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Scientific note

Feasibility studies on future phycological research in polar regions

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***Abstract*:** Cyanobacteria and algal communities are essential in the process of initial primary succession following landscape deglaciation. Near the glacial front, a shallow wetland zone is maintained by melting ice. Here, algal mats and crusts quickly develop. The ground in all of these wet habitats is cold due to the close presence of the glacial front and permafrost. Cyanobacteria and algal populations which survive and expand in such extreme cold and unstable environments display special ecological and physiological acclimatization-adaptation characteristics, which enable them to succeed during the initial colonization phase. In this text, it is proposed to use these young microbial ecosystems as feasibility studies for developing the necessary methodology to assess the algal response to climate change. Cyanobacteria and algal communities are the most appropriate model microorganisms for such study because of their global universality, environmental sensitivity, fast reproductive potential and relatively easy experimental manipulation. We propose the microbial studies on three different mutually complementary levels:

- Study of diversity, structure and life strategies of Cyanobacteria and algae participating in the initiation of primary succession.
- Study of primary production, nitrogen fixation and nutrient utilization in natural and nutritionally-manipulated experimental set-ups.
- Study of physiological response of Cyanobacteria and algae to temperature change.

The processes of primary succession have been widely studied by Japanese, as well as Czech, scientists in polar regions during recent times. In this paper, we review phycological studies which have been carried out in the Antarctic and the Arctic, and sounded on feasibility studies in this field.

key words: Cyanobacteria, algae, primary succession, primary production

Introduction

Algae, especially the blue greens (Cyanobacteria), are omnipresent in polar desert, semidesert and humid landscapes in impressive variety (Hirano, 1979, 1983; Broady, 1982, 1984, 1989, 1996; Ohtani, 1986; Ohtani and Kanda, 1987; Ohani *et al.*, 1991; Elster *et al.*, 1995, 1997, 1999; Elster and Svoboda, 1996; Elster, 2001). One of the prominent features of the polar biome is its youthfulness. Cyanobacteria and algal

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communities are essential in the process of initial primary succession following landscape deglaciation, and thus facilitate the establishment of higher plants, since Cyanobacteria fix and provide nitrogen from the atmosphere, the only element which cannot be obtained through weathering processes (Chapin *et al.*, 1992).

During the last few decades, some polar icecaps have been shrinking and their outlet glaciers releasing virgin terrain to recolonization (Bergsma *et al.*, 1985). Near glacial fronts, a wet zone is maintained by the melting ice in summer and numerous glacial streams drain the meltwater down slopes and valleys. New, often only temporary, (semi-aquatic) habitats are formed where algal mats and crusts quickly develop. The ground in all of these wet habitats is cold due to the close presence of permafrost. When, in early autumn, temperatures drop below freezing, the shallow streams and wet soil quickly freeze or freeze-dry (Broady, 1989; Elster *et al.*, 1995).

Following the ongoing glacial retreat at Sverdrup Pass, Ellesmere Islands (79°N) Cyanobacteria, Xanthophyceae, Chlorophyceae and Conjugatophyceae-Zygnematales, (represented mainly by their filamentous forms), initiate the process of primary succession on the fresh ice-free landscape (Elster and Svoboda, 1996; Elster *et al.*, 1997). The organisms take advantage of the continuous supply of low nutrient concentrations in the glacial meltwater. Occupying some of the coldest habitats known, this group of algae tolerate low temperatures, as was shown by Elster and Svoboda (1995) in a series of field manipulation experiments involving the construction of artificial glacial streams.

Elster and Svoboda (1996) and Kubečková *et al.* (2001) classified the algae that participate in the initiation of primary succession (a ruderal strategy), *i.e.* those growing within the first 200 m away from the glacial front, as being of a 'generalist genotype'. This group experiences broad conditions of optimal and sub-optimal growth and have developed special adaptations which help them to survive the dry-freezing, low-temperature conditions and high irradiance (Davey, 1989; Vincent *et al.*, 1993a, b; Hawes *et al.*, 1992; Vincent and Quesada, 1995; Elster and Svoboda, 1996).

Cyanobacteria and algal populations, which survive and expand in such extreme cold environmental conditions, display special ecological and physiological acclimatization-adaptation characteristics that enable them to succeed during the initial colonization phase. However, no specific or unique 'mechanisms' inherent to these cold-tolerating, even cold-preferring, forms has been detected yet. But the physiological performance of these species tends to be optimal at low temperatures. A number of reports (Becker, 1982; Cohen *et al.*, 1988; Vincent and Howard-Williams, 1989; Howard-Williams and Vincent, 1989; Wada and Murata, 1990; Thompson *et al.*, 1992a, b; Matsumoto *et al.*, 1993; Vincent *et al.*, 1993a, b; Kirst and Wiencke, 1995; Nagashima *et al.*, 1995; Kanda and Komarkova, 1997; Elster, 2001) have summarized the ecophysiological principles of acclimatization-adaptation to low temperature into the following points:

- Positive carbon budget and survival under low temperatures are mainly due to low respiratory rates.
- Changes in lipid cell membrane composition may be another feature characteristic for acclimatization or adaptation to low temperature. The degree of unsaturation and the length of fatty acids chains, the phospholipid composition, and the presence of sterols may serve as an indicator of cold acclimatization.

Concept of study

In this paper, we propose to study a newly-deglaciated, sensitive micro-ecosystem—an algal system which restores life to deglaciated terrain—as an indicator of climate change. The following ideas have been developed from previous experiences with microbial primary succession after glacial retreat, gained from studies such as: Program of Japanese Antarctic Research Expedition (JARE) at Syowa Station, Japanese Arctic Research Program at Spitzbergen (Svalbard), Canadian-Czech Cooperative Research Program and Canadian-Japanese Cooperative Research Program on Ellesmere Island, High Canadian Arctic, Ellesmere Island, and finally, in the Polish-Czech Cooperative Research Project, King George Island, Antarctica. Therefore it is proposed to establish a Japanese-Czech research project which will be concerned with taxonomical, ecological and physiological studies of Cyanobacteria and algae participating in the processes of primary succession.

In the topics outlined below, we would like to take advantage of the temperature sensitivity of young microbial assemblages developing on newly-deglaciated terrain in order to indicate and predict climate changes. The studies will cover various field and laboratory manipulation experiments of light, temperature, and nutrients in Cyanobacteria and algal communities participating in primary succession, and determine and observe subsequent changes in species diversity and abundance in the treated communities. Similar manipulations will be designed for single species at the level of unialgal strain cultures. If there are significant differences in species composition and/or performance (*e.g.* productivity and physiology), the results may have a predictive value with respect to any warming or cooling of regional climate. The possible role of these microbial systems of primary succession as predictors of response to climatic changes will be studied on three different mutually complementary levels.

Study of diversity, structure and life strategies of cyanobacteria and algae participating in initiation of primary succession

In streams fed by glacial meltwater and running down the ground moraine slope in the central part of Sverdrup Pass, Ellesmere Island, an interesting phenomenon has been described (Elster and Svoboda, 1996; Elster *et al.*, 1997)—in the first 160 m away from the glacial front, an algal community has produced a large, macroscopically-visible biomass. This productive zone occurred in an area of less-vegetated 'new' moraine (sparse in vascular plants), 'new' in the sense of being deglaciated during the last 100 years, and still under the direct influence of the glacier front. The lower portion of one stream studied, which ran through an 'old' moraine, *i.e.* deglaciated some 6000 years ago, had a higher species diversity (and was more dominated by mosses and vascular plants) but lower biomass. Three ecologically complementary life strategies were recognized as operating in the stream. The first life-strategy group, represented by filamentous *Klebsormidium, Tribonema, Zygnema* and *Spirogyra*, produced floating mats in the stream. The second life-strategy group, *Phormidium, Nostoc, Dichothrix, Scytonema* and *Coleodesmium*, produced mucilaginous films and flocculi on submersed stones and sandy banks. These species produced macroscopically-visible biomass and showed weak seasonal trends but adopted a specialized life strategy, which has been described also from Antarctic periphyton by Vincent and Howard-Williams (1986), Hawes (1989), Davey (1989) and Hawes *et al*. (1992). The first algal component of this visible biomass was characterized as having a small number of vegetative cells which survive the winter period. These algae will start to propagate and reach maximum biomass in 2 to 3 months after the stream break-up in spring. These are ecological opportunists which grow only during the short polar summer. The second algal component shows an opposite life strategy. This component was characterized as having low productivity but vegetative cells which can survive the dark winter as dry frozen mats. This 'perennial' biomass provides a large inoculum for growth the following summer, thus allowing for a vigorous perennial population. The overwintering mass of cells rapidly reaches a high metabolic activity after spring rehydration. Both these life strategy groups initiate the process of primary succession on newly-deglaciated landscapes. Species of the third life-strategy group, mostly unicellular metaphytons, are suspended among the 'higher' plants, mosses, submersed vascular plants and algal mats. They occurred mostly in the section of the stream in the 'old' moraine. This group of algae showed sharp seasonal trends (Elster and Svoboda, 1996).

Methods:

From a glacial stream in freshly-deglaciated locality, algal samples will be collected randomly along and in the glacial stream a few times during the summer season. In the laboratory, a representation of each taxon (computer image analyses) observed in unsorted mixed samples will be classified for Index of Species Abundance, Species Occurrence and Species Frequency. These data will be further used for determination of algal diversity and seasonality along the stream. These ecological parameters will be calculated for various algal life-strategy groups, for ecologically different parts of the stream, and for species producing visible biomass and occurring occasionally.

The main aim of this part of the study is the precise determination of species diversity, structure and life strategy of those Cyanobacteria and algae which occupy newly-deglaciated landscapes, and a comparison of these data over time.

Study of primary production, nitrogen fixation and nutrient utilization in natural and nutritionally-manipulated experimental set-ups

As mentioned above, the studied glacial stream at Ellesmere Island demonstrates a striking phenomenon. The algal biomass (abundance and standing crop) increases and then diminishes with distance away from the glacial front. The lower section of the stream shows again a clean gravel bottom, which is virtually free of any aquatic life (Elster and Svoboda, 1996). The results of water analyses show that the stream algae utilize the minerals supplied by the melting glacier, mainly the nitrogen, up to the point of depletion. With no more nutrients available nitrogen, algal growth stops.

To verify the trend of nitrogen depletion and algal biomass diminishing along the glacial stream, three 100 m long, 7.5 cm wide, troughs were constructed in parallel to the studied stream in Sverdrup Pass (Elster and Svoboda, 1995). At the beginning of the season, before a more significant algal biomass could develop, the concentration of nitrogen supplied from the glacial water had not changed along the length of the trough. Later, however, when the troughs were populated with algae, N concentration showed a marked depletion (Elster and Svoboda, 1995).

To emphasize this result, it is worth mentioning some complementary experiments. Two fibre-glass nets (2.5×25 cm), placed to facilitate algal incubation, along with many others, in the natural and artificial streams, were lifted from the natural stream and placed one each at the lower end of two of the troughs. These nets became fully covered by filamentous algae. Algae on one of the nets, in one trough with a nitrogen-enriched medium, stayed green and the net even increased its biomass. In contrast, algae on the net placed in a trough with no N added died and were washed away within two weeks (Elster and Svoboda, 1995).

From the preliminary studies in the natural stream, as well as from data collected from artificial troughs, we have learned that *nitrogen* (mainly nitrate-nitrogen coming with the glacial melt water) is the major limiting factor in the revegetation of a newly-deglaciated landscape (Elster and Svoboda, 1995). We further need to find out how much nitrogen is derived from the stream bed due to slow decomposition of the old organic substrate, mosses and vascular plants. We also do not know the rate of nitrogen fixation of Cyanobacterial mats, which are common and produce a high biomass in the glacial stream. The ratio between dissolved inorganic and organic nitrogen in the glacial water is also unknown. Some results from the Antarctic (Howard-Williams *et al.*, 1989; Greenfield, 1992) report that 30–50% of the glacier-derived nitrogen was in the form of dissolved organic nitrogen, and the major proportion of this was identified as urea. From the Antarctic results, we postulate that the imported dissolved organic nitrogen also decreased in absolute terms along the glacial streams in our case too. We are convinced that both inorganic and organic nitrogen (mainly urea) were removed by the stream microbiota, thus initiating and facilitating the process of primary colonization. Moreover, phosphorus is also an important nutrient in sense of algae growth. The dissolved orthophosphate requirement for algal succession has to be tested as well.

Methods:

a) Natural stream: A glacial stream in a newly-chosen, freshly-deglaciated locality will be divided into sections starting from the glacial front. In each section of the stream, immediately after snowmelt, 60 fiber-glass nets (2.5×25 cm) will be positioned; these will be soon invaded by algae. The nets will then be removed from each section four times per season, dried and the relative productivity and cumulative production of algae will be determined. In addition, species composition and its quantitative distribution (computer image analyses) will be determined on some nets. Water samples for mineral and organic nutrient content will also be collected in each section and analyzed. Rate of flow, stream speed, pH, alkalinity, and irradiance, will be measured along the stream as well.

The form and source of nitrogen in the glacial stream will be determined, especially the ratio between inorganic nitrogen (nitrate, ammonia) and organic nitrogen (urea) that comes with the glacial water. Furthermore, there will be quantification of the nitrogen originating from the decomposition of 'palaeo-soil' (at Ellesmere Island, centuries-old soil is buried under the outlet glaciers) and recent organic soil, and quantification of the nitrogen originating from on-going nitrogen fixation.

b) Artificial streams: Three parallel troughs will be constructed in the vicinity of the natural stream being concurrently studied. These troughs will be made of white, 3″ diameter, PVC pipes, cut lengthwise (to become troughs), built above the ground moraine, and 100 m long. Glacial meltwater cascading down the glacial wall will fill up a 45 gallon (*c.* 200 *l*) drum, then be conveyed further down by a flexible 1½″ (3.8 cm) hose to a mechanical filter, before being supplied at a rate of 100 m*l* per second to each trough, the water supply being controlled by regular ½″ (1.3 cm) taps. The bottom of each trough will be lined with previously-weighed, fiberglass-nets, each 25×2.5 cm, in order to facilitate algal incubation. The nets, over 400 per trough, will be gradually sampled and replaced. On the nets in these troughs, algal primary production (chlorophyll *a* concentration, ash-free biomass, wet biomass) and water nutrient (nitrogen) concentration will be measured under controlled conditions. The first trough will be supplied with glacial water and enriched with a nutrient medium (all nutrients—concentration of nitrogen at the start of the trough will be 100 μgl^{-1}). The second trough will carry glacial water enriched by nitrate-nitrogen only. The third trough, serving as a control, will be supplied only with glacial water (see Elster and Svoboda, 1995). Analyses of dissolved nutrients, determination of ash-free biomass, chlorophyll and species diversity (computer image analyses) will be done at 1 m, 35 m and 100 m from the start of each experimental trough. Nitrogen fixation and decomposition will be measured on nets along the troughs.

The experiments will help us to determine the productivity and nutrient (nitrogen) consumption in natural and artificial stream ecosystems with particular interest regarding their sensitivity to temperature, simulating climate warming. A secondary aim will be verification of previous results (Elster and Svoboda, 1995) which suggested that nitrogen depletion is the main controlling factor in the diminution of algal biomass along other glacial streams in the region.

Study of physiological response of Cyanobacteria and algae to temperature change

Methods:

a) *'In situ'* cultivation experiments: Two mini-cultivation platforms (Málek *et al.*, 1969; Elster, *et al.*, 2001) are planned to be constructed in the vicinity of the glacial front. Each of these mini-cultivation units will have approximately 2 m^2 (100×200 cm) of cultivation area, partitioned by narrow bars into 25 cm wide segments over which water would cascade. Under the platform, a pump will provide continuous circulation for the algal suspension. Each cultivation unit will be inoculated by a suspension of Cyanobacteria and algae from the nearby natural stream, a nutrient-full medium being used for their cultivation. The algal suspension will be regularly filtered away to obtain biomass measurements and the medium will be adjusted to certain pre-set standards. The pump will be powered by a small hydro-power unit, installed in the glacial stream waterfall. In both cultivation platforms, temperature of algal cultivation suspension will be manipulated by heating the water in the collection tank beneath the unit, or one cultivation unit table will be white (cool) while the other will be black (warm). In these experiments, algal species diversity, dominance (computer image analyses), changes at the cytomorphological level (cell volume and size, thickness of cell wall, number size and shape of chloroplasts, presence-absence of pyrenoids, starch globules and other storage material, quantification and identification of assimilation-storage products—soluble and insoluble organic compounds), and the viability of cells (vital staining epifluorescence) will be studied. Simultaneously, productivity (photosynthetic capacity and photochemical yields-variable fluoresce, oxygen evolution and ash-free biomass) will be regularly measured. In addition to these analyses, biochemistry of the cultivated algal biomass (photosynthetic pigment content and composition–HPLC and membrane lipid content; gas chromatography, HPLC, TLC) will also be carried out. Physical (temperature, irradiance) and chemical (pH, nutrient concentration, alkalinity) parameters of the cultivation suspensions will also be continually monitored.

The technique of continuous algal cultivation (algal farming) will be attempted for the first time under relatively-favorable, polar conditions of low, or manipulated, temperature and continuous daylight. An additional bonus might be that knowledge of the algal community response to such a temperature regime could provide indicators of climate change at the ecological and ecophysiological level.

b) *'In vitro'* experiments: Isolated uni-algal strains from newly-deglaciated habitats (plankton from moraine pools, periphyton of glacial streams and pools, soil algae) will be cultivated in controlled light-temperature conditions in order to assess their ecophysiological limits (growth rate, doubling time). The experiments will be done as cross-gradient, semi-continual, cultivations on cultivation equipment under elevated light-temperature conditions. The most convenient Cyanobacteria and algae species will be used for detailed cytomorphological, physiological and biochemical studies, similar to those mentioned before, but at the species level.

To discover the acclimatization/adaptation strategies of Cyanobacteria and algae to elevated temperatures. Knowledge of an acclimatization/adaptation rate could help us to understand the ecological roles of single species (r and K strategies) among the processes of primary succession, as well as their ecophysiological response to climatic change.

Conclusions

These feasibility studies will be integrated into the research programs of Global Change and Terrestrial Ecosystem (GCTE), International Tundra Experiment (ITEX) and Regional Sensitivity to Climate Change in Antarctic Terrestrial Ecosystems (RiSCC). The studies will focus on developing the methodology for an assessment of algal response to climate change. Because of their global universality, environmental sensitivity, fast reproductive potential and relatively easy experimental manipulation, Cyanobacteria and algae can be recommended as the most suitable of model microorganisms. The project, if successful, may develop into a long-term program based at two or three alternative polar, or even alpine, sites.

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