7 samples have been found positive with coccidian- the sample from *Plectrophenax nivalis* containing oocysts of *Isospora* (Fig. 2.3.19.) and *Anser brachyrhynchus* and *Branta leucopsis* containing unsporulated oocyst of the unidentified coccidia (Fig. 2.3.20.).

Fig. 2.3.19. Oocyst of *Isospora* (objective magnification 400x, light microscopy).

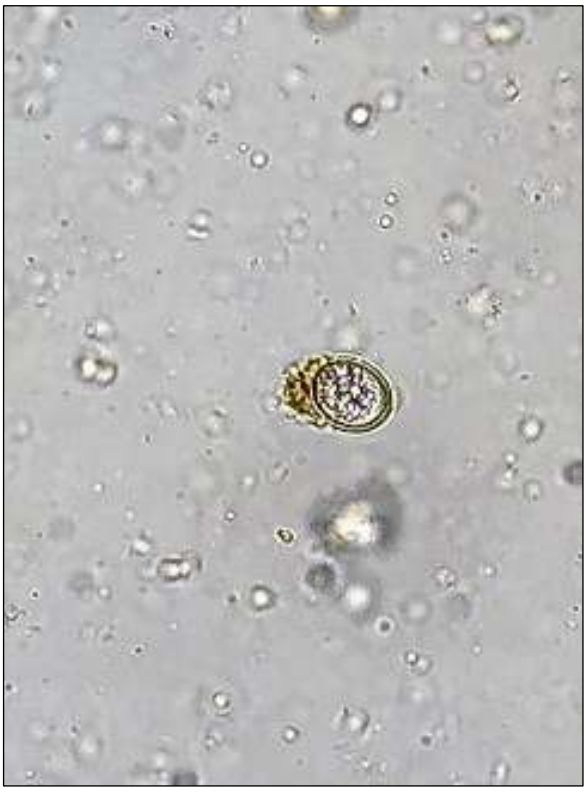


Fig 2.3.20. Unsporulated oocyst of coccidia (objective magnification 400x, light microscopy).

References

[1]Kovacs, K.M., Lydersen, C. (eds.) (2006): Birds and mammals of Svalbard. Norwegian Polar Institute, Trømso.

[2]De Gussem, M. (2007): Coccidiosis in poultry: Review on diagnosis, control, prevention and interaction with overall gut health. 3rd edn. Blackwell Publishing, Ames.

[3]Shikani, H., Weiss, L. (2014): Human Cryptosporidiosis. A Clinical Perspective. In: Caccio, S.M., Widmer, G. (eds.): Cryptosporidium: Parasite and Disease. Springer-Verlag, Wein, 383-421.

[4]van Gool, T., Dankert, J. (1995): Human microsporidiosis: Clinical, diagnostic and therapeutic aspects of an increasing infection. Clinical Microbiology and Infection, 1 (2), 75-85.