LIMNOLOGICKÉ NOVINY

LIMNOLOGICAL NEWS

Číslo 1	Duben 2020	ISSN 1212-2920

DOKTORSKÁ PRÁCE NOMINOVANÁ NA CENU SEFS 2021

Exploring the diversity of snow algae using polyphasic approach

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Snow algae cause blooms in slowly melting snowfields in mountain and polar regions. Although they are excellent models for the study of life in extremely cold environments, their taxonomical diversity, geographical distribution and variety of physiological strategies used to cope with their harsh environment are only partially understood. The outcome of my thesis resulted in ten publications in peer-reviewed journals. A few these stories will be shown here.

The snowfield colouration due to algae can be red, pink, orange, golden-brown, green or grey (**Figure 1**). The rarest snow coloration is blue, caused by cyanobacteria (Kol 1955). The factors responsible for snow colourations are a combination of the pigment contents per cell and the cell concentration per snow volume, with blooms generally becoming macroscopically visible once cell counts exceed one thousand 'giant' red cells (Remias et al. 2016) or six thousand tiny vegetative cells per ml of meltwater (Hoham & Duval 2001).

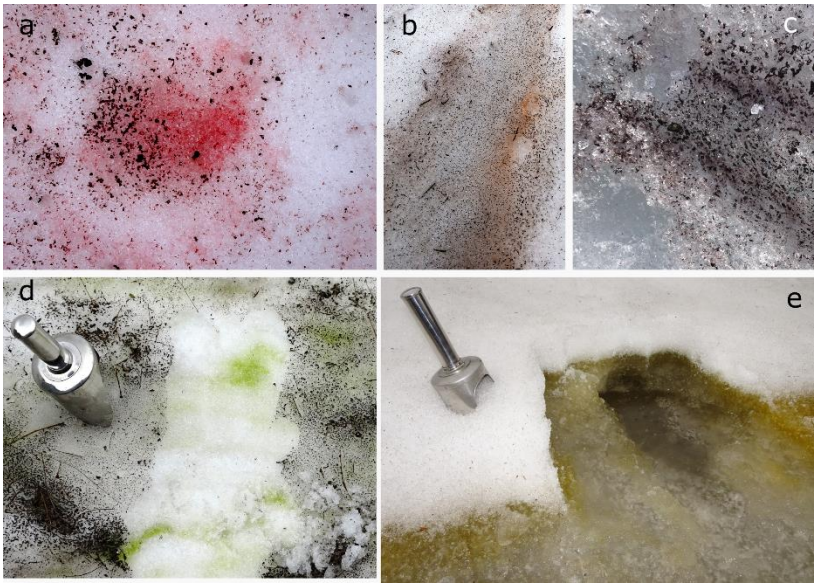

Figure 1. Snow and glacial algal blooms: (a) red snow (Tyrolean Alps, Austria); (b) orange snow (Krkonoše Mts, Czech republic); (c) grey snow or purple ice (a glacier, Swiss Alps); (d) green snow (High Tatra Mts, Slovakia); (e) golden-brown snow (Svalbard, Norway).

This thesis includes new data on less-known or poorly-described green microalgae, and taxonomic descriptions of novel green and golden algae species (*papers I, III, IV–VI*). Several algal species are reported to cause the macroscopic phenomenon of orange and red to pinkish snow, including *Chlamydomonas nivalis* (Wille 1903; Kol 1968; Leya 2004), *Chloromonas* (*Cr.*) *nivalis* (Matsuzaki et al. 2018), *Cr. brevispina* (Hoham et al. 1979), *Cr. polyptera* (Remias et al. 2013), *Smithsonimonas abbotii* (Kol 1942), *Chlainomonas* (*Cl.*) *kolii* (Novis 2002)

and *Cl. rubra* (Hoham 1974). In our research, we focused on **molecular diversity** and **geographical distribution** of the main algae responsible for causing the so-called 'watermelon' snow phenomenon (*paper I*). This is what people called *Chlamydomonas nivalis*. We explored field samples from Arctic, Antarctic, North and South America and Europe. At Arctic conditions, we can even find orange and red tinted spots occurring next to each other. Light microscopy showed that the cells in both bloom types are either made of red or orange spherical cysts. No culturable isolates of these investigated algae are available yet. Based on 18S rDNA and *rbc*L phylogenetic analyses, one of the main algae causing the phenomenon of red snow was shown to represent a single, monophyletic lineage, independent from other algae in Chlamydomonadales. Therefore, the new genus *Sanguina* (*S.*) was described, with two closely related species, *S. nivaloides* and *S. aurantia.* Using molecular methods, the cosmopolitan distribution of red snow forming *S. nivaloides* in polar and alpine regions was demonstrated (**Figure 2**). It is a diverse species, with at least 18 ITS2 rDNA haplotypes. No geographic structuring was found indicating an effective dispersal strategy with the cysts being dispersed all around the globe. The orange snow causing *S. aurantia* was recorded so far in the arctic sites and at lower latitudes with subarctic climate.

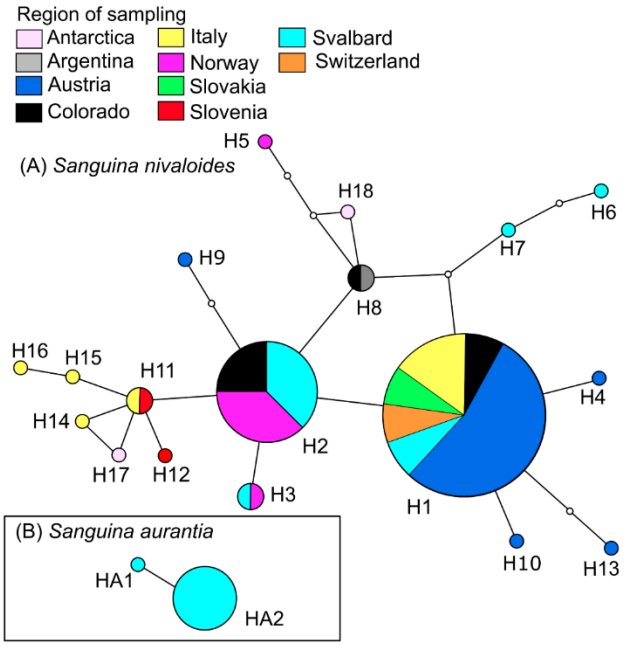

Figure 2. Geographic distribution of ITS2 rDNA haplotypes detected in this study: (A) *Sanguina nivaloides*, (B) *Sanguina aurantia*.

The lack of laboratory cultures for many snow algae also causes gaps in the knowledge about their physiology. **Fatty acid** and **pigment** profiling provide new insights into the **adjustments of metabolic pathways** in these algae. To briefly introduce the two metabolic compounds: Lipids are the main structural components of biological membranes, the main storage site for energy, and are involved in cell signalling. Microalgae deposit neutral lipids in organelles referred to as lipid bodies or oil globules, while phospholipids and glycolipids are part of bio-membranes. Three major classes of photosynthetic pigments occur among algae: chlorophylls, carotenoids (carotenes and xanthophylls) and phycobilins. Under certain scenarios, e.g. when cells are exposed to high light condition or nitrogen depletion, some microalgae have an ability to produce secondary carotenoids (e.g. astaxanthin) (Leya et al. 2009). In our research, we wanted to answer following question: *Are there is any differences in adaptation strategies between populations, between different life cycle stages of one species, or between closely related taxa in terms of pigments and lipid profiles?* The thesis compared for the first time the pigment and fatty acid profiles of two contrasting life cycle stages in one *Chloromonas* species (*paper III*). Field collected cysts of *Cr. hindakii* were astaxanthin rich, while vegetative flagellates stayed green. However, both stages were almost consistent in their fatty acid profile, with polyunsaturated fatty acids (PUFAs) dominating (65.8% and 58.1%, respectively, of total lipids), the content of saturated acids did not exceed 23%. PUFAs dominated neutral lipids, phospholipids, and glycolipids. These pigment findings correspond with the proposed main ecophysiological differences between immotile cysts and motile flagellates (Remias 2012). On the other hand, fatty acid desaturation is one of the core adaptations to extreme temperature conditions in melting snow to keep membrane fluidity, independent of algal life cycle stage (Morgan-Kiss et al. 2006). Our analyses of pigment profiles showed that the intracellular ratio of astaxanthin to chlorophyll significantly differs by one up to two magnitudes between three unrelated main taxa causing snow blooms: *Cr.* spp. (*papers III and IV*), *Cl.* sp. and *S. nivaloides* (*paper II*). These results correspond with findings that the latter two species are restricted to exposed habitats in term of irradiance, where a high accumulation of astaxanthin in extraplastidal lipid bodies plays an inevitable

role in multifunctional stress defence. In contrast, members of the former genus may occupy latitudinal gradients from forested to alpine sites; these cells rely more on heat dissipation in the xanthophyll cycle (Remias et al. 2010). Closely related taxa (*Cr. nivalis* and *Cr. nivalis* subsp. *tatrae* (**Figure 3**)) or even two genetically identical populations (for ITS2 rDNA; *Cl.* sp.) shared ecological requirements (high alpine sites) but differed slightly in the abundance of secondary carotenoids (*papers II and IV*). It is assumed that cell aging (maturation of cysts over the course of the season) also plays a role in the extent of astaxanthin accumulation (Hoham & Duval 2001).

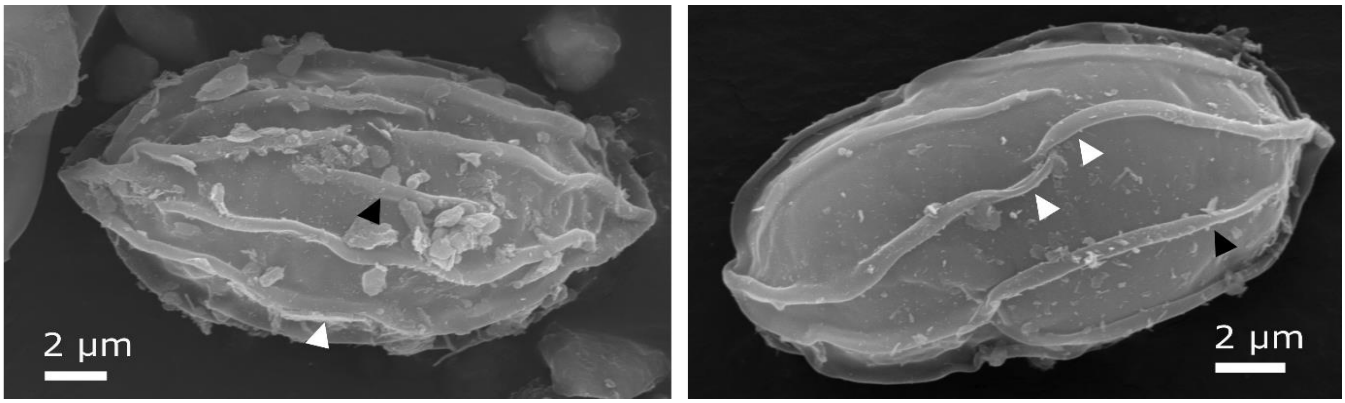

Figure 3. SEM micrographs of mature aplanozygotes of *Chloromonas nivalis* subsp. *tatrae* subsp. nov.

The relationship between the rate of photosynthesis and light intensity can be described by 'light curves'. There are several ways to measure photosynthetic performance. A direct method utilises oxygen evolution monitoring, while an indirect method utilises the measurement of rapid light curves using variable chlorophyll fluorescence (Strasser et al. 2000, Stibal & Elster 2007; *papers II–V*). In the latter, a parameter called the electron transport rate, or ETR, is closely related to the photosynthetic activity measured by oxygen evolution or oxygen dioxide uptake (Beer et al. 1998). Relative ETR (rETR) approximates the rate of electrons pumped through the photosynthetic chain (Ralph & Gademann 2005). Photon energy captured by a chlorophyll-a molecule can either drive photosynthesis, be converted to heat, or be emitted as chlorophyll fluorescence. The latter provides the most appropriate means for screening the dynamic processes occurring in the photosystem II (PSII) reaction centres (Ralph & Gademann 2005) and we used it as a proxy of **light preferences of PSII.** For quantitatively comparing light curves, several characteristic parameters such as α (the slope of the linear part of the curve), I_k (the light saturation coefficient) and P_{max} (the maximum rate when light becomes saturating; $rETR_{max}$) are calculated (Ralph et al. 2002; *papers II–V*). We wanted to answer following questions: *Do the light preferences reflect the conditions in the original habitat? Do populations from high light conditions become photoinhibited at higher irradiances than those from low light conditions?* The first ecophysiological comparison of populations of the same cryoflora species from two different mountain ranges was done for *Chlainomonas* sp. thriving in melting snow at ice-covered high alpine lakes (High Tatra Mts. in Slovakia (**Figure 4**), Tyrolean Alps in Austria). The photosynthetic rates of the alga from both sites were consistent in their response to irradiance, with photoinhibition not noticed up to high light intensities of 1,300 µmol photons m^{-2} s^{-1} (*paper II*), corresponding to previous findings for this species based on oxygen evolution measurements (Remias et al. 2016). However, the former population (sampled later in the season) needed only half the irradiance than the latter (collected earlier in the season) to become saturated (*paper II*). This can be explained by the advanced snowmelt and associated irradiance stress experienced later in the season (*paper II*). While many snow and glacial algae are exposed at the surface to high visible and UV irradiance levels, other populations thriving deep under the snow surface cope with low photosynthetic active radiation levels for reaching the light saturation point. Above timberline in the

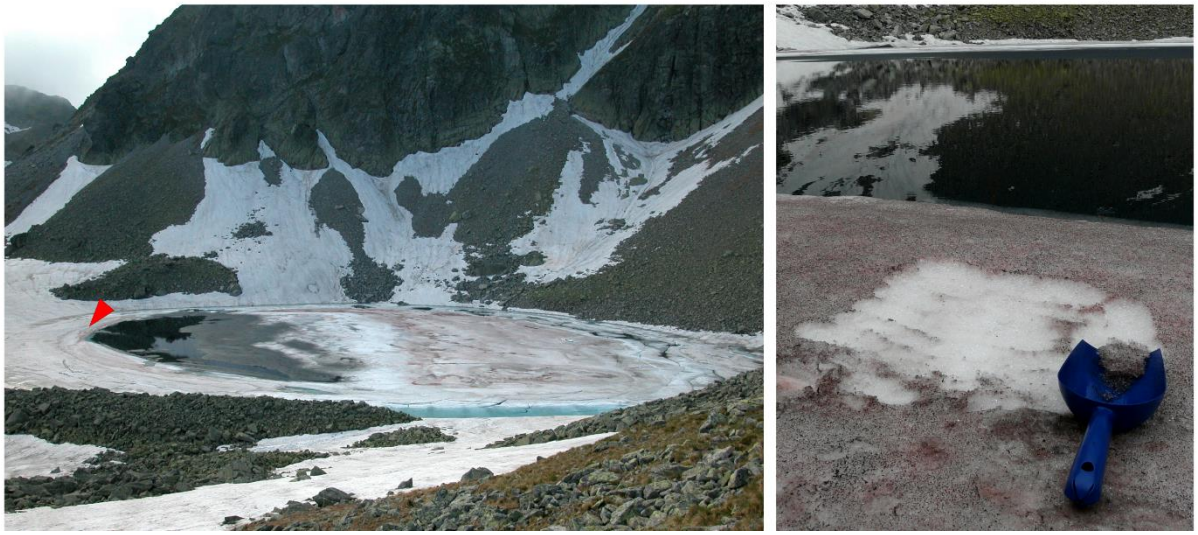

Figure 4. Red snow colouration caused by *Chlainomonas* sp. at Ľadové Lake, the High Tatra Mts., Slovakia (mid-June 2016).

Tyrolean Alps, this was the case for *Scotiella cryophila* K-1 collected from depths of 20 to 40 cm. Our results indicated presence of a cellular 'memory' of light conditions in the field since the cells showed photoinhibition already at irradiances for 70 µmol PAR m^{-2} s^{-1} on (*paper V*). Still, detailed mechanisms of physiologic adaptations among life cycle stages remain unexplained: drastic cellular changes must take place, e.g. during photo-acclimation from low light conditions deep below the snow surface to high-light exposure on the surface – a change happening within a few weeks. Interestingly, we documented the high intraspecific photophysiological plasticity of photosystem II for cyst samples of one *Chloromonas* species collected from high and low light conditions in Krkonoše and High Tatra Mts (*paper III*) (**Figure 5**).

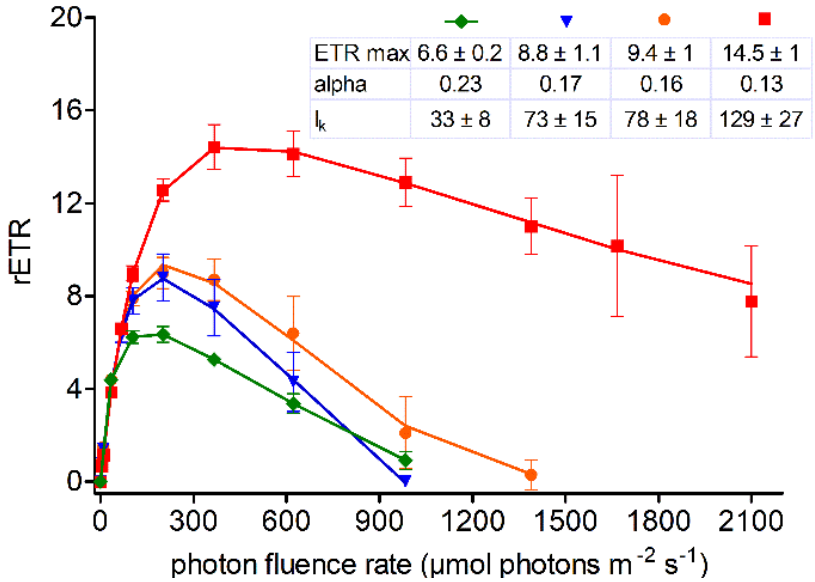

Figure 5. Rapid light curves showing the intraspecific photosynthetic flexibility of *Chloromonas hindakii* sp. nov. : vegetative flagellates (green diamonds, strain grown in the lab) and non-motile field-collected cysts from low light sites (blue triangles and orange circles) and a high light site (red boxes).

Concerning environmental samples, only virtually monospecific field blooms (**Figure 6**) or isolated single cells from snow are suitable for Sanger sequencing (Matsuzaki et al. 2015; *papers I–V*). In contrast, mixed communities are ideal targets for state-of-the-art high-throughput (i.e. metagenomic) amplicon sequencing, e.g. using the Illumina system (*paper VII*). Both approaches provide different results, leading to insights into the biodiversity and biogeography of snow algal communities (using culture independent methods). One of the first **metagenomic studies of snow algal assemblages** was based on a single marker. Lutz et al. (2015) investigated the partial 18S rDNA of eukaryotic communities at three different Arctic sites. Only four algal phylotypes were detected. Similarly, partial 18S rDNA high throughput sequencing (HTS) was applied to the so far under-investigated cryoflora at a nival site above 5,000 m a.s.l. in the Chilean Andes (Vimercati et al. 2019). The authors focused on microbial communities in thin blades of hardened snow known as 'nieves penitentes', and the most abundant operation taxonomic unit (OTU) was 100% identical with '*Chlamydomonas nivalis*' CCCryo RS_0015-2010 (the

type specimen of *S. nivaloides*, *paper I*), while the second and third most common OTUs belonged to two still undescribed species of *Chloromonas*. In our study, the application of this HTS was evaluated for the first time for the characterization of snow algal communities in the European Alps. We proposed an updated approach for such projects to assist in accurate biodiversity analyses. First, short reads data from Illumina should be accompanied by the generation of new long Sanger sequence libraries from local samples to compensate for a lack of reference data in public databases. Second, we showed that several snow dwelling *Chloromonas* species shared more than 99 % identity in the hypervariable V3-V4 region of the 18S rRNA gene (*papers V and VII*), therefore section of this marker is not specific enough for the resolution of closely related *Chloromonas* species. In contrast, the use of a hypervariable marker ITS2 rDNA for metagenomic sequencing allowed to assign the OTUs on species level. On the other hand, there is scarcity of ITS2 rDNA sequences in the public databases. Consequently, two-molecular marker approach is recommended to compare differences in the taxonomic composition based on a combined use of a conservative marker (e.g. 18S rDNA) and of a hypervariable marker (e.g. ITS2 rDNA). Third, one of the weak points of most studies conducting environmental sequencing of snow algal assemblages is the lack of any light microscopy of the harvested samples. This creates an information gap that could be useful for later correct OTU affiliation. Therefore, light microscopy base guidance of HTS samples is inevitable task. Finally, for accurate assignment of OTUs to taxonomy, a combination of manual inspection of automated assignments and secondary structure prediction of ITS2 rDNA is needed.

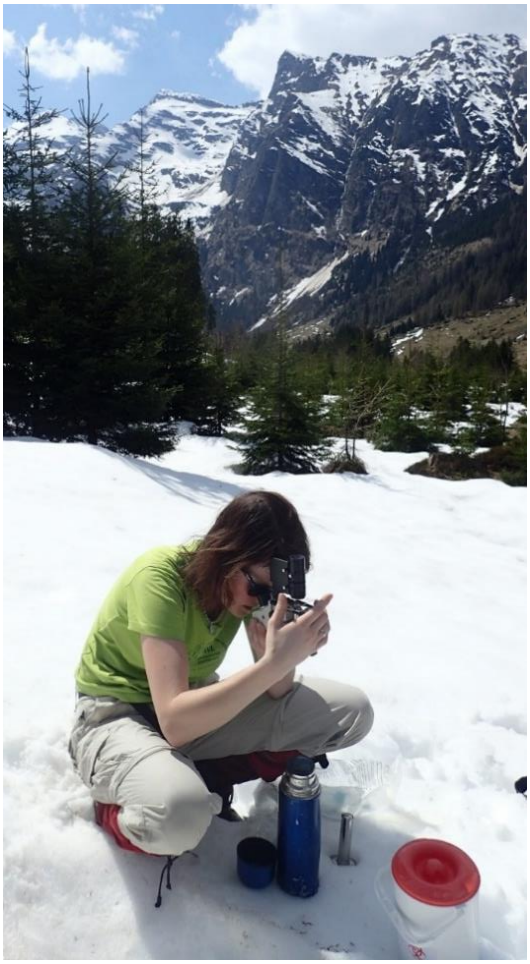

Figure 6. Observation of snow algal populations *in situ* by a portable field microscope is a successful strategy for a selection of the best sampling spots. Lower Tauern, Austria, (April 2018).

<u>For further reading (selected publications):</u>

Procházková L, Leya T, Křížková H & Nedbalová L (2019): *Sanguina nivaloides* and *Sanguina aurantia* gen. et spp. nov. (Chlorophyta): the taxonomy, phylogeny, biogeography and ecology of two newly recognised algae causing red and orange snow. *FEMS Microbiology Ecology* 95(6): fiz064.

Procházková L, Remias D, Holzinger A, Řezanka T & Nedbalová L (2018): Ecophysiological and morphological comparison of two populations of *Chlainomonas* sp. (Chlorophyta) causing red snow on ice-covered lakes in the High Tatras and Austrian Alps. *European Journal of Phycology* 53(2): 230–243.

Procházková L, Remias D, Řezanka T & Nedbalová L (2019): Ecophysiology of *Chloromonas hindakii*, sp. nov. (Chlorophyceae), causing orange snow blooms at different light conditions. *Microorganisms* 7(10): 434.

Procházková L, Remias D, Řezanka T & Nedbalová L (2018): *Chloromonas nivalis* subsp. *tatrae*, subsp. nov. (Chlamydomonadales, Chlorophyta): re–examination of a snow alga from the High Tatra Mountains (Slovakia). *Fottea* 18(1): 1–18.

Remias D*, **Procházková L***, Holzinger A & Nedbalová L (2018): Ecology, cytology and phylogeny of the snow alga *Scotiella cryophila* K-1 (Chlamydomonadales, Chlorophyta) from the Austrian Alps. *Phycologia 57(5):* 581–592.

Remias D*, **Procházková L***, Nedbalová L & Andersen R A (2020): Two new *Kremastochrysopsis* species, *K. austriaca* sp. nov. and *K. americana* sp. nov. (Chrysophyceae). *Journal of Phycology* 56(1): 135–145.

Lutz S*, **Procházková L***, Benning LG, Nedbalová L & Remias D (2019): Evaluating amplicon high–throughput sequencing data of microalgae living in melting snow: improvements and limitations. *Fottea* 19(2): 115–131.

* These authors equally contributed to this work.

PDF práce: https://is.cuni.cz/webapps/zzp/detail/85473

Úspěšný coming out odborné skupiny Rybníky ČLS na Hejného semináři

Tradiční mokřadní seminář, pořádaný na paměť Slavomila Hejného (1924–2001) a Dagmar Dykyjové (1914–2011) u příležitosti světového dne mokřadů (2. 2. 1971 byla podepsána Ramsarská úmluva o mokřadech), letos dosáhl své „plnoletosti". U jeho zrodu – krátce po úmrtí bývalého ředitele Botanického ústavu AV ČR a významného hydrobotanika – stáli jeho spolupracovníci Jan Květ, Jan Pokorný a Hana Čížková z bývalého hydrobotanického pracoviště v Třeboni, u jehož kořenů stála také D. Dykyjová. O vznik hydrobotanického oddělení se zasloužil právě ředitel Hejný, který v době normalizačních čistek „uklidil" celou řadu špičkových odborníků (tehdy ovšem tzv. „nespolehlivých kádrů") do třeboňských bažin, kde jim umožnil nerušený výzkum... Tým D. Dykyjové a J. Květa tak významně přispěl k poznání mokřadů Třeboňska a také k vyhlášení Biosférické rezervace UNESCO (1977) a CHKO Třeboňsko (1979).

Zatímco „dětství" mokřadního semináře probíhalo každoročně v Třeboni, během „puberty" začal ob rok „dojíždět" do Českých Budějovic, kde letos 4.–5. února proběhl již **18. seminář z ekologie mokřadů a hydrobotaniky** – opět na Zemědělské fakultě JU. Nově se k tradičním pořadatelům semináře připojila i ČLS. Jelikož odborná skupina Rybníky hledala vhodnou platformu k oslovení odborné veřejnosti a rybniční tématika se na programu mokřadních seminářů pravidelně objevovala, bylo naše zapojení do přípravy programu přirozenou volbou. Neformální formát semináře bez vložného umožňuje jak pravidelnou účast studentů JU, tak akademiků i pracovníků z praxe – např. ochrany přírody či podniků povodí. Významnou část odborného programu prvního dne semináře tak letos tvořily rybniční příspěvky – od revitalizací, přes odbahňování až po nové poznatky z rybniční hydrobiologie a ornitologie. Na semináři jsme prezentovali mj. první výsledky širokého týmu PřF a FROV JU, HBÚ BC AVČR a ENKI v rámci tříletého projektu GAČR (*Rybníky jako modely pro studium diversity a dynamiky planktonu hypertrofních mělkých jezer*), které nabízí v lecčems nový pohled na rybniční ekosystémy, možnosti jejich rybářského obhospodařování a ochrany. A především se podařilo tento nový pohled nabídnout i kolegům mimo „limnologickou bublinu" a navázat neformální kontakty, které budou potřebné pro úspěšné fungování odborné skupiny ČLS. Z tohoto pohledu se tedy první zviditelnění odborné skupiny Rybníky docela povedlo a – i podle ohlasů mezi účastníky – splnilo účel.

Poděkování patří především oběma dlouholetým obětavým organizátorům mokřadního semináře, Hance Čížkové a Honymu Květovi, za udržování tradice a povedenou akci, jakož i za vstřícnost a poskytnutý prostor pro rybniční hydrobiologii.

- Jaroslav Vrba -

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Všem jubilantům přejeme pevné zdraví, mnoho životního elánu a vše nejlepší do dalších let a také stálou věrnost a přízeň ČLS!

Za ČLS: doc. RNDr. Petr Znachor, Ph.D.

LIMNOLOGICKÉ NOVINY, č. 1/2020 ISSN 1212-2920

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