Faculty of Biological Sciences
University of South Bohemia

Institute of Botany
Academy of Sciences of the Czech Republic

Adaptation of algae to extreme environments

Mgr. Jana Kvíderová

PhD thesis

Supervisor: RNDr. Jaromír Lukavský, CSc.

Consultants: ing. Josef Elster, CSc., RNDr.Ondřej Prášil, CSc.

Botanical PhD theses 2004/2 **České Budějovice and Třeboň**

Kvíderová J., 2004: Adaptation of algae to extreme environments. Doctoral thesis, University of Southern Bohemia, Faculty of Biological Sciences, České Budějovice, Czech Republic.

Supervisor: RNDr. Jaromír Lukavský, CSc.
Consultants: Ing. Josef Elster, CSc.
RNDr. Ondřej Prášil, CSc.

Annotation: Properties of a new unit for crossed gradients of temperature and light were evaluated. Ecological requirements for temperature and light of temperate and polar strains of *Stichococcus* (Trebouxiophyceae, Chlorophyta) were compared.

Acknowledgements: This thesis was supported from grants INCO-COPERNICUS, – ME419, MŠMT 1231 00004, K 60 051 14 of the Academy of Science of Czech Republic, EU project - COBRA (The conservation of a vital European scientific and bacterial resource: microalgae and Cyanobacteria) No. QLRT-2000-01645, and Czech-Japan Project - Contact ME 576 Ecological and physiological adaptation of algae to low temperature and low irradiance.

I'm very grateful to J. Lukavský, J. Elster and O. Prášil for critical comments and suggestions, to Dr. J. Simmer and Mrs. M. Kovářová for statistical analyses proposals, and Mrs. M. Kašpárková and Mrs. V. Titlová, CCALA technicians, for preparation of the cultures. I thank my parents and grandmother for support and my brother Hawkeye for solving all problems with IT technology. Finally, I thank Hal (named after its "colegue" form A. C. Clark's book 2001: A space odyssey) for keeping and processing the data.

Hereby I declare that I worked up this thesis myself only with help of literature listed in References and people listed in Acknowledgements.

November 11, 2004, Třeboň

Jana Kvíderová

List of enclosed papers

1. KVÍDEROVÁ J. & LUKAVSKÝ J. (2001): A new unit for crossed gradients of temperature and light. – In: ELSTER, J., SECKBACH, J., VINCENT, W.F., LHOTSKÝ O. (eds.): Algae and extreme environments: Ecology and physiology. – Nova Hedvigia Beih. 123: 541 – 550.
2. KVÍDEROVÁ, J. & LUKAVSKÝ, J. (2003): The cultivation of *Phaeodactylum tricornutum* in crossed gradients of temperature and light. – Algological Studies 110: 6 – 80.
3. KVÍDEROVÁ J. & LUKAVSKÝ, J. (submitted): The ecological characteristics of *Stichococcus* (Chlorophyta) strains isolated from polar and temperate regions. Algological Studies.

Statement of co-author contributions to individual papers enclosed in this doctoral thesis.

1. KVÍDEROVÁ J. & LUKAVSKÝ J. (2001): A new unit for crossed gradients of temperature and light. – In: ELSTER, J., SECKBACH, J., VINCENT, W.F., LHOTSKÝ O. (eds.): Algae and extreme environments: Ecology and physiology. – Nova Hedvigia Beih. 123: 541 – 550.

   Jana Kvíderová measured all data, worked out mathematical models of temperature and irradiance gradients. Jaromir Lukavsky supplied data about cultivation methods. Both authors prepared the manuscript.

2. KVÍDEROVÁ, J. & LUKAVSKÝ, J. (2003): The cultivation of *Phaeodactylum tricornutum* in crossed gradients of temperature and light. – Algological Studies 110: 6 – 80.

   Jana Kvíderová performed all experiment and prepared the manuscript. Jaromír Lukavský assisted in design of the experiments and corrected the text of the paper.

3. KVÍDEROVÁ J. & LUKAVSKÝ, J. (submitted): The ecological characteristics of *Stichococcus* (Chlorophyta) strains isolated from polar and temperate regions. Algological Studies.

   Jana Kvíderová performed all experiment and prepared the manuscript. Jaromír Lukavský assisted in design of the experiment and corrected the text of the paper.

Hereby I declare that the contributions mentioned above correspond to the reality.

November 11, 2004, Třeboň

Jana Kvíderová

I agree with this statement of co-author contributions to individual papers enclosed in this doctoral thesis mentioned above.

November 11, 2004, Třeboň

Jaromír Lukavský

Contents

Abbreviations used in text:

AA	aminoacid
APC	allophycocyanin
ATP	adenosine triphosphate
CCM	CO_2 concentrating mechanism
Chl a	chlorophyll a
cyt b_6/f	cytochrome b_6/f
Gy	10^9 years
LHC II	light-harvesting complex 2
My	10^6 years
NADPH	nicotinamidadeninedinucleotidephosphate
OEC	oxygen evolving complex
PAR	photosynthetic active radiation
PBS	phycobilisome
PC	phycocyanin
PE	phycoerythrin
PQ	plastoquinone
PSI	photosystem 1
PSII	photosystem 2
RC	reaction centre
RUBISCO	ribuloso-1,5-bisphosphatecarboxylaseoxidase
UV	ultraviolet

1. Introduction

Eukaryotic microalgae and prokaryotic cyanobacteria belong to the oldest organisms on the Earth. Cyanobacteria appeared on the planet early, approximately 3.5 Gy ago. Their lineage separated from other Eubacteria and their structure did not change further in the course of evolution. For long time, they were the only organisms capable of oxygenic photosynthesis; the present oxygen atmosphere is the result of their metabolism (SCHOPF 1993). They precipitated large amounts of CO_2, so almost all limestones are of biogenic origin (HSU 1992, FERRIS et al. 1994, FALKOWSKI et RAVEN 1997, ZAVRAZIN 2002). Existence of simple unicellular eukaryotic organisms is proven in fossils 1.9 Gy old. Folios organisms resembling algae were found in sediments 1.3 Gy old. These microscopic and macroscopic algae do not possess any structures for determination of their taxonomic position in individual classes. Fossil records indicate presence of the Chlorophyta, Rhodophyta and Dinophyta in the end of the Precambrian, some 600 My ago. Unmistakable evidence of the fossil diatoms is dated about 120 My ago. This does not preclude the possibility that they have been presented earlier, perhaps in the form of organisms without silicified walls (VAN DEN HOEK et al. 1995).

During their existence, the algae and the cyanobacteria had to adapt to various conditions, from almost anoxic to present oxygen-rich atmosphere (the evidence is the enzyme RUBISCO that has its affinity to CO_2 and O_2 that is not advantageous in present composition of the atmosphere and results in various mechanisms of CO_2 concentration, ŠETLÍK et al. 2000), to varying average temperature of the planetary surface, to changes in intensity and spectral composition of light resulting from the changes in composition of the atmosphere and to changes associated with movement to other biotopes.

According to environmental conditions, the algae and the cyanobacteria change their morphology and physiology. The changes can be caused by many environmental factors, i.e. temperature, irradiance, nutrient availability, presence of toxic compounds, pH, etc., and their combinations. The morphological changes were observed e.g. in *Heterococcus*, *Scenedesmus quadricauda* or *Xanthonema* (LUKAVSKÝ 1982, DARLING et al. 1987, BROADY et al. 1997), so the work of taxonomists becomes more complicated. However, it is possible to experimentaly determine changes in morphology of individual species, and even limits of this variability, necessary for the determination during cultivation in marginal environmental conditions. From ecological point of view, knowledge of photosynthesis efficiency under extreme

conditions is highly important because the photosynthesis efficiency significantly affects the carbon and oxygen cycles of our planet.

The Polar Regions are the largest ecosystem; polar seas belong to the most productive regions of the World Ocean. In these regions, very extreme environmental conditions prevail and the algae and the cyanobacteria have to cope with various environmental stresses, e.g. with low temperature, continuous irradiance including UV, desiccation and freezing etc. Algal and cyanobacterial strains isolated from the Polar Regions are especially suitable for testing of physiological resistance to different types of stresses and for studies of mechanisms of adaptation - acclimatisation.

The interest in studies of adaptability or capability of acclimatisation of psychrophilic and psychrotrophic microorganisms lasts for many years. These microorganisms are source of compounds valuable for biotechnology, e.g. enzymes for processes operating at low temperatures or polyunsaturated fatty acids (STIBOR et POTOCKÝ 2000). During cultivation, specific adaptations to laboratory conditions occur. During the last years, the interest has become deeper because the technologies enabling exploration of planets and moons of the Solar System reached the stage when we can seriously investigate the possibility of extraterrestrial life (FRIEDMANN 1993a,b, ESA SP-1231 1999, ESA SP-496 2001, ESA SP-518 2002, CELNIKIER et TRÂN THANH VÂN 2003, ESA SP- 545 2004).

It is presumed that the cyanobacteria and algae that manage to survive in extreme conditions found in the Polar Regions are hypothetically also capable of further adaptation - acclimatisation to harder environmental conditions. These cyanobacterial and algal strains that possess broad capability of adaptation-acclimatisation could be interesting e.g. for Mars terraforming (FRIEDMANN et al. 1993a).

1.1. Extreme environments and microorganisms

1.1.1. Extreme environments

No textbook of phycology or microbial ecology has defined the term "extreme environments" completely. Our life, based on carbon compounds flowing in water, has limits based on physical and chemical laws. The most apparent example is the requirement of liquid water, so the imposed limits are the melting and boiling points of water. Some organisms have found the way how to survive in temperatures under the melting point or above the boiling point but they cannot live in other environments. Then, the extreme environment is defined as an environment that requires considerable changes in organisms that live there (BOSTON 1999).

KRISTJASSON et HREGGVIDSSON (1995) define the extreme conditions as those out of normal from human point of view, i.e. temperatures in range 4 to 40 °C, pH 5 to 8.5, salinity from freshwater to marine. This division is relative. According to these criteria, it is possible to find a very well adapted organism for which the "extreme" (from human point of view) conditions are not extreme. Even if we suppose that the extreme conditions are those different from prevailing average in general (the so called mesophilic conditions), we must consider that the criteria of the mesophilic conditions change in time, e.g. the mesophilic conditions in Archean differ from the present ones. FRIEDMANN (1993a) describes the real extreme environments as those when the conditions reach limits to which no organism can adapt successfully, in accordance with the definition of BOSTON (1999).

ELSTER (1999) suggests establishing two categories of extreme environments in phycology:

a) objectively extreme but stable environments

   In these habitats, the cyanobacteria and the algae live near the limit of their physiological potential. Their life processes provide an example of adaptation to those environments and the successive development and selection of genotypes occurred. These special habitats are inhabited by their own well-adapted flora, e.g. psychrophilic or thermophilic algae. Their survival depends on equilibrium of physico-chemical factors, slight disturbation can cause extinction of a species or a community (FRIEDMANN 1993a). According to GOMBUSHINA et KRUMBEIN (1999), these habitats are normal, i.e. with stable, proper and utilisable physical and chemical parameters for

metabolism and reproduction of any species. This is also defined as an environment where any change is small.

b) "marginal" unstable types of environments

The seasonal and diurnal variation of this environment can impose severe conditions. Thus, the algae have to overcome a series of environmental patterns to survive in such habitats, e.g. environmental conditions in polar streams, in cryptoendolithic communities of hot and polar deserts. These patterns can differ in periodicity, amplitude, synchrony and regularity and may initiate a number of different adaptations. These "marginal" conditions conform to "poikiloenvironment" of GOMBUSHINA et KRUMBEIN (1999), characterised by accidental, very delayed or rarely occurring conditions for optimum of metabolism or reproduction of the given species.

1.1.2. Organisms in extreme environments

According to individual environmental factors, we distinguish several categories of organisms living in extreme environments, i.e. extremophiles. These categories are not strictly limited; one organism can belong to more than one. The limits for definition of the extremophile rise from the limits given by KRISTJASSON et HREGGVIDSSON (1995), see chapter 1.1.1. According to the definition of GOMBUSHINA et KRUMBEIN (1999), the extremophile is an organism highly adapted to narrow environmental conditions.

1.1.2.1. Thermophilic microorganisms

Thermophiles grow best at temperatures above 40 °C. They live in hot springs, in sun-heated soils and in geothermal regions. Growth of hyperthermophilic bacteria and Archaea is the fastest in range 80 to 100 °C, they do not grow at temperatures below 60 °C. Archaea and bacteria were found near hydrothermal vents at 115 °C (WALSH et SECKBACH 1999). In Cyanobacteria, the upper growth limit is approximately 73 to 74 °C; the genus *Synechococcus* is the most thermophlilic (OREN et SECKBACH 2001). *Cyanidium caldarium*, an acidophilic Rhodophyte, is the best-studied thermophilic eukaryotic autotroph whose optimum growth temperature is 45 °C. The alga does not compete with any known microorganism in its niche, it seem possible that *Cyanidium* grows at the most limiting conditions. The upper limit for thermophilic fungi lies near 60 °C (ROBERTS 1999).

1.1.2.2. Psychrophilic microorganisms

The optimum growth temperature of psychrophiles is lower then 15 °C and the upper growth limit lies below 20 °C. On the other hand, psychrotrophes, living also at 0 °C, have their optimum growth temperature above 15 °C and their upper temperature limit of surviving reaches 40 °C in some cases (MORITA 1975). They live in polar or mountain soils and water, even in sea ice (WALSH et SECKBACH 1999). If water freezes, all living processes must be stopped, so the melting point is the lower limit of growth and the organisms are subjected to freeze-thaw cycles. Typical examples are organisms of polar or mountain regions, e.g. an Antarctic heterotrophic flagellate *Heteromita globulosa.* Snow can be coloured by kryoseston microorganisms even in temperate region (e.g. KOL 1975, LUKAVSKÝ 1993). Among kryoseston, we often find *Chlamydomonas nivalis*, *Chloromonas brevispina*, and *Raphidonema* sp. (LUKAVSKÝ 1993).

1.1.2.3. Acidophilic microorganisms

Their growth optimum lies below pH 5. One of the best-known organisms is *Thiobacterium ferredoxans*, a bacterium oxidising iron and sulphur. Six organisms growing at pH near 0 are known - *Cyanidium caldarium* (a red alga), *Acontium cylatium*, *Cephalosporium* sp., *Trichosporon cerebriae* (fungi), *Picrophyllus oshimae*, and *Picrophyllus torridus* (prokaryotes, ROBERTS 1999). Green flagellates, e.g. *Dunaliella acidophila* and *Chlamydomonas acidophila* are able to grow in low pH between 2 and 3 (PICK 1999, VISVIKI et PALLADINO 2001). Although the cyanobacteria are not usually observed at pH below 4 -5 (OREN et SECKBACH 2001) the acid tolerant ones do exist (STEINBERG et al. 1998)

1.1.2.4. Alkalophilic microorganisms

They grow better at pH above 9 and their growth is slower or is stopped at neutral pH (or pH below 6.5). They were isolated from soil, excrements and deep-sea sediments (WALSH et SECKBACH 1999). The champion among them is the cyanobacterium *Plectoneama nostocorum* growing at pH 13 (ŠMIGÁŇ et GREKSÁK 2000). Typical alkalophilic photosynthetic eukaryotes are some diatoms, e.g. *Nitzschia frustulum* (OREN et SECKBACH 2001).

1.1.2.5. Halophilic microorganisms

The environment of halophiles is characterised by extreme salinity, above 7 %, and by precipitation of dissolved minerals. Halophilic bacteria and cyanobacteria mostly occur in hypersaline lakes and lagoons (WALSH et SECKBACH 1999). It has been shown that bacteria can survive in old alkalic salt deposits and also many eukaryotes can survive in hypersaline environments (HENLEY 2001, HENLEY et al. 2002). Halophilic and halotolerant algae were reviewed by GLIMOUR (1990). *Dunaliella salina* is the well-known halophilic flagellate (GINZBURG 1987) used for biotechnological mass-scale carotene production, e.g. in lagoons in Western Australia (BOROWITZKA 1991). *Arthronema africanum* grows in nature in saturated NaCl solution, but it is rather halotolerant because its growth optimum is at 0.5 and 1.5 ‰ salt solution (KOMÁREK et LUKAVSKÝ 1988).

1.1.4.6. Xerophilic microorganisms

Although water is essential for life, some organisms are capable of surviving with minimum requirement for liquid water. The cyanobacteria and algae of cold and hot deserts are found either in soil or inside or on the surface of rocks (e.g. PALMER et FRIEDMANN 1990). Low nutrient availability also accompanies the water deficiency. Organisms adapt to these conditions by different ways, e.g. by miniaturisation, by binding to surface, by storage polymers (MORGAN et DOW 1986). The green algae *Pleurococcus* (*Desmococcus*) and *Trentepohlia umbrina* can survive on the tree bark almost without water for major part of the year; rain falls on the bark only during period without leaves.

1.1.2.7. Barophilic microorganisms

Deep oceans are the best-studied environment of the barophiles. Bacteria and Archaea can range from barotolerant (i.e. capable of growth at high pressures, but grow better at normal atmospheric pressure of 0.1 MPa) to obligatory barophilic (i.e. grow only at high pressures). Another possible environment lies deep under the Earth surface where pressure reaches 20 to 50 MPa (WALSH et SECKBACH 1999). Fungus *Magnaporthe grisea* can create pressure of 8 MPa during invasion to a host cell, eukaryotic communities of continental shelf grow at 20 MPa (ROBERTS 1999). According to GOLD (1999), barophiles are the original living organisms from which organisms on the surface evolved (see also CÍLEK et MARKOŠ 2000a). However, this theory has been disproved by findings that these barophiles have secondary adapted to the environment of hydrothermal vents. However, the origin of living organisms in

the barophilic environment cannot be excluded, but in this case, a period of low temperature is necessary for evolution at the molecular and cellular levels (FORTETERRE 2000).

1.1.2.8. Anaerobic organisms

They are found in anoxic environments in surface and water layers of mud (ROBERTS 1999). Some chemoautotrophic bacteria and archaea live in the atmosphere composed of pure CO_2 or NH_3 (SIEGEL 1999). No strictly anaerobic eukaryotic phototrophic organism is known (ROBERTS 1999) although flagellates migrating for nutrient uptake to anaerobic conditions near bottom of a floodplain pool were observed (e.g. PITHART et al. 1997).

1.2. Adaptation of microorganisms to extreme environments

The reaction to stress conditions (and thus even to the extreme ones) is the basic characteristics of all living organisms. The organisms cope with stresses differently - they can run away, survive the hostile conditions in stage of spores or seeds, or adapt and survive.

The stress reaction can be described by curve published by PROCHÁZKA et al. 1998. Immediately after beginning of incidence of stress factor (stressor), cell structure and functions are disturbed (alert stage). If the stress intensity does not exceed the level when death of cell or organism occurs, compensating mechanisms are mobilised (restitution stage). The resistance of the organism increases (hardiness stage) up to maximum level (resistance stage). The resistance does not need to last constantly. During the long-term and intensive stress, the resistance can decrease again (exhaustion stage). The course of the stress reaction does not depend only on the intensity and duration of the stress but also on the genetically fixed suppositions of the response that can be generally described as the adaptation capability.

Several terms are used for adjustment of organism to stressor but their definitions differ from each other and often overlap. ELSTER (1999) defines following terms for the algae:

Adaptation - genetically fixed responses to outer environmental conditions

Acclimatisation - response to sporadic extremes of the environment that is not genetically fixed

Acclimation - response to laboratory conditions (e.g. laboratory cultivation)

PROCHÁZKA et al. (1998) use the term *acclimation* for temporary increase of resistance, acquired in the course of stress, not only in laboratory conditions.

The most studied stress factors that contribute to extremisation of environment for the algae are temperature, irradiance, water deficiency and toxicity. In my doctoral thesis, I focus on adaptation of algae to low temperatures and low irradiances, so known adaptation mechanisms to these factors are described comprehensively.

1.2.1. Temperature

Individual species of the Algae are capable of surviving only in relative narrow temperature range between the lower and the upper growth limits of 30 to 40 °C. In algae from very stable habitats, e.g. hot springs or polar ocean, this range can be lowered to approximately 10 °C. Growth temperature characteristics (lower growth limit - optimum –

upper growth limit) lie in range from 0 °C, typical for some cryoseston algae (HOHAM 1975), to temperatures near 75 °C, characteristic for cyanobacteria of hot thermal springs (WARD et CASTENHOLZ 2000). Limits of surviving can exceed these values (ELSTER 1999).

While optimum growth temperature of mesophilic algae lies around 20 – 30 °C and these algae can not grow at temperature near 0 °C, some cyanobacteria and algae are able to grow in temperatures around the melting point of water. Typical representatives of psychrophilic and psychrotolerant algae are kryosestonic species (HOHAM 1975, ROUND 1985) and species of mountain and the Polar Regions. The Antarctic ice algae can grow even at temperatures below 0 °C in ice crystals (ROUND 1985, SOUTH et WHITTICK 1987).

GOUNOT et RUSSEL (1999) summarise adaptations to low temperatures at cellular level. Temperature shift from normal to low (high) temperatures leads to lag-phase of growth curve. Then, the growth is restored but the growth rate is lower. At low temperature, the growth rate of psychrophiles and psychrotolerants is higher then in mesophiles, this is also valid for the metabolic rates.

The dependency of metabolism rate on temperature can be described by Arrhenius plot. The decrease of temperature results in the decrease in metabolic rate. Usually, the slowest reaction becomes the rate-determining factor of the complex chemical reaction (e.g. photosynthesis). At specific temperature, the rate of membrane bound electron transport reaction breaks (so-called Arrhenius discontinuity) and from this point facing to the low temperatures, the decrease of the rate is faster. These breaks are caused by lipid phase transitions of the membrane (RAI et GAUR 2001).

During shifts to low temperatures, the changes in fatty acid composition of membranes result in higher fluidity of membranes, i.e. in increase of ratio of unsaturated fatty acids, cis-double bonds, methyl branching, and shortening of fatty acid chains, in order to preserve important membrane-bound functions, e.g. passive transport or electron transfer. The lipid composition can be affected in two ways. The first way, the direct one, concerns to physical properties of the lipid bilayer that increase or decrease the fluidity (e.g. KOTÍK 1996). The second way, the non-direct one, affects the enzymes of the fatty acids biosynthesis in such way that results in changes in acyl chains of lipids in existing membranes (fast changes) or in composition of products of fatty acid synthase for subsequent insertion into new membrane lipids (slow changes, RUSSEL 1984).

Preservation of structure and function of various proteins during low temperature is also another problem faced by microorganisms. In the water-dissolved proteins, the hydrophobic

regions are responsible for stabilisation of the protein. These regions are created by α-helixes or β-sheets in the core of the protein. On the surface, polar and ion interactions occur (JAENICHE 1990). During lowering of the temperature, the strength of the polar interactions increases but the strength of hydrophobic ones decrease. The compact protein structure decays and individual subunits dissociate (PRIVALOV 1979). All cellular proteins are affected, so the proteins of organisms adapted to cold must be fold is such way as to maintain their function and to prevent dissociation (WALLES et al. 1999).

The enzymes are proteins that are highly sensitive to temperature. The complete catalytic cycle of enzyme consists of three basic parts where weak interactions sensitive to temperature occur: identification and binding of substrate, conformation change of the enzyme leading to the transition state and formation of the product, and release of the product (e.g. MARSHALL 1997, VOET et VOET 1995). The sensitivity is different for various enzymes even isolated from the same species (LOPPES et al. 1996) and different enzymes have developed different responses to low temperature. In comparison with pure enzymes isolated from mesophiles, the enzymes isolated from cold adapted organisms are more active in lower temperatures (LOPPES et al. 1996). On the other hand, activity of RUBISCO was lower in psychrophilic algae than in mesophiles and the optimum activity temperature was similar in psychrophiles and mesophiles. The lower enzyme activity was probably compensated by higher amount of RUBISCO subunits in the psychrophilic alga (DEVOS et al. 1998).

An increase of temperature above the optimum damages the organisms by different ways, e.g. by inhibiting proteosynthesis, by leakage of intercellular compounds caused by membrane failure, by changes in cell wall structure, by formation of groups of coccoid cells (in *Arthrobacter glacialis*), and by activation of some catabolic respiratory and autolytic enzymes (GOUNOT et al. 1977). So, the thermolability of enzymes determines the upper limit of surviving, the cold dissociation defines the lower one.

If the organisms are exposed to temperatures above the growth optimum, heat shock proteins (HSPs) are synthesised (NEIDHART et al. 1984). The structure of HSPs is highly conserved and their function is similar in all organisms. HSPs serve in cell as molecular chaperons, i.e. facilitate correct protein folding and degradation of unfold or denaturised proteins. If the cells are exposed to the heat shock, the requirement for HSPs increases for folding of the newly synthesised polypeptides and degradation of the heat denaturatised proteins. In psychrophiles, the induction of the HSPs synthesis occurs at lower temperature than in mesophiles. The HSPs synthesis is also induced by other stresses, e.g. by the presence

of toxic compounds, but sudden exposure of the microorganisms to low temperatures does not induce their synthesis.

During temperature shift to low temperatures, reactions of the cold shock occur and cold shock proteins (CSPs) are synthesised during the initial lag-phase. CSPs were found in mesophilic, thermophilic and psychrophilic microorganisms. CSPs differ from other stress proteins. There are two classes of CSPs. Class I CSPs are present at low concentration in the cell at normal temperature and their concentration increases more then 10 times after the cold shock. Class II CSPs are presented in the cell at specific level that grows up less then 10 times after the low temperature shock. Some of the CSPs are necessary for restoration of growth in low temperatures. They operate on transcription and translation levels (GOUNOT et RUSSEL 1999).

The response to cold shock slightly differs in psychrophiles and mesophiles. In the cold-adapted microorganisms, the synthesis of housekeeping proteins is not inhibited, the number of CSPs is higher and increases with stress intensity, and the level of CSPs genes expression is lower. Special group of cold acclimation proteins (CAPs) is necessary for growth in cold environments together with CSPs but the function of CAPs in the cell has not been elucidated yet (ROBERTS et INNIS 1992, BERGER et al. 1997).

The molecular mechanism of the cellular thermometer is not known. Three possible sensors are proposed (PANDOFF et al. 1998):

Ribosomes - cold shock reduces the translation capacity, concentration of AA-$_t$RNA is high and the A-site of the ribosome is blocked. During heat shock, AA-$_t$RNA supply is depleted by the higher translation rate and the A-site is empty.

Membranes - concentration of (p)ppGpp directly or indirectly influences the intensity of the cold shock response. Nucleotides also form connection among events occurring inside the cell and those in membrane where they participate in lipid synthesis. Temperature dependence of synthesis, phosphorylation and dephosphorylation of proteins was observed in the membrane and in cytosol. The temperature dependence of the phosphorylation of lipopolysaccharides is included in the cold adaptation reactions.

Nucleic acids - DNA superhelix structure changes in dependence on the temperature, the negative supercoiling increases in lower temperatures.

WEBB et SHERMAN (1984) regard protein DnaK as the cell thermometer. DnaK is monomeric protein of molecular weight of 69 kDa, necessary for cell growth and division.

The protein acts as chaperon. The concentration of the substrate for DnaK (unfolded proteins) increases with temperature, and leads to substrate binding to DnaK. Depletion of free DnaK induces the heat shock response.

In nature, the psychrophilic microorganisms are subjected to freeze-thaw cycles. The response to these changes depends on the physiological state of the cell, the rate of cooling, freezing and thawing and on the chemical composition of freezing medium (MACLEOD et CALCOTT 1975, SMITH 1995). Growing cells are less resistant than spores. If bacteria, like higher plants, are exposed to low temperature treatment, they are more resistant to frost (LARCHEL 1988).

Cells protect themselves against influence of the temperatures near freezing point by various ways in order to prevent formation of ice crystals that could damage cell membranes. Antifreeze proteins were documented in many organisms but their homologues have not been described yet in bacteria (GOUNOT et RUSSEL 1999). Ice nucleation in bacteria is ascribed to presence of protein that is composed of highly repetitive sequences and is present in outer cell membrane where it acts as a template for the ice nucleation. In this way the desiccation is prevented, the water source near cell surface is separated, ice particles are spread out from the cell and the damage induced by ice formation inside the cell is prevented (LEE et al. 1995). Other molecules are often synthesised by Arctic and Antarctic microorganisms. Freezing invokes osmotic shock that can be averted by accumulation of compounds like polyalkohols or sacharides in algae, or AAs in fungi (GOUNOT et RUSSEL 1999).

Temperature is also one of the main factors that control rate of photosynthetic reactions. The influence of the temperature on photosynthesis has been described by DAVISON (1991). The temperature range in which the photosynthesis occurs is -7 °C to +75 °C (CASTENHOLTZ 1969, DAVEY 1989). The lower limit is given by the presence of liquid water, the upper one by the thermal stability of PSII (ŠETLÍK et al. 2000). Q_{10} (multiplication factor for the metabolic rate at T + 10 °C) of photosynthesis is usually close to 2.0 (SUKENIK et al. 1987). The photosynthesis rate increases continuously to optimum temperature, after that it falls down steeply (ŠETLÍK et al. 2000). If the photosynthesis is not limited by light, limitation of electron transport or carbon metabolism is possible. The carbon metabolism could be limited by carbon fixation by RUBISCO and its activase (SUKENIK et al. 1987), or by the rate of sucrose synthesis that regulates amount of inorganic phosphate and so the rate of ATP regeneration (STITT et al. 1987). Temperature also influences other enzymes and other physical processes that could limit the photosynthesis. These processes include diffusion,

carbonylanhydrase activity and active transport of CO_2 or HCO_3^- across cytoplasmatic and chloroplast membranes. Further, the inorganic carbon availability modifies the shape of the photosynthetic curve. In higher temperatures, photorespiration must be taken into account. The algae, although belonging to C-3 plants, often possess the CO_2 concentrating mechanism (CCM) that reduces the photorespiration. The CCMs do not use reactions described in C-4 plants.

If the photosynthesis is limited by light, the efficiency of light capturing decreases with increased temperature in Antarctic algae. Subsequently with increasing temperature, the irradiance necessary for reaching the compensation point increases. The rate of net photosynthesis, measured at subsaturated irradiance, declines with increasing temperature, or increases more slowly than in light saturated photosynthesis.

Phenotypic acclimatisation of photosynthesis differs from short-term effects of low temperatures. In higher plants grown in low temperature, both the maximum rate of photosynthesis and the resistance to photoinhibition induced by low temperatures increase, the sensitivity to temperature declines and the optimum temperature for photosynthesis shifts to lower values. In many cases, the temperature acclimatisation of photosynthesis can be related to the changes in RUBISCO activity, of other Calvin cycle enzymes and of the electron transport chain. Alga *Laminaria saccharina*, grown at 15 °C, had more PSII reaction centres than alga cultivated at 5 °C and larger photosynthetic unit (PSU based on ratio Chl a:PSII). In red alga *Chondrus cripus*, the amounts of PSI and PSII are influenced by temperature, but ratios Chl a:P700, PC:APC, PE:APC are significantly higher at 20 °C then in 5 °C (DAVISON 1991). Photoinhibition was caused by low electron transport rates. Similar observations in the green alga *Chlorella* and cyanobacterium *Anabaena* (GRIGOREVA et al. 1989) prove that the temperature acclimatisation of photosynthetic apparatus is a widespread phenomenon.

Physiological adaptation is given by strong selection pressure on the photosynthetic apparatus. Algae from low-temperature environments reach higher photosynthetic rates in lower temperatures; their optimum and upper temperature limits of photosynthesis are lower then in algae of warmer regions (KUEBLER et al. 1991). The physiological basis of photosynthesis adaptation often resembles temperature acclimation for the given species. Photosynthetic enzymes isolated from algae grown at different temperatures differ in their kinetic characteristics (LOPPES et al. 1996, DEVOS et al. 1998).

Only a few biophysical studies have focused on photosynthesis of low temperatures. The electron transport rate, like the other metabolic processes, is influenced by temperature. The temperature has dual effect. The steep dependence on temperature results from high activation barrier of the transport reactions and light damage occurs if the capacity of photosynthetic apparatus for light energy utilisation or dissipation is exceeded. In PSII reaction, two points of temperature regulation were identified, one on the acceptor and second on the donor sites. The steep dependence of electron transport rate form Q_A to Q_B on temperature was measured in temperate plants. Q_A^- oxidation rate in wild temperate species ranged 150 and 250 μs, the rate slowed down to 50 to 100 ms at 0 °C (JOLIOT 1974, GLEITER et al. 1990, KANAZAWA et al. 1992). This reaction probably limits whole electron transport. In Arrhenius plotting, there is break at 4 °C when change of phase of lipid membrane occurs (GLEITER et al. 1990).

The temperature dependence of S-state transitions was studied using the spinach PSII particles. All S-states had significant activation barriers, among them the $S_3 \rightarrow S_0$ transition, leading to O_2 release, was the most important (RENGER et HANSSUM 1992). This transition is the slowest and probably limits the electron transport. At 20 °C, the rate is 1.66 ms and slows down to 4 ms at 1 °C. Similar results were obtained in thermophilic cyanobacterium *Synechococcus vulcanus* (KOIKE et al. 1987).

Cyt b_6/f complex, carrying electrons from PSII to PSI is also often considered to be the rate limiting stage of the electron transport. The reaction has steep temperature dependence in temperate species. The rate ranges from 2 to 10 ms at 20 °C. At 4 °C, the measured rate was 66 ms. It seems that this stage is strong control point of the low-temperature photosynthesis (WHITMARSH et CRAMER 1979, KRAMER et CROFTS 1993). Limitation of photosynthesis, in low temperature, leads to photoinhibition (see chapter 2.3.2).

In psychrophilic organisms, the existence of lower activation barriers is proposed. However, measurements of flash induced changes need not essentially provide adequate information about steady-state conditions. In continuous light, rates of individual reaction can be affected by changes in metabolite levels, stromatal and lumenal pH, membrane electrical potential and mutual connections of regulation mechanisms (PRÁŠIL p.c.).

Even in spite of slowering of the processes, the photosynthesis is less sensitive to low temperatures then the respiration so even small temperature change results in change in growth rate. It enables net primary production even in low temperatures (PRÁŠIL, p.c.) and that is reason why the polar seas are usually the most productive parts of world oceans (LARCHEL 1988).

1.1.2. Light

For life to exist, a source of energy is necessary. On the Earth surface, the radiance is the most important energy source for life because it drives photosynthesis, the only process that can covert the light energy to the energy of chemical bonds. For photosynthesis, the most important part of electromagnetic radiation is from 400 to 700 nm, the photosynthetic active radiation (KUBÍN 1973, PROCHÁZKA et al. 1998). The radiation of other wavelengths also affects living organisms, e.g. UV-B and the ionising radiation (ROTHSHIELD 1999); far-red light induces germination of seeds of *Lactuca sativa* or flowering. In algae, the light (not only red, but also even yellow and green) can affect all growth and developmental stages - division, thalus branching and growth, formation of gametes and spores and their germination (PROCHÁZKA et al. 1998).

Photosynthetic active radiation

The PAR availability, its spectral composition and intensity strongly vary in time. In all waters, the red light diminishes quickly, while the blue-green and blue light prevail. The intensity can vary from minute intensities in cryptoendolithic communities (ca 0.1 $\mu mol.m^{-2}.s^{-1}$, NIENOW et al 1988a) up to full sunlight (>2000 $\mu mol.m^{-2}.s^{-1}$, ŠETLÍK et al 2000). UV radiation, penetrating through the damaged ozone layer to the surface becomes serious ecological problem because it affects nitrogen and carbon fixations by algae and cyanobacteria so cycles of these elements in biosphere are disturbed.

The algae compete for radiation with one other and in addition, with all light-absorbing and scattering components in water environment. This competition, in both aquatic and terrestrial ecosystems, results in vertical distribution of species and is driving force for development of light harvesting pigments (ELSTER 1999). The photosynthetic apparatus forms a homeostatic regulator that ensures that the light energy is optimally distributed between both photosystems to obtain maximum photosynthetic efficiency of light energy utilization. This is valid for quality and even for quantity of incoming light.

Absorption of specific wavelengths in water influences the pigment composition of algae. In deep waters, the algae possess more pigments absorbing in green and blue regions, e.g. siphonoxanthin (green algae), fucoxanthin (brown algae), and biliprotein pigments (red algae and the Cyanobacteria). Changes in pigment composition were also observed in the algae and the cyanobacteria cultivated in lights with different spectral composition already at the

beginning of the 20th century, e.g. in *Oscillatoria rubescens* and *Calothrix* sp. (GAIDUKOV 1903a,b, GROSSMAN et al. 1994). This phenomenon is called chromatic adaptation. MARSAC (1977) divided the cyanobacteria into three groups according to their reaction to the spectral composition. Group I cyanobacteria change the size and the number of PBS, together with photosystem stoichometry, but no changes in PBS absorption characteristics occur. Group II cyanobacteria can change the level of PE in PBS; group III cyanobacteria can change the levels of PE and PC.

The chromatic adaptation was mainly studied in cyanobacterium *Calothrix* sp. strain PCC 7601. If the cells grew under red light, large amounts of PC and small amounts of PE accumulated. On the contrary, when the green light was used, low levels of PC and high levels of PE were observed, so the organism could use available light efficiently. GROSSMAN et al. (1994) proposed molecular model of chromatic adaptation. In the red light, neither repressor of cpcB2A2, encoding for the PC subunits, nor transcription activator cpeBA, encoding PE subunit are active. This situation causes increased cpcB2A2 transcription and low level of cpeBA transcription. In the green light, both regulators become active, resulting in increased cpeBA transcription and cpcB2A2 repression, and transcripts of cpeBA accumulate. It is proposed that the control of the regulation elements includes the regulation cascade whose elements could be the histidin kinases that are the most widespread in signal transduction in many prokaryotes.

In natural environments, not only changes in spectral composition, but even in intensity occur. Irradiances range from very low, ca 0.1 $\mu mol.m^{-2}.s^{-1}$, to very high, up to 6000 $\mu mol.m^{-2}.s^{-1}$. The change in intensity activates changes in cell ultrastructure. Photosynthetic microorganisms modify their chemical composition, cell structure and chloroplast organisation. For acclimatisation to low intensities, increased number of thylakoids, increased chloroplast volume and package of more thylakoids inside of the organelle are characteristic. For high light acclimatisation, reduction of surface density of thylakoid membranes, decreased chloroplast volume and accumulation of storage compounds are typical (BERNER et SUKENIK 1998).

Two types of photosynthetic apparatus adaptations are distinguished, short-term and long-term adaptations. The short-term adaptation, the so-called “state transition”, is a reaction to changes in irradiance lasting no longer then minutes. The mechanism is described in e.g. FUJITA et al. (1994), PROCHÁZKA et al. (1998) and ALLEN (2003). In algae and higher plants, when the absorbed energy is transferred preferentially to RC PSII (e.g. because of the spectral

composition of the absorbed irradiance or because of the initially high absorbtion cross-section of PSII), the major part of PQ pool becomes reduced. The kinase for phosphorylation of LHCII, localised in thylakoid membranes, is then activated. The kinase is probably an enzyme of molecular weight of 64 -65 kDa. After the phosphorylation, the LHCII separates from PSII and moves closer to PSI. As a result, the energy balance between PSII and PSI is created (state 2). However, if excessive transfer of energy to PSI occurs, more of the PQ molecules are oxidised. The kinase is deactivated and phosphatase activity comes into play that is independent on redox potential. Dephosphorylated LHCII undocks from PSI and moves to PSII (state 1). The transition from the state 1 to state 2 involves phosphorylation of approximately 30 % of LHCII.

In the cyanobacteria, the ratio of energy transferred from PBS to PSII decreases and the ratio transferred to PSI during irradiation by light absorbed preferentially by phycobilisomes. This state is called state 2. The irradiation by light absorbed by Chl a causes the opposite reaction, called state 1. Three models of regulation of energy distribution between the photosystems are proposed, but none fully explains observed data. The first model resembles the model of regulation in algae and green plants when the PBS migrates between photosystems. The second model assumes electron flow regulation from PSII to PSII without PBS separation from PSII. The third model suppose separation of PBS from PSII but its transition to PSI does not occur (FUJITA et al.1994).

During the long-term adaptation, the changes in PSI:PSII ratio occur. These changes can last hours to days. When the green algae grow under low irradiance, the PSI:PSII ratio is close to 1. In high irradiance, the PSI:PSII ratio is less then 1 (ELSTER 1999). FUJITA et al. (1994) observed similar dependencies in the cyanobacteria and the red algae. The detailed mechanism of PSI:PSII ratio regulation remains unclear. The probable signal for the regulation is ATP:NADPH ratio in cytosol. Also, the ratio of opened and closed RC of PSI and PSII corresponds with the PSI:PSII ratio. The change in the ratio is caused by changes in PSI synthesis. It is possible that the same signal system that triggers short-term adaptation is responsible for signal transduction in the long-term adaptation. FUJITA et al. (1994) described simplified model of photosynthetic RC stoichiometry regulation. If the PSII turnover exceeds PSI turnover or the cell needs more ATP, the electron flow through cyt b_6/f increases. The signal reaction, cyt b_6 oxidation, activates a factor in signal pathway that releases Chl a for PSI formation. At the same time, repressed synthesis of PSI proteins is unblocked and PSI

assembly is stimulated. The chromatic adaptation (mentioned above) can be also included in the long-term adaptations.

In excessive irradiation, the decrease of photosynthetic rate, photoinhibition, occurs. The photoinhibition is caused by modification of D1 protein in PSII. In moderate temperatures, the photoinhibition is observed only when the rate of D1 damage exceeds the rate of its repair. The damaged molecule has to be replaced completely. The rate of damage is proportional to irradiance, whereas the rate of repair depends on cell capability to degrade old D1, to synthesise and to insert the new protein. Even under normal conditions, the half-life of D1 protein is estimated to 30 minutes (PROCHÁZKA et al. 1998).

Two types of PSII photoinhibition are described - at the acceptor and donor sites of PSII (DEMMING-ADAMS et ADAMS 1992). The acceptor side photoinhibition is caused by blocking of electron transport by overreduction of Q_A (STYRING et al. 1990). The overreduction enables formation of chlorophyll triplets. As the triplet states relax, the singled oxygen is formed (DE LAS RIVAS et al. 1993, HIDEG et al. 1994) that can damage proteins in its vicinity, mainly proteins of the RC PSII (KIM et al. 1993). During the donor site photoinhibition, the reactions at the donor site of PSII are inhibited. Highly oxidative types of acceptors, like $P680^+$ and Y_Z^+, usually reduced by OEC, are formed. When the OEC is damaged, these acceptors can remain active for long time and can cause the oxidative damage to PSII (JEGERSCHÖLD et al. 1990).

Theoretically, the rate of repair should be lower in low temperature. The cells living in lower temperatures should be photoinhibited in lower irradiances that would not cause photoinhibition in higher temperatures (POWELS et al. 1983, BRADBURY et BAKER 1984, GREER et al. 1986, SMILLIE et HETHERINGTON 1988). Sometimes, this phenomenon is called "photoinhibition of low temperatures". In lower temperatures, it is necessary to take into account also the photoinhibition of PSI. Illumination of *Cucumis sativus* leaves in low temperatures by relatively high irradiances leads to decrease of PSI activities, probably by damage of Fe-S acceptors (SONOIKE et al. 1995). It was also found that the presence of unsaturated lipids accelerates recovery of PSII after the photoinhibition but does not affect the photoinhibition rate in low temperatures (MOON et al. 1995).

UV radiation

Other important part of the incoming radiation is the UV region. The UV radiation is defined as the region of wavelengths shorter than 400 nm, and is further divided into UV-A

(400-320 nm), UV-B (320 -280 nm) and UV-C (280-180 nm). During evolution, the UV radiation played double role - as mutagen and as selection agent, influencing evolution of bases of nucleic acids, ecology, biochemistry and evolution of the first living forms. According to models of evolution of atmosphere composition, partial pressure of oxygen was relatively stable around 10^{-8} Pa before atmospheric ozone accumulation, 3.5 - 2.5 Gy ago. Then, 2.4 Gy ago, the partial pressure increased considerably and relatively stable increment followed up to present value of 0.0201 MPa in the beginning of Cambrian, and maybe even earlier. The first organisms were obligatory and facultatively anaerobic bacteria that developed defence against UV radiation, probably by UV absorbing pigments and DNA repair by photoreactivation (ROTHSHIELD 1999).

Today, local increases of incoming UV radiation, caused by ozone depletion in stratosphere, is observed namely in Antarctica and Arctica. One of the most important effects of increased UV is the influence on the primary producers, the plants, the algae and the cyanobacteria. The diminution of phytoplankton was documented in the Weddel Sea, Antarctica (DÖHLER 1988), changes in vertical distribution of macroalgae were found near Svalbard, Arctica (BISCHOF et al. 1998). The effects of UV radiation were observed in DNA where hydroxylation of cytosine, formation of bound cytosine-thymine, connection of DNA with proteins instead with DNA, breaks of DNA chain and denaturation of DNA occurred. Creation of photoproducts between adjacent bases is the main damage. The effects on metabolism were also studied. The increased amount of UV-B radiation results in limitation of protein synthesis, decline in protein content, and decrease in rates of carbon and nitrogen metabolisms (DÖHLER 1988, 1994). These effects can be caused by decline in supply of ATP and carbon skeletons for AA synthesis, damage of synthesis and activity of key enzymes in metabolism, variability in arrangement of AA, lipids and fatty acids, alike as inhibition of the regulatory mechanisms (DÖHLER 1994). UV-B increases non-photochemical quenching and the effective quantum yield of photosynthesis is disturbed (BISCHOF et al. 1999, 2002).

The organisms can protect against UV radiation damage by different ways. The most simple defence is migration from irradiated places, e.g. vertical migration of phytoplankton that also come be involved in protection against excessive PAR irradiance (SOUTH et WHITTICK 1987). Another possibility is attenuation of irradiance before entering into cell. The protection can be provided by pigments absorbing UV radiation, sporopolenin (XIONG et al. 1995) production of antioxidants that neutralise effect of radicals formed by photoreactions. The materials in water column can also shield the cells. DNA damage is repaired primarily by

two mechanisms - excision repair and photoreactivation (ROTHSHIELD 1999). DÖHLER et al. (1995) proved induction of synthesis of some HSPs by UV radiation; the molecular weight of the proteins was 45 kDa.

The algae living in areas with increased incoming UV radiation, i.e. in polar and mountain ecosystems, are less sensitive to the damage and have developed ways of protection. Screening of photosynthetic apparatus sensitivity to UV-B radiation in mountain and lowland algae proved that major portion of algae sensitive to UV were isolated from soil in both ecosystems and from lowland plankton (XIONG et al 1996). These algae have small cells or trichal thalus, naked zoospores occur in their life cycle. Majority of UV resistant algae were isolates from mountains and places exposed to sun. The resistant algae have bigger cells, or grow in large colonies or coenobia that also provide effective shading of inner parts of the cell (XIONG et al. 1995). Some resistant Algae can contain mycosporine-like AA (KARSTEN et al. 1998). Picoplankton, consisting primarily of the cyanobacteria, is less sensitive (XIONG et al. 1995, LAURION et VINCENT 1998). The cyanobacteria have large amounts of pigments absorbing in UV and blue region of spectrum (QUESADA et al. 1999). ARAOZ et HADER (1999) observed increase in PE synthesis after UV irradiation. The response to UV radiation is specific according to the environment and locality the picoplanktonic cyanobacteria are not affected.

1.2.3. Other conditions contributing to extremisation of environment

Other conditions contributing to extremisation of environment are pH, water deficiency, toxicity and excess of salts.

pH

pH affects the form and uptake of carbon by the Algae and on the contrary, CO_2 consumption causes alkalisation of medium (PEŠLOVÁ et al. 1990, SHIRAVA et al. 1993). At pH < 6.5, CO_2 prevails; at pH in range 6.5 to 10.5, HCO_3^- predominates; and at pH > 10.5, the majority of inorganic carbon occurs in form of CO_3^{2-}. Acid rains lower pH that results in dissolution and following increase of concentrations of aluminium, manganese, zinc, iron and other metals. The Algae are then subjected not only to direct effects of pH, but also to metal toxicity. Acid environment can be formed near hydrothermal vents, geysers etc. Mechanisms of acidity and alkalinity tolerances have not been satisfactorily resolved yet. It is not known

how acidophiles and alkalophiles keep intercellular pH different from their surrounding and how membranes and membrane proteins are modified against these conditions. Acidophiles and alkalophiles belong predominantly to Archaea whose cell membrane consists of rare ether lipids that do not degrade easily, are resistant to temperature and mechanical damage, and tolerate high concentration of salts. Many organisms have tetraether lipids whose arrangement in the membrane causes impermeability of the membrane to protons (ŠMIGÁŇ et GREKSÁK 2000).

Water deficiency

Majority of metabolic and chemical processes occur in water environment. For water Algae, the desiccation is more dangerous then for terrestrial species. The cyanobacteria are better adapted to drought and desiccation. LIPMAN (1941) isolated living cyanobacteria from herbarium specimens 78 years old. Algal cells tolerating the desiccation include zygotes, akinetes, cysts and spores. After desiccation and rehydration, cells posse thick cell wall and produce mucilage.

The physiological response to water deficiency is considered separately from osmotic and ionic effects. The cells subjected to desiccation have to cope with increasing concentration of dissolved salts that further affects osmotic and electrochemical potential of the cell. There are two types of metabolic adjustments during the water stress: ionic regulation and synthesis of osmotic active compounds. Ionic regulation is the consequence of decrease of outer water potential. Water is attracted out of the cell, turgor decreases and the cell shrinks. The changes in cell volume are lessened by ion transport across the membrane, especially of Na^+.

The osmotically active compounds play main role in maintaining positive turgor. These compounds accumulate in osmotically stressed cells and do not inhibit enzymatic activity and metabolic functions. The osmotically active compounds include disacharides, sucrose and threalose, glucosylglycerol, glycine-betaine or glutamate-betaine. Maintainance of membrane stability is improved in the presence of sacharides and its fluidity is increased by special type of fatty acid. Specific hydrophobic proteins could keep the stable cell environment and could preclude aggregation and denaturation of proteins (ELSTER 1999).

Toxicity and salts

Water deficiency, toxicity and excess of salts are tightly connected. Unlike of water deficiency, the excess of salts is a typical local factor that occurs in tidal zone, in mineral

springs etc. Apart from natural environments, there are many other localities of anthropogenic origin with increased salt concentration, e.g. mines. Concentration of NaCl and other salts, heavy metals and nutrients can reach high levels. The Algae tend to accumulate salts or heavy metals inside their cells. High concentrations of salts evoke the same response like water deficiency and the stress reactions are similar (ELSTER 1999).

The algae developed various strategies for uptake of mineral compounds that are necessary for the cell in low concentrations, and how to prevent themselves against their excessive abundance in the surrounding environment or how to lessen their toxic effects. In these reactions, chelation systems are important; the chelation systems include derivates of some AAs, citric acid, malic acid and phytochelatines, polypeptides binding heavy metals (ONDŘEJ 1999).

Halophilic organisms that grow even in 5M NaCl solution possess halophilic proteins that are stable only in high salt concentrations. These proteins consist of high amount of acidic AA and low amount of basic ones. The AA composition enables adequate balance of hydrophobic-electrostatic interactions, alike as appropriate hydration. Like in all extreme environments, halophiles are primarily archaea whose membrane composition is similar to other extremophiles (ŠMIGÁŇ et GREKSÁK 2000).

1.3. Extreme environments in the Polar Regions

1.3.1. The Polar Regions

The Polar Regions are defined by forest boundary, and average mid-summer isotherm around 10 °C. On the Northern Hemisphere, the boundary includes northern coast of Europe, Asia and North America. Majority of the region is Northern Ocean. On the Southern hemisphere, the Polar Regions consist of Antarctica and island along its coast.

Compared to Antarctica, the Arctic is warmer and more humid. It is connected to Atlantic Ocean by oceanic currents. The Gulf Stream influences the climate, so about 120 species of higher plants are found at latitude of 80° N (Svalbard, SKULBERG 1996). The north-southern arrangement of mountains of surrounding continents (the Ural Mountains, the Rocky Mountains) also helps warming.

The Antarctica is separated from the temperate and tropic waters by West Windrift flowing around the whole continent. At 58° S, the Antarctic convergence occurs where the composition and temperature of water change in a narrow range (40 - 50 km). With exception of coastal regions of the Antarctica, the climate is continental. The Antarctica is covered by continental glacier of average height of 2.3 km. No higher plants grow on the continent; only two species were found on the surrounding islands (*Deschampsia antarctica* and *Colobanthus quitensis*).

In both regions, temperatures range from - 88 °C (Vostok station, Antarctica) to +10 °C in summer. Range of rainfall depends on geographic position of a place, from almost 0 mm/year in polar deserts (e.g. Ross Desert, Ellesmere Island) to 250 mm/year (Low Arctic). Irradiance is very variable: in fact, in summer the Polar Regions obtain daily more energy than tropics (FOGG 1998).

Conditions in a given place (microclimate) can differ significantly from the climate of whole region. Nanoclimatic measurements in endolithic ecosystem show that the stone surface can be warmer then surrounding air by about 5 °C (FRIEDMANN et al. 1987, MCKAY et al. 1988, NIENOW et al. 1988b, OHTANI et al. 1991a). The maximum irradiance in the endolitic community ranges 0.1 to 150 $\mu mol.m^{-2}.s^{-1}$ (NIENOW et al. 1988a). According to FRIEDMANN et al. (1987), appropriate conditions for biological activity are at temperatures above -10 °C and irradiances above 100 $\mu mol.m^{-2}.s^{-1}$.

The Polar Regions are source of organisms adapted to cold, low precipitation, various irradiances and large input of UV radiation. Conditions in Dry Valleyes (Mc Murdo Station, Antarctica) resemble the condition on Mars and could serve as model conditions for searching of life on that planet or its terraforming (ANDERSEN et al. 1994, DORAN et al. 1998).

1.3.2. The cyanobacteria and the algae of the Polar Regions

Organisms of the Polar Regions had to adapt to low temperatures, autotrophic organisms moreover to various irradiances and to water deficiency in polar deserts. The algae and the cyanobacteria are the most important primary producers of polar region. ELSTER (2002) distinguishes three categories of algal communities according to water gradients: planktic, hydroterrestric and terrestric (soils, surface and inside of stones, epiphytes, phycobionts of lichens and endolithic communities). They can survive in permafrost (FRIEDMANN 1994) for thousands and millions of years (GILICHINSKI et al. 1992, STONE 1999a) and VOROBYOVA et al. (1997) proposed a model of deep cold biosphere.

1.3.2.1. Planktic communities

Permanently frozen lakes

Permanently frozen lakes are found in flat regions of Antarctica, in Land of Queen Maud, Victoria Land and at the coast of Indian Ocean. The permanently frozen lakes are covered by ice layer 1 - 2 m thick and the temperature inversion occurs; the warmest water lies near the bottom (4 - 6 °C). Near the surface, the water freezes to the ice layer. On the surface, the ice sublimes and the ice-layer changes continually. Light penetrating through the ice enables life in the bottom and cyanobacteria of genera *Leptolyngbya* and *Nostoc* were found, sometimes even diatoms and desmids occur (LIKENS 1964, KOMÁREK et RŮŽIČKA 1966, PARKER et WHARTON 1985, VINCENT 1988).

The lakes were found even under the glacier in central Antarctica. Considering the thickness of the glacier, it is not possible to suppose living photosynthetic organisms in these lakes. Lake Vostok is an example of such lake with a liquid water layer thick of 600 m with 100 m of deposits under 4 km of ice. Borehole was stopped approximately 250 m above water level to prevent contamination. New technologies developed for the lake research could be used also in exploration of Jupiter's moon Europa (CÍLEK et MARKOŠ 2000b, STONE 2000b).

Ice cyanobacteria and algae

Ice cyanobacteria and algae are found in ice floes in the Polar Regions. These organisms grow in spaces between ice crystals; temperature of the environment is stable, ranges around -1.75 °C and changes do not exceed 0.5 °C. They are adapted to low irradiance. Diatoms dominate, but green flagellates, Dinoflagellates, and Cryptophyta were also recorded. Majority of species is not planktic. It is assumed that the Algae are heterotrophic in winter months but the experimental evidence is lacking (ROUND 1985, SOUTH et WHITTICK 1987, SMITH et al. 1988).

1.3.2.2. Hydroterrestrial communities

Glacial streams

Polar streams can be seasonal or permanent and are most abundant in glacial regions. The seasonal streams originate from melting glaciers and differ in length and slope and are affected by distance from the forehead of the glacier and altitude. Close to the moraine, the cyanobacteria predominate (e.g. *Phormidium autumnale*, *Scytonema myochrous*) with increasing distance, trichal green algae prevail (*Klebsormidium*, *Ulothrix mucosa* - ELSTER et SVOBODA 1996). According to ELSTER et SVOBODA (1995), nitrogen is depleted at the further end, as has been proved by studies at Ellesmere Island (Arctica) and King George's Island (Antarctica).

Cyanobacteria and algae form mats on the surfaces of stones, sand, mud, humid soil on surfaces of submerged mosses and higher plants. Thickness of the mats differs, their morphology is variable but their pigment organisation is similar. Under surface rich of carotenoids, there is a layer rich in Chl a and PC.

The main representatives of Cyanobacteria belong to orders Chroococcales, Oscillatoriales and Nostocales. The Algae belong to classes Chrysophyceae, Xanthophyceae, Chlorophyceae, Charophyceae, Ulvophyceae and Zygnemaphyceae (ELSTER 2002).

The water often spills on the surface and various types of seepages, pools and lakes come into existence and are flooded by melting water at the beginning of the summer season; they differ in specific microflora (ELSTER et al. 1994).

Kryoseston

Cryosestonic species, i.e. species growing in snow, are widespread in places with permanent or regular snow cover. More than 110 species of kryosestonic algae have been described (e.g. KOL 1968, JAVORNICKÝ et HINDÁK 1970, KOMÁREK et al. 1973). Arctic and Antarctic representatives are described in e.g. KOL (1972), KOL et EULORA (1974). These algae and cyanobacteria are adapted to temperatures around 0 °C (HOHAM 1975) and high irradiances. The algae can also hide from the high irradiance in deeper layers of melting snow. The way of migration of flagellate forms is obvious, but remains unknown for coccoid forms. The life cycle proceeds in one week. The red, orange or green coloration of snow is caused mainly by genera *Chlamydomonas* and *Chloromonas*, less frequently by genera *Koliella*, *Raphidonema* and others (HOHAM et DUVAL 2001). In the Czech Republic, cryoseston was described for the first time in the Krkonoše Mountains in 1976 (FOTT et al. 1978) and in Šumava Mountains in 1992 (LUKAVSKÝ 1993). It is a question if the kryoseston had been disregarded or if its presence is result of recent changes, e.g. snow eutrophication by industrial pollutants.

Springs

Geothermal springs are common in the Polar Regions. Their temperature and flow rates are stable all the year round. Their temperature is slightly above 0 °C, but sometimes reaches even 25 °C (BANKS et al. 1998). The springs are inhabited by special microflora (LANGANGEN 2000).

The secondary springs arise when the water from melting glaciers, immerse under the surface, flows on the permafrost and rises in various locations. Their temperature ranges around 0 °C, the springs freeze and desiccate in winter. According to observation at Ellesmere Island, cyanobacterium *Tribonema minus* emerges firstly in the beginning of spring and is followed by green Algae. In Antarctica, *Schizothrix frigida* and some species of genus *Nostoc* were found (ELSTER et al. 1994).

Minor limnic biotopes

Minor limnic biotopes, cryoconite holes and supraglacial pools are newly discovered polar biotopes. The holes are 1-2 m deep and permanently frozen, not always to the bottom. They are found in all areas of the Polar Regions, even near poles. In summer, the surrounding rock is heated and a water layer is formed at the bottom. These biotopes are inhabited by the

cyanobacteria and the algae, the cyanobacteria of oscillatorian type prevail (VINCENT 1988, KOMÁREK p.c.).

1.3.2.3. Terrestrial communities

Soil cyanobacteria and algae

Soil cyanobacteria and algae have been less studied up to now in spite of being the only primary producers in polar deserts. Diversity of species and number of representatives are variable and depend on environmental conditions. High number of species were found in newly deglaciated moraines, the near melting glacier supplies the locality by moisture and nutrients. Polar microflora consists of Cyanobacteria, Xanthophyceace, Bacillariophyceae and Chlorophyceae. Some of the isolated species have not been described yet (ELSTER et al. 1999). In Antarctica, the Cyaobacteria are presented in higher number of species, probably due to harsh condition. The microflora is richer near rookeries and streams that is caused by better nutrient supply (OHTANI et al. 1991b).

The soil algae are part of layers covering the surface other parts are formed by fungi, lichens and mosses (ELSTER 2002). The soil crust plays important role in soil genesis, stabilisation of soils, soil friability, fertilisation and build-up of organic content (JOHANSEN et SHUBERT 2001).

Lichens

Symbiosis of an alga or a cyanobacterium with a fungus is one of possible adaptation to extreme environments, especially to water and nutrient deficiency. Phytobionts are most often green Algae, about 30 species are known, e.g. *Trebouxia*, *Trentepohlia*, and cyanobacterial genus *Nostoc* (ROUND 1985, SOUTH et WHITTICK 1987).

Litophytic (aerophytic) communities

In conditions of polar deserts, and not only there (e.g. FRIEDMANN 1980, BÜDEL 1999), algal communities become endolithic. A cavernous substrate is necessary, it could be limestone, sandstone or pudding stone. The communities survive under surface of stones, their upper layer starts about 5 mm under the surface. Three types of relationship between organism and its substrate are distinguished. Chasmoendolithic microorganisms live in

cleavages and corrugations on the surface of a rock, cryptoendolithic communities exist in rock pores. Euendolithic organisms create their environment actively by etching the rock.

The cryptoendolithic communities of polar deserts are the best-studied (GARTY 1999). About 100 species of algae and cyanobacteria from 70 genera were described as endolithic (VAN THIEN et GARBARY 1999). FRIEDMANN et al. (1988) distinguish two types of eukaryotic communities - lichen dominated communities and communities of type *Hemichloris*. In cyanobacterial communities, they describe three types - *Gloeocapsa*, *Hormathonema-Gloeocapsa*, and *Chroococcidiopsis* ones.

The conditions inside the rock are sufficient for retaining of life in outside unfriendly environments. Water remains liquid in supercooled state at -3.8 °C (MEYER et al. 1988), the maximal irradiance ranges from 0.1 to 150 $\mu mol.m^{-2}.s^{-1}$ (NIENOW et al. 1988a). Mathematical models of temperature regime according to NIENOW et al. (1988b) show that the rock surface has to face to the north and the altitude should not exceed 2.5 km during colonisation of rocks in Antarctica.

The productivity of the cryptoendolithic communities is very low, the major part of the production is used for surviving in extreme conditions (FRIEDMANN et al. 1993b) and according to radiocarbon dating, these communities are thousands of years old (BONANI et al. 1998).

Increased dosages of UV radiation, caused by ozone depletion, have large impact on these communities. The UV radiation penetrate deeper in the rock and can endanger surviving of the algae and the cyanobacteria in regions with sufficient PAR irradiation, i.e. above the compensation point (VAN THIEN et GARBARY 1999).

Epiphytic cyanobacteria and algae

Species composition and number were documented at the Syowa Station (Antarctica). Algae and Cyanobacteria were found among leaves and stem near moss surface. Majority of 23 species was formed by Cyanobacteria (16), but representatives of diatoms (4) and green Algae (3) were also found. Species composition and number of algae and cyanobacteria varied according to location. It seems that the sufficiency of water is the main limiting factor. Mucilage sheath preventing from loss of water plays important role in more arid conditions (OHTANI 1986, OHTANI et KANDA 1987).

1.5. Extreme conditions and astrobiology

Astrobiology, or exobiology, is a science studying extraterrestrial life. The range of investigations includes all living processes beyond the Earth, from microbial life in our Solar system to signals of intelligent beings in distant parts of the Milky Way Galaxy. In addition the astrobiology interferes with "terrestrial" biology by asking basic questions, e.g. are all living processes based on biochemistry of L-stereoisomers? Is DNA the only molecule suitable for keeping of the genetic information? If yes, is our genetic code the only possible one? (FRIEDMANN 1993b).

The extreme environments on the Earth can serve as a model for conditions on other planets. These models are useful from several reasons that FRIEDMANN (1993b) states for the Mars, but that are valid for other planets or for moons of Jovian planets (planets like the Jupiter):

1. They help understand processes that occurred on other planets (moons) in the past.
2. They serve as the basis for development of life detection technologies.
3. They form the basis for obtaining necessary information for planning of terraforming methods (changes of original conditions to terrestrial ones).

The recent research shows that the Sun is not the only star having its planetary system (the actual list of found planets is available at EXTRASOLAR PLANET ENCYCLOPAEDIA website), and even the Earth is not the only planet where life can exist. The first exploration of planets and moons of the solar system in the sixties and the seventies of the 20th century showed the planets and big number of moons with conditions that had not been suitable for life according to known criteria. In time of Pioneers and Voyagers, the only possible type of life was the one that had been observed and studied on the surface. The existence of ecosystems near deep-sea hydrothermal vents was unknown, and even was not expected. The next, more thorough exploration in the nineties of the 20th century by more technically advanced and cheaper probes and biological research in extreme environment suggests that the life, even in microbial forms, could exist beyond the Earth. The celestial bodies that can bear the life include the Mars and Europa (Jupiter's moon), maybe Venus and Titan (Saturn's moon).

Already 100 years ago, the Mars was presumed to have conditions required for origin of life. Although the present concepts about Martian organisms are entirely different from those from the end of the 19th century, according to the newest findings, liquid water can exist on or close to the martian surface even in present time (MALIN et EDGETT 2000, KUZNETZ et GAN

2002). If the recent NASA rovers confirm this hypothesis, the Martian ecosystem shall more resemble the ecosystem of polar deserts on the Earth where organisms can live. If the microorganisms already existed on the Martian surface, they could probably form the cryptoendolithic communities or can be found in the soil (CHELA-FLOES 1998) or in deep subsurface. It should be possible to use knowledge about microorganism fossilisation in extreme environments and help develop methods of searching of life by manned expedition to the Mars.

The next suitable candidate for life search is Europa (and maybe other Jovian ice moons). The moon was discovered in 1609 by Galielo Galilei. Approximately after 400 years, probe Galileo allowed its detailed observation. The moon consists of rocks and the surface is covered by ice 120 - 170 km thick (SOHL et al. 2002). The surface is disturbed by corrugations. It is possible that there is liquid water under the ice layer. This hypothesis is supported by observation of the magnetic field by the space probe (KIVELSON et al. 2000). The energy for heating could be supplied from tides. In the water, ecosystems could develop resembling the terrestrial ecosystems near hydrothermal vents or ecosystems of Antarctic permanently frozen lakes that serve as a model for development of technologies of sterile boreholes and sampling (CHELA-FLOES 1998, STONE 1999b). A suggested project of probe for investigation of the ice surface of Europa (GERSHMAN et al. 2003) was redesigned and proposed as Icy Moon Orbiter (NASA 2004).

Venus and Titan could also be interesting for astrobiology, even though the probability of origin and evolution of life there is smaller than in the case of Mars and Europa. The ecosystems of Venus could be found in high atmosphere or under the surface (SCHULZE-MAKUCH et IRWIN 2002). Titan can serve as model of early stage of planetary development (RAULIN et OWEN 2002) and will be explored by probe Cassini-Huygens in late 2004 (LEBRETON et MATSON 2002, MATSON et al. 2002).

It is possible that the life has evolved on every suitable planet or moon. The assumption for further evolution of organisms capable of communication beyond their native planet is the long-term stability of suitable conditions. This seems to be disturbed easily, e.g. by irregular eruptions of novas or supernovas, by impacts of large meteorites or comets, or by γ bursts of binaries that sterilise entirely surrounding space. The Earth probably had exceptional position because no possible star of that type has been discovered yet in our close vicinity. Although the Sun will exists at least next 5 Gy (KLECZEK 2002) the life on the Earth will die out after

approximately 1 Gy when the increased solar irradiance will cause massive global greenhouse effect (WARD et BROWNLEE 2004).

2. Aims of the doctoral thesis

The aims of the doctoral thesis are following:

Aim 1: Characterisation of properties of the newly developed unit for crossed gradients of temperature and light for cultivation in the extreme environments and the development of methods suitable for ecological characteristics evaluations

Aim 2: Comparison of ecological characteristics of strains of genus *Stichococcus* isolated from polar and temperate regions

3. The summary of results

The evaluation of properties of the new unit for crossed gradients of temperature and light and cultivation procedures has been crucial for the subsequent experiments. From the time of its original description (HALLDAL et FRENCH 1958), the unit has been used and modified several times (e.g. YARISH 1976, YARISH et al. 1979, LUKAVSKÝ 1982, ALBERTANO et al. 1994). The new unit has improved thermoregulation control; the precise temperature gradient is achieved by pulse heating of the aluminium cultivation plate that is continuously cooled at one of its sides. The temperature range can be set from -4.5 to 45 °C however for individual experiments, a gradient of temperature spanning 20 to 25 °C is recommended. The temperature gradient is linear in all temperature setting (KVÍDEROVÁ et LUKAVSKÝ 2001).

Different light sources were tested: fluorescent tubes, compact lights, halogen incandescent lamps and sodium vapour lamps. The irradiation intensity can reach up to 1500 $\mu mol.m^{-2}.s^{-1}$, when the sodium vapour lamps are used, that is comparable with irradiances encountered in field. However, the maximum irradiances, e.g. of 6000 $\mu mol.m^{-2}.s^{-1}$ in the mountains (WILLIAMS et al. 2003), are out of the range of the unit yet. Generally, linear light souces, e.g. fluorescence tubes, are recommended for better light homogeneity. The shape of the light gradient depends on the light source and its height above the plate. Steeper light gradients can be achieved by strip filters applied on the Perspec cover or directly on the cultivation vessel (KVÍDEROVÁ et LUKAVSKÝ 2001). Moreover, the irradiance output of compact lights can be regulated by a computer program, so modelling of the diurnal light cycles could extend the possibilities of experimental setting of the unit. However, the diurnal cycles of temperature cannot be simulated due to temperature inertia of the plate.

The reliability of the unit was proved by cultivation of a marine diatom *Phaeodactylum tricornutum*. The growth limits and optima found by the cultivation in our unit for crossed gradients ccorresponded to known values and were used for optimalisation of conditions for marine biassays (KVÍDEROVÁ et LUKAVSKÝ 2003).

In order to allow simultaneous testing of up to ten algal or cyanobaterial strains in one experiment in the crossed gradients, the method of cultivation in the microplates (8x12 cm, FB, 96 wells) was applied. The reliability of this method was tested by cultivation of model strains where temperature and irradiance limits and optima are known (KVÍDEROVÁ, unpublished data). The method became the basis of several bachelor and master theses (STIBAL 2001, ŠABACKÁ 2001, MACHOVÁ 2004, STIBAL 2004, ŠABACKÁ 2004, ZAPOMĚLOVÁ

2004) and experiments (STIBAL 2003, NEDBALOVÁ, in prep.). The cultivation in the microplates was also used for testing of the effect of various compounds on algal growth at various combinations of temperature and light (SHUKLA et ELSTER, p.c.).

For comparison of reaction of polar and temperate algae to temperature and light, six strains of the cosmopolite *Stichococcus* (Chlorophyta) were selected. This genus belongs to primitive trichal green algae but its further taxonomic position is not clear (VAN DEN HOEK et al. 1995, LEE 1995) because molecular taxonomy studies of genus *Stichococcus* are rare. Recent results propose that *Stichococcus* belongs to Trebouxiophyceae (KATANA et al. 2001). According to WDCM (2003), more 100 strains have been isolated from a wide spectrum of localities, from tropical strains isolated in Florida to strains of polar soils in Arctica and Antarctica. Their habitats include periphyton, plankton, and soil localities, so this genus is supposed to have a large ecological and physiological plasticity and could be suitable for study of mechanisms of adaptation-acclimatisation. Three selected strains, *S. bacillaris* Hindák 1984/15, *S. exiguus* Komárek 1962/1 and *S. mirabilis* Pringsheim/Praha Ac. A 146, originated from temperate zone and the other three ones, *S. bacillaris* Elster 1998/28, *S. exiguus* Elster 1998/31 and *S.* sp. Kováčik 1988/9, from the Polar Regions.

The temperature growth limits and optima reflected the microclimate temperature in their original localities. According to their response to the temperature, the strains *S. bacillaris* Elster 1998/28, *S. exiguus* Elster 1998/31, both isolated from polar soil in Ny Ålesund, Svalbard, and *S. exiguus* Komárek 1962/1, isolated from snow detritus in High Tatra Mountains, Slovakia, were considered psychrotrophic, i.e. tolerating low temperature. The psychrophiles are able to survive at temperatures near zero, but their optima are higher then 20 °C (ELSTER 1999). The growth optima of polar and snow strains were similar to the other *Stichococcus* strains isolated from polar and mountain environments (HOHAM 1975, STIBAL 2003).

The ecological characteristics of strains *S. bacillaris* Hindák 1984/15, isolated from Haffner See, Austria, *S. mirabilis* Pringsheim/Praha Ac. A 146, isolated from a temperate freshwater ecosystem, and *S.* sp. Kováčik 1988/9, isolated from thermal Troll Springs, Svalbard, corresponded to the definition of a mesophilic microorganism that was not surviving at temperatures near 0 °C and its growth optimum is higher then 20 °C (ELSTER 1999). The optimal temperatures of the temperate strains *S. bacillaris* Hindák 1984/15 and *S. mirabilis* Pringsheim/Praha Ac. A146 range 20 to 25 °C reflecting the water temperature in original location. However in the temperate Lake Ammersee (Germany), the maximum cell

density of the autotrophic picoplankton that included *Stichococcus minutissimus* appeared in at temperature range 9 to 20 °C (CHANG 1998). The lower growth temperatures can be caused by lower water temperatures in the Lake Ammersee ranging 3.2 to 21 °C during the year (WORLD LAKE DATABASE 2003) then in Haffnersee (HAFNERSEE 2003). The species also could be out-competed by other algae or cyanobacteria that grow faster in higher temperatures. In this case, the conditions of maximum abundance in the lake do not correspond to optimum growth conditions estimated from the laboratory experiments. The polar strain *S.* sp. Kováčik 1988/9 is probably adapted to the temperatures in thermal springs, which are stable and range 9.5 to 25.9 °C in individual springs (BANKS et al. 1998).

The irradiance requirements were not crucial in distinguishing polar and temperate strains, or did not correspond to the microenvironment of the original localities. However the average values of irradiances are lower in the Polar Regions but are still comparable in all localities. The average daily irradiance reaches approximately 1500 $\mu mol.m^{-2}.s^{-1}$ in the temperate region and approximately 1000 $\mu mol.m^{-2}.s^{-1}$ in the Polar Regions at the surface (during vegetative season). The actual values at the noon should be higher (e.g. FRIEDMANN 1988, BISCHOF et al. 2002). However, the irradiance gradient used in the experiment did not reach maximum irradiances encountered in both ecosystems, so the difference in reaction to high light cannot determined.

On contrary, these ecological characteristics could be influenced by long-term adaptation to culture collection condition, i.e. 15 °C and 45 $\mu mol.m^{-2}.s^{-1}$, and to pre-cultivation (20 °C, 400 $\mu mol.m^{-2}.s^{-1}$). According to PRÁT (1970), the alga *Chlorella pyrenoidosa* Sorokin TX 71105 was not physiologically changed even after 17 years in the culture. This acclimation, i.e. adaptation to culture collection conditions (ELSTER 1999), does not occur in all strains. The experimental strains kept their ecological characteristics almost unchanged for more then one year (KVÍDEROVÁ, unpublished data) and the growth characteristics are consistent with other observations with newly isolated strains (e.g. STIBAL 2003). However, the acclimation process cannot be entirely excluded due to long-term keeping in the culture collection conditions.

The forthcoming research will be focused on comparison of photosynthetic performance of psychrophilic, psychrotolerant and mesophilic *Stichococcus* strains, on changes in membrane fatty acid composition, on stress protein synthesis and on differences in ultrastucture at low temperatures. Results of these experiments will be compared with genetic analyses of mutual

relationships of the experimental strains. In these experiments, strains from the culture collection and newly isolated ones from the Arctica and Antarctica will be included.

4. References

ALBERTANO, P., KOVÁČIK, L., GARDAVSKÝ, A. (1994): Cross-gradient cultures of filamentous cyanophytes. – Giorn. Bot. Ital. 127: 855 – 856.

ALLEN, J.F. (2003): State transitions – a question of balance. – Science 299: 1530 – 1532.

ANDERSEN, D.T., DORAN, P., BOLSHIYANOV, D., RICE, J., GALCHENKO, V., CHERYCH, N., WHARTON, R.A., MCKAY, C.P., MEYER, M., GARSHENK, V. (1994): A preliminary comparison of 2 perennially ice-covered lakes in Antarctica – analogs of past Martian lacustrine environments. – Adv. Space Res. 15 (3): 199 – 203.

ARAOZ, R., HADER, D.P. (1999): Phycoerythrin synthesis is induced by solar UV-B in the cyanobacterium Nostoc. – Plant Physiology and Biochemistry 37 (3): 223 – 229.

BANKS, D., SLETTEN, R.S., HALDORSEN, S., DALE, B., HEIM, M., SWENSEN, B. (1998): The thermal springs of Böckfjord, Svalbard: Occurrence and major ion hydrochenistry. – Geothermics 27 (4): 445 – 467.

BERGER, F., MORELLET, N., MENU, F., POTIER, C. (1997): Cold shock and cold acclimation proteins in the psychrotrophic bacterium *Arthrobacter globiformis* SI55. – J Bacteriol. 178: 2999 – 3007.

BERNER, T., SUKENIK, A. (1998): Photoacclimation in photosynthetic microorganisms: An ultrastructural response. – Israel Journal of Plant Sciences 46 (2): 141 – 146.

BISCHOF, K., HANELT, D., TUG, H., KARSTEN, U., BROUWER, P.E.M., WIENCKE, C. (1998): Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). – Polar Biology 20 (6): 388 – 39 5.

BISCHOF K., HANELT, D., WIENCKE, C. (1999): Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. – Plant Biology 1 (4): 435 – 444.

BISCHOF, K., HANELT, D., AGUILERA, J., KARSTEN, U., VÖGELE, B., SAWALL, T., WIENCKE, C. (2002): Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. – Marine Biology 140: 1097 – 1106.

BONANI, G., FRIEDMANN, E.I., OCAMPO-FRIEDMANN, R., MCKAY, C.P., WOELFLI, W. (1998): Preliminary report on radiocarbon dating of cryptoendolithic microorganisms. – Polarforschung 58 (213): 199 – 200.

BOROWITZKA, L.J. (1991): Development of Western Biotechnology's algal beta-carotene plant. – Biores. Technol. 38: 251 – 252.

BOSTON, P (1999): The search of extremophiles on Earth and beyond. –http://www. astrobiology.com/adastra/extremophiles.html

BRADBURY, M., BAKER, N.R. (1984): A quantitative determination of photochemical and non-photochemical quenching during the slow phase of chlorophyll fluorescence curve of bean *Phaseolus vulgaris* leaves. – Biochim. Biophys. Acta 765: 275 - 281.

BROADY, P.A., OHTANI, S., INGERFELD, M. (1997): A comparison of strains of *Xanthonema* (= *Heterothrix*, Tribonematales, Xanthophyceae) from Antarctica, Europe and New Zealand. – Phycologia 36 (2): 164 – 171.

BÜDEL, B. (1999): Ecology and diversity of rock-inhabiting cyanobycteria in tropical regions. – Eur. J. Phycol. 34: 361 – 370.

CASTENHOLZ, R.W. (1969): Thermophilic blue-green Algae and the thermal environment. – Bacteriol. Rev. 33: 476 – 504.

CELNIKIER, L.M., TRÂN THANH VÂN, J. eds. (2003): Frontiers of life. Proceedings of the XIIth Rencontres de Blois, The Gioi Publishers, pp 342.

CHELA-FLOES, J. (2002): A search for extraterrestrial eucaryotes: Physical and paleontological aspects. – Origins of life and Evolution of the Biosphere 28 (4-6): 583 – 596.

CÍLEK, V., MARKOŠ, A. (2000a): Hluboká horká biosféra [Hot deep biosphere]. – Vesmír 79 (130): 253 – 258.

CÍLEK, V., MARKOŠ, A. (2000b): Hluboká horká biosféra a zemský povrch [Hot deep biosphere and Earth surface]. – Vesmír 79 (130): 323 – 326.

DARLING, R.B., FRIEDMANN, E.I., BROADY, P.A. (1987): *Heterococcus endolithicus* sp. Nov. (Xanthophyceae) and other terrestrial *Heterococcus* species from Antarctica: Morphological changes during life history and response to temperature. – J. Phycol. 23: 598 – 607.

DAVEY, M.C. (1989): Effect of freezing and dessication on photosynthesis and survival of terrestrial Antarctic Algae and Cyanobacteria. – Pol. Biol. 10: 29 – 36.

DAVISON, I. R. (1991): Environmental Effects on Algal Photosynthesis – Temperature. – J. Phycol. 27: 2 – 8.

DE LAS RIVAS, J., SHIPTON, C., PONTICOS, M., BARBER, J. (1993): Acceptor side mechanism of photoinduced proteoloysis of the D1 protein in photosystem II reaction centers. – Biochemistry 32: 6944 – 6949.

DEMMING-ADAMS, B., ADAMS, W.W., III (1992): Photoprotection and other responses of plants to high light stress (review). – Ann. Rev. Plant Physiol. and Plant Mol. Biol. 43: 599 – 626.

DEVOS, N., INGOUFF, M., LOPPES, R., MATAGNE, R.F. (1998): RUBISCO adaptation to low temperatures: A comparative study in psychrophilic and mesophilic unicellular algae. – J. Phycol. 34: 655 – 660.

DÖHLER, G. (1988): Effect of UV-B (280 – 320 nm) radiation on the ^{15}N-nitrate assimilation of some Algae. – Plant Physiol. (Life Sci. Adv.) 7: 79 – 84.

DÖHLER, G. (1994): UV-effects on the nitrogen metabolism of marine phytoplancton and adaptation to UV radiation. –In: BIGGS, R.H, JOYNER, M.E.B. (eds.): Stratospheric ozone depletion/UV-B radiation in the biosphere. Springer-Verlag, Berlin, Heidelberg, New York, p. 163 – 174.

DÖHLER, G., HOFFMANN, M., STAPPEL, U. (1995): Pattern of proteins after heat shock and UV-B radiation of some temperate marine diatoms and the Antarctic *Odontella weissflogii*. –Bot. Acta 108: 93-98.

DORAN, P.T., WHARTON, R.A., DES MARAIS, D.J., MCKAY, C.P. (1998): Arctarctic paleolake sediments and the search for extinct life on Mars. –J. Geophys. Res. – Planets 103 (E12): 28481 – 28493.

ELSTER, J., KOMÁREK, J., SVOBODA, J. (1994): Algal communities of polar wetlands. –In: Scripta Fac. Sci. Nat. Univ. Masaryk. Brun. 24: 13 – 24.

ELSTER, J., SVOBODA, J. (1995): *In situ* simulation and manipulation of a glacial stream ecosystem in the Canadian High Arctic. – In: JENKINS, A., FERRIER, R.C., KIRBY, C. (eds.): Ecosystem manipulation experiments: scientific approaches, experimental design and relevant results. Office for the official publication of the European communities, Luxembourg, p. 254 – 267.

ELSTER, J., SVOBODA, J. (1996): Algal diversity, seasonality and abundance in, and along glacial stream in Sverdrup Pass, 79°N, Central Ellesmere Island, Canada. – Mem. Natl Inst. Pol. Res., Spec.Issue 51: 99-118.

ELSTER, J. (1999): Algal versatility in various extreme environments. – In: SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht Boston London, p. 215 – 227.

ELSTER , J., LUKEŠOVÁ, A., SVOBODA, J., KOPECKÝ, J., KANDA, H. (1999): Diversity and abundance of soil Algae in the polar desert, Sverdrup Pass, central Ellesmere Island. – Polar Record 35 (194): 231 - 254.

ELSTER, J. (2002): Ecological classification of terrestrial algal communities in polar environments. - In: BEYER, L., BÖLTER, (eds.): Geoecology of Antarctic ice-free coastal landscapes. – Ecologicsl Studies, Vol. 154, Springer-Verlag, Berlin, Heidelberg, pp. 303 – 326.

ESA-SP 1231: BRACK, A., FITTON, B., RAULIN, F., eds. (1999): Exobiology in the Solar System & Search of Life on Mars. ESA Publication Division, ESTEC, Noordwijk, pp. 188.

ESA SP-496: EHRENFREUND, P., ANGERER, O., BATTRICK, B., eds. (2001): Proceedings of the first European workshop on exo-astrobiology, Frascati, Italy, 21 – 23 May 2001. ESA Publication Division, ESTEC, Noordwijk, pp. 444 pp.

ESA SP-518: LACOSTE, H., ed. (2002): Proceedings of the second European workshop on exo-astrobiology, Graz, Austria, 16 – 19 September 2002. ESA Publication Division, ESTEC, Noordwijk, pp. 625 pp.

ESA SP-545: HARRIS, R.A., OUWEHAND, L., eds. (2004): Proceedings of the third European workshop on exo-astrobiology – Mars: the search for life, Madrid, Spain, 18 – 20 November 2002. ESA Publication Division, ESTEC, Noordwijk, pp. 339 pp.

EXTRASOLAR PLANET ENCYCLOPAEDIA. - www. obspm.fr/encycl/encycl.html (June 2004)

FALKOWSKI, P.G., RAVEN, J.A. (1997): Aquatic photosynthesis. – Blackwell Science, 375 pp.

FERRIS, F.G., WIESE, R.G., FYFE, W.S. (1994): Precipitation of carbonate minerals by microorganisms – implications for silicate weathering and the global carbon-dioxide budget. – Geomicrobiology Journal 12: 1 – 13.

FOGG, G.E. (1998): The biology of polar habitats. Oxford University Press, Oxford, New York, Tokio, pp. 274.

FORTETERRE, P. (2000): Origin and evolution of organisms living at temperatures near the boiling point of water. – In: Frontiers of life. Abstracts. 12èmes Rencontres de Blois, p. 17.

FOTT, B., REJMÁNEK, M., ŠTURSA, J. (1978): Prvý nález červeného sněhu v Krkonoších [the first rekord of red snow in the Krkonoše (Giant) Mountains]. – Opera Corcontica 15: 109 – 112 .

FRIEDMANN, E.I. (1980): Endolithic microbial life in hot and cold deserts. – Origins Life Evol. B. 10: 223 – 235.

FRIEDMANN, E.I., MCKAY, C.P., NIENOW, J.A. (1987): The cryptoendolithic microbial environment in the Ross Desert of Antarctica: Satellite-transmitted continuous nanocilmate data, 1984 to 1986. – Polar Biol. 7: 273 – 287.

FRIEDMANN, E.I., HUA, M., OCAMPO-FRIEDMANN, R. (1988): Cryptoendolithic lichen and cyanobacterial communities of the Ross Desert, Antarctica. – Polarforschung 58 (213): 251 – 259.

FRIEDMANN, E.I. (1993a): Extreme environments, limits of adaptation and extinction. – In: GUERRERO, R., PEDRÓS-ALIÓ, C. (eds.): Trends in microbial ecology, Spanish Society for Microbiology, p. 9 – 12.

FRIEDMANN, E.I. (1993b): Extreme environments and exobiology. – Giornale Botanico Italiano 127 (3): 369 – 376.

FRIEDMANN E.I., HUA M., OCAMPO-FRIEDMANN R. (1993a): Terraforming Mars: Dissolution of carbonate rocks by Cyanobacteria. – Journal of the British Interplanetary Society 46: 291 – 292.

FRIEDMANN, E.I., KAPPEN, L., MEYER, M.A., NIENOW, J.A. (1993b): Long-term productivity in the cryptodenolithic microbial community of the Ross Desert, Antarctica. – Microb. Ecol. 25: 51 – 69.

FRIEDMANN, E.I. (1994): Permafrost as microbial habitat. –In: GILICHINSKI, D. (ed.): Viable microorganisms in permafrost, Russian Academy of Sciences, Puschino, p. 21 – 26.

FUJITA, Y., MURAKAMI, A., AIZAWA, K., OHKI, K. (1994): Short-term and long-term adaptation of photosynthetic appararus: Homeostatic properties of thylakoids. – In: BRYANT, D.A. (ed.): The molecular biology of Cyanobacteria, Kluwer Academic Publishers, p. 677 – 692.

GAIDUKOV, N., (1903a): Die Farbenvänderung bei den Prozessen der Komplementëren chromatischen Adaptation. – Ber. Deut. Botan. Ges. 21: 517 – 522.

GAIDUKOV, N. (1903b): Weitere Untersuchungen über den Einfluss farbigen Lichtes auf die Färbung des Oscillatarien. – Ber. Deut. Botan. Ges. 21: 484 – 492.

GARTY, J. (1999): Lithobionts in the Eastern Mediterranean. –In: SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht Boston London, p. 258 – 276.

GERSHMAN, R., NILSEN, E., OBERTO, R. (2003): Europa Lander. - Acta Astronautica 52 (2-6): 253 – 258.

GILICHINSKI D.A., VOROBYOVA, E.A., EROKHINA, L.G., FYODOROV-DAVYDOV, D.G., CHAIKOVSKAYA, N.R. (1992): Long-term preservation of microbial ecosystem in permafrost. – Adv. Space Res. 12 (4): 255 – 263.

GINZBURG, M. (1987): *Dunaliella*: a green alga adapted to salt. – Adv. Bot. Res. 14: 93 – 183.

GLEITER, H.M., HAAG, E., RENGER, G. (1990): Temperature dependence of the PS II reaction pattern detected by flash induced fluorescence changes. – In: BALTSCHFFSY (ed.): Current reseach in photosynthesis, Vol. 1, Kluwer Academic Publishers, pp. 479 - 482.

GLIMOUR, D. (1990): Halotolerant and halophilic microorganisms. – In: EDWARDS, C. (ed.): Microbiology of extreme environments, Open University Press, Milton Keynes, p 147 – 177.

GOLD, T. (1999): The deep hot biosphere. - Copernicus Springer Verlag, pp. 235.

GOMBUSHINA et KRUMBEIN (1999): The poikilotrophic micro-organism and its environment. Microbial strategies of establishment, growth and survival. –In: SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht Boston London, 177 – 185.

GOUNOT, A.M., NOVITSKI, T.J., KRUSHER, D.J. (1977): Effect of temperature on macromolecular composition and fine structure of psychrophilic *Arthrobacter* species. – Can. J. Microbiol. 23: 357 - 362.

GOUNOT, A.M., RUSSEL, N.J. (1999): Physiology of cold-adapted microorganisms. – In: MARGESIN, R., SCHINNER, F. (eds.): Cold-adapted organisms. Ecology, physiology, enzymology and molecular biology. Springer, Berlin, Heildelberg, New York, p. 33 – 55.

GREER, D.H., BERRY, J.A., BJÖRKMAN, O. (1986): Photoinhibition of photosynthesis in intact bean leaves: role of light and temperature, and requirement for chloroplast-protein synthesis during recover. – Planta 168: 253 – 260.

GRIGOREVA, Y.S., ZNAK, N.Y., GLADYSHEVA, E.E., GEKHMAN, A.V. (1989): Temperautre dependence of delayed fluorescence in algae adapted to different temperatures. – Fiziol. Rast. 36: 391 – 398.

GROSSMAN, A.R., SCHAEFER, M.R., CHIANG, G.G., COLLIER, J.L. (1994): The response of cyanobacteria to environmental conditions: Light and nutrients. – In: BRYANT, D.A. (ed.): The molecular biology of Cyanobacteria, Kluwer Academic Publishers, p. 641 – 675.

HAFNERSEE (2003): www.umwelt.ktn.gv.at/seenbericht2001/hafnersee/mitte.html

HALLDAL, P., FRENCH, C.S. (1958): Algal growth in crossed gradients of light intensity and temperature. – Physiol. Plantarum 11: 401 – 420.

HENLEY, W.J. (2001): Algae and cyanobacteria under desiccation and ionic stress. – In: ELSTER, J., SECKBACH, J., VINCENT, W.F., LHOTSKÝ O. (eds.): Algae and extreme environments: Ecology and physiology. – Nova Hedvigia Beih. 123: 443 – 452.

HENLEY, W.J., MAJOR, K., HIRONAKA, J.L. (2002): Response to salinity and heat stress in two halotolerant chlorophyte algae. – J. Phycol. 38: 1 – 11.

HIDEG, E. SPETEA, C., VASS, I. (1994): Singlet oxygen production in thylakoid membranes during photoinhibition as detected by EPR spectroscopy. – Photosyn. Res. 39: 191 – 199.

HOHAM, R.W. (1975): Optimum temperatures and temperature ranges for growth of snow Algae. – Arctic and Alpine Research 7 (1): 13 – 24.

HOHAM, R. W., DUVAL, B. (2001): Microbial ecology of snow and freshwater ice with emphasis on snow algae. – In: JONES, H.G., POMEROY, J.W., WALKER, D.A., HOHAM, R.W. (eds.): Snow Ecology: An interdisciplinary Examination of Snow-covered Ecosystems, Cambridge University Press, Cambridge, U. K., pp. 166-226.

HSU, K.J. (1992): Is Gaia endothermic? – Geological Magazine 129: 129 – 141.

JAVORNICKÝ, P., HINDÁK, F. (1970): *Cryptomonas frigoris* spec. nova (Cryptophyceae), the new cyst forming flagellate from the snow of the High Tatras. – Biológia 25: 241 – 250.

JEANICHE, R. (1990): Protein structure and function at low temperature. – Phil. Trans. Roy. Soc. London 326B: 535 – 553.

JEGERSCHÖLD, C., VIRGIN, I., STYRING, S. (1990): Light -dependent degradation of the D1 protein on photosystem II is accelerated after inhibition of water splitting system. – Biochemistry 29: 6179 – 6186.

JOHANSEN, J.R., SHUBERT, L.E. (2001): Algae in soils. – In: ELSTER, J., SECKBACH, J., VINCENT, W.F., LHOTSKÝ O. (eds.): Algae and extreme environments: Ecology and physiology. - Nova Hedvigia Beih. 123: 297 - 306.

JOLIOT, A. (1974): In fluorescence rise from 36 μson following a flash at low temperatures (+2 to -60 °C). – In: AVRON, M. (ed.): Proceedings of the third international congress on photosynthesis, Elsevier Scientific Publishing Company, Amsterodam, p. 315 – 322.

KANAZAWA, A., KRAMER, D., CROFTS, A. (1992): Temperature dependence of PS2 electron transfer reactions measured by flash-induced fluorescence changes. – In: MURATA, N. (ed.): Research in photosynthesis, Vol. II, Kluwer Academic, Dordrecht, p. 131 – 134.

KARSTEN, U., FRANKLIN, L.A., LUNING, K., WIENCKE, C. (1998): Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalga *Chondrus crispus* (Rhodophyta). – Planta 205: 257 – 262.

KATANA, A., KWIATOWSKI, J., SPALIK, K., ZAKRYŚ, B., SZALACHA, E., et SZYMAŃSKA, H. (2001): Phylogenetic position of *Koliella* (Chlorophyta) as inferred from nuclear and chloroplast small subunit rDNA. - J. Phycol. 37: 443 - 451.

KIM, C.S., HAN, G.H., KIM, J.M., JUNG, J. (1993): *In situ* susceptobilities of photosystem I and II photosensitized inactivation *via* singlet oxygen mechanism. – Photochem. Photobiol. 57: 1069 – 1074.

KIVELSON, M.G., KHURANA, K.K., RUSSELL, C.T., VOLWERK, M., WALKER, R.J., ZIMMER, C., (2000): Galileo Magnetometer Measurements: A Stronger Case for a Subsurface Ocean at Europa. – Science 289: 1340 – 1343.

KLECZEK, J. (2002): Velká encyklopedie vesmíru [Great encyclopedia of the Universe]. Academia, Praha, pp. 584.

KOIKE, H., HANSSUM, B., INOUE, Y., RENGER, G. (1987): Temperature dependence of S-state transition in a thermphilic cyanobacterium *Synechococcus vulcanus* Copeland measured by absorption changes in the ultraviolet region. – Biochim., Biophys. Acta 893: 524 – 533.

KOL, E. (1968): Kryobiologie. Biologie und Limnologie des Schenes und Eises. I. Kryovegetation. - In: ELSTER, H.J., OHLE, W. (eds.): Die Binnengewässer, vol. 24. Schweizerbart'sche Verlagsbuchhandlung. Suttgart, pp. 1 – 216.

KOL, E. (1972): Snow Algae from Signy Island (South Orkney Islands, Antarctica. – Annales Historico-naturales Musei Nationalis Hungarici 64: 63 – 70.

KOL, E. (1975): Cryobiological researches in the High Tatra I. – Acta Botanica Academiae Scientiarum Hungaricae 21 (1-2): 61 – 75.

KOL, E., EUROLA, S. (1974): Red snow Algae from Spitsbergen. – Astarte 7: 61 – 66.

KOMÁREK, J., HINDÁK, F., JAVORNICKÝ, P. (1973): Ecology of green kryophilic algae from Belanské Tatry Mountains (Czechoslovakia). – Arch. Hydrobiol./Suppl. 41 Algol. Stud. 9: 429 – 449.

KOMÁREK, J., LUKAVSKÝ, J. (1988): *Arthronema*, a new cyanophyte genus from Afro-Asian deserts. - In: ANAGNOSTIDIS, K., GOLUBIČ, S., KOMÁREK, J., LHOTSKÝ, O. (eds.): *Cyanophyta (Cyanobacteria)*, morphology, taxonomy, ecology. Proc. of the 10th Symp. of the IAC, Greece (Hellas) 1986. – Arch.Hydrobiol., Stuttgart, Suppl. 80 Algolog. Stud. 50-63: 249 – 267.

KOMÁREK, J. RŮŽIČKA, J. (1966): Freshwater algae from a lake in proximity of the Novolazerevskaya Station, Antarctica. – Preslia 38: 237 – 244.

KOTÍK, A. (1996): Struktura a funkce biomembrán [Structure and function of biomembranes]. – Masaryk University, Brno, pp. 173.

KRAMER, D.M., CROFTS, A.R. (1993): The controlled reduction of high and low potential chains of the *bf* complex by plastoquinol. – Biochim. Biophys. Acta 1184: 193 – 201.

KRISTJASSON, J. .K., HREGGVIDSSON, G.O. (1995): Ecology and habitats of extremophiles. – World Journal of of Microbiol. & Biotechnology 11: 17 – 25.

KUBÍN, Š. (1973): Zdroje fotosynteticky účinného záření a metody jeho měření [Sources of photosynthetically active radiation and methods of its measurement]. –Academia, Praha, pp .235.

KUEBLER, J.E., DAVISON, I.R., YARISH, C. (1991): Photosynthetic temperature adaptation in the red Algae *Lomentaria baileyana* and *Lomentaria orchadensis*. – Br. Phycol. J. 26: 9 – 19.

KUZNETZ, L. H., GAN, D. C. (2002): On the existence and stability of liquid water on the surface of Mars today. - Astrobiology 2: 183 – 195.

KVÍDEROVÁ J. & LUKAVSKÝ J. (2001): A new unit for crossed gradients of temperature and light. – In: ELSTER, J., SECKBACH, J., VINCENT, W.F., LHOTSKÝ O. (eds.): Algae and extreme environments: Ecology and physiology. – Nova Hedvigia Beih. 123: 541 – 550.

KVÍDEROVÁ, J., et LUKAVKÝ, J. (2003): The cultivation of *Phaeodactylum tricornutum* in crossed gradients of temperature and light. - Algological Studies 110: 67 – 80.

KVÍDEROVÁ J. & LUKAVSKÝ, J. (submitted): The ecological characteristics of *Stichococcus* (Chlorophyta) strains isolated from polar and temperate regions. Algological Studies.

LANGANGEN, A. (2000): Charophytes from the warm spring of Svalbard. – Polar Research 19: 143 – 153.

LARCHEL, W. (1988): Fyziologická ekologie rostlin [Physiological ecology of plants]. – Academia, Praha, pp. 361.

LAURION, I., VINCENT, W. F. (1998): Cell size versus taxonomic composition as determinants of UV- sensitivity in natural phytoplankton communities. – Limnol. Oceanogr. 43: 1774 – 1779.

LEBRETON, J. P., MATSON, D. L. (2002): The Huygens probe: Science, payload and mission overview. – Space Science Reviews 104: 59 - 100.

LEE, M.R., LEE, R.E. Jr., STRONG-GUNDERSON, J.M., HINGES, S.R. (1995): Isolation of ice nucleating active bacteria from the freeze-tolerant frog *Rana sylvatica*. – Cryobiology 32: 358 – 365.

LIKENS, G.E. (1964): An unusual distribution of algae in an Antarctic lake. – Bull.Torrey Bot. Club 91: 213 –217.

LIPMAN, C.B. (1941): The successful revival of *Nostoc commune* from a herbarium speciement eighty-seven years old. – Bull.Torrey Bot. Club 68: 664 - 666.

LOPPES, R., DEVOS, N., WILLEM, S. (1996): Effect of temperature on two enzymes from a psychrophilic *Chloromonas* (Chlorophyta). – J. Phycol. 32: 276 – 278 .

LUKAVSKÝ, J. (1982): Cultivation of chlorococcal algae in crossed gradients of temperature and light. – Arch. Hydrobiol./Suppl. 60,4 (Algological Studies 29): 517 – 528.

LUKAVSKÝ, J. (1993): First record of cryoseston in the Bohemian Forest Mts. (Šumava). – Arch.Hydrobiol./Suppl.97, Algological Studies 69: 83 – 89.

MACHOVÁ, K. (2004): Ekofyziologie dvou druhů rodu *Tribonema* a společenstev perifytonu dočasných tůní [Ecophysiology of two genera of the genus *Tribonema* and communities of temporal pools, Mgr. thesis, in Czech]. Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic, pp. 37.

MACLEOD, R.A., CALCOTT, P.H. (1976): Cold shock and freezing damage to microbes. -In: GRAY, T.R.G., POSTAGEN, R. (eds.): The survival of vegetative microbes. Cambridge University Press, Cambridge, p. 81 – 109.

MALIN, M.C., EDGETT, K.S. (2000): Evidence for recent groundwater seepage and surface runoff on Mars. – Science 288: 2330 – 2335.

MARSAC TANDEAU, N. DE (1977): Occurance and nature of chromatic adaptation in cyanobacteria . – J. Bacteriol. 130: 82-91.

MARSHALL, C.J. (1997): Cold-adapted enzymes. – TIBTech 15: 359 – 364.

MATSON, D. L., SPILKER, L. J., LEBRETON, J. P. (2002): The Cassini/Huygens mission to the Saturnian system. – Space Science Reviews 104 (1-2): 1 – 58.

MCKAY, C.P., NIENOW, J.A., MEYER, M.A., FRIEDMANN, E.I. (1988): Continuous nanoclimate data (1985-1988) from the Ross Desert (McMundo Dry Valleys) cryptoendolithic microbial ecosystem. – Antarctic Research Series 61: 201 – 207.

MEYER, M.A., HUANG, G.-H., MORRIS, G.J., FRIEDMANN, E.I. (1988): The effect of low temperatures on Antarctic endolithic green Algae. – Polarforschung 58 (2/3): 113 – 119.

MOON, B. Y., HIGASHI, S.I., GOMBOS, Z., MURATA, N. (1995): Unsaturation of the Membrane-Lipids of Chloroplasts Stabilizes the Photosynthetic Machinery Against Low-Temperature Photoinhibition in Transgenic Tobacco Plants. – PNAS 92: 6219 – 6223.

MORGAN, P., DOW, C.S. (1986): Bacterial adaptation for growth in low nutrient environment. – In: HERBERT, R.A., CODD, (eds.): Microbes in extreme environments, Academic Press, New York, p. 187 – 214.

MORITA, R.Y. (1975): Psychrophillic bacteria. – Bacteriological Reviews 39: 146 – 167.

NASA (2004): www.jpl.nasa.gov

NEDBALOVÁ, L. (in prep.): Growth optima of snow algae.

NEIDHART, F.C., VAN BOGELEN, R.A., VAUGHN, V. (1984): The genetics and regulation of heat-shock proteins. – Annu. Rev. Genet. 18: 295 – 329.

NIENOW, J.A., MCKAY, C.P., FRIEDMANN, E.I. (1988a): The cryptoendolithic microbial environment in the Ross Desert of Antarctica: Light in the photosynthetically active region. – Microb. Ecol. 16: 271 – 289.

NIENOW, J.A., MCKAY, C.P., FRIEDMANN, E.I. (1988b): The cryptoendolithic microbial environment in the Ross Desert of Antarctica: Mathematiacl models of the thermal regime. – Microb. Ecol. 16: 253 – 270.

OHTANI, S. (1986): Epiphytic Algae on mosses in the vicinity of Syowa station, Antarctica. – Mem. Natl. Inst. Polar Res., Spec. Issue 44: 209 – 219.

OHTANI, S., AKIYAMA, M., KANDA, H. (1991a): Analysis of Antarctic soil Algae by the direct observation using the contact slide method. – Nankyoku Shiryô (Antarctic Record) 35: 285– 295.

OHTANI, S., KANDA, H. (1987): Epiphytic Algae on the moss community of *Grimmia lawiana* around Syowa station, Antarctica. – Proc. NIPR Symp. Polar Biol. 1: 255 – 264.

OHTANI, S., KANDA, H, INO, Y. (1991b): Microclimate studies in the Yukidori Valley, Langhovde, Antarctica in 1988-1989 (Extended abstract). – Proc. NIPR Symp. Polar Biol. 4: 186 – 188.

ONDŘEJ, M. (1999): Genetika rostlin [Genetics of plants]. – Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic, pp. 125.

OREN, A., SECKBACH, J. (2001): Oxygenic photosynthesis in extreme environments. – In: ELSTER, J., SECKBACH, J., VINCENT, W.F., LHOTSKÝ O. (eds.): Algae and extreme environments: Ecology and physiology. - Nova Hedvigia Beih. 123: 13 – 31.

PALMER, R.J., FRIEDMANN, E.I. (1990): Water relatioship and photosynthesis in the cryptoendolithic microbial habitat of hot and cold deserts. – Microb. Ecol. 19: 111 – 118.

PANDOFF, J.M., THAMMAVONGS, B., GUEGUEN, M., BOUTIBONNES, P. (1998): Cold stres responses in mesophilic bacteria. – Cryobiology 36: 75 – 83.

PARKER, B.C., WHARTON, R.A. (1985): Physiological ecology of bluegreen algal mats (modern stromatolites) in Antarctic oasis lakes. – Arch. Hydrobiol. Suppl. 71: 331 – 348.

PEŠLOVÁ, J., POKORNÝ, J., KOMÁREK, J. (1990): Adaptation of 21 microalgae species to low level of molecular CO_2. – Algological studies 59: 97 – 109.

PICK, U. (1999): *Dunaliella acidophila* – A Most Extreme Acidophilic Alga. – In: SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht Boston London, p. 465 - 478.

PITHART, D., PECHAR, L., MATTSSON, G. (1997): Summer blooms of raphidophyte *Goniostomum semen* and its diurnal vertical migration in a floodplain pool. – Algological Studies 85: 119 – 133.

POWLES, S.B., BERRY, J.A., BJÖRKMAN, O. (1983): Interaction between light and chilling temperatures on the inhibition of photosynthesis in chilling-sensitive plants. – Plant Cell Environ. 6: 117 – 123.

PRÁT, S. (1970): Problémy sbírek kultur [Problems of the culture collections]. - Biologické listy 35: 81-89.

PRIVALOV, P.L. (1979): Stability of proteins. – Adv. Prot. Chem. 33: 167 – 241.

PROCHÁZKA, S., MACHÁČKOVÁ, I., KREKULE, J., ŠEBÁNEK, J. et al. (1998): Fyziologie rostlin [Physiology of Plants]. – Academia, Praha, pp. 484.

QUESADA, A., VINCENT, W.F., LEAN, D. R. S. (1999): Community and pigment structure of Arctic cyanobacterial assemblages: the occurrence and distribution of UV-absorbing compounds. – Fems Microbiology Ecology 28: 315 – 323.

RAI, L.C., GAUR, J.P. (eds.) (2001): Algal adaptation to environmental stresses. Physiological, biochemical and molecular mechanisms. – Springer-Verlag Berlin Heilderberg, 421pp.

RAULIN, F., OWEN , T. (2002): Organic chemistry and exobiology on Titan. - Space Sciences Reviews 104: 377-394.

RENGER, G., HANSSUM, B. (1992): Studies on the reaction coordinates of the water oxidase in PSII membrane fragments from spinach. – FEBS Lett. 299: 28 – 32.

ROBERTS, D.MCL. (1999): Eucaryotic cells under extreme conditions. – In: SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht Boston London, p. 165 - 173.

ROBERTS, M.E., INNIS, W.E. (1992): The synthesis of cold shock proteins and cold acclimation proteins in the psychrophilic bacterium *Aquaspirillum arcticum*. – Curr. Microbiol. 25: 275 – 278.

ROTHSHIELD, L.J. (1999): Microbes and radiation. – In: SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht Boston London, p. 551 – 562.

ROUND, F. (1985): The ecology of Algae. –Cambridge University Press, Cambridge London New York New Rochelle Melbourne Sydney, pp. 653.

RUSSEL, N.J.(1984): Mechanisms of thermal adaptation in bacteria: Blueprints for survival. – Trends in Biochem. Sci. 9: 108 – 112.

SCHULTZE-MAKUCH, D., IRWIN, L. N. (2002): Reassessing the possibility of life on Venus: Proposal for an astrobiology mission. – Astrobiology 2: 197 – 202.

SCHOPF, J.W. (1993): Microfossils of the Early Archean Apex Chert: New evidence of the antiquity of life. – Science 260, 640 – 646.

SHIRAIWA, M., GOYAL, A., TOLBERT, N.E. (1993): Alkalization of the medium by unicellular green Algae during uptake of dissolved inorganic carbon. – Plant Cell Physiol. 34: 649 – 657.

SIEGEL, B.Z. (1999): Growth of organisms in ammonia: *Kakabekia*, a microbial enigma. – In: SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht Boston London, p. 91 – 96.

SKULBERG, O.M. (1996): Terrestrial and limnic algae and cyanobacteria. – In: Elvebakk, A., Prestrud, P. (eds): A Catalogue of Svalbard Plants, Fungi, Algae and Cyanobacteria. Part 9, Norsk Polarinstitutt Skrifter 198, p. 383 – 395.

SMILLIE, R.M., HETHERINGTON, S.E. (1988): Stress tolerance and stress-induced injury in crop plants measured by chlorophyll fluorescence *in vivo*: chilling, freezing, ice cover, heat and high light. – Plant Physiol. 72: 1043 – 1050.

SMITH, M.G. (1995): Survival of *E. coli* and *Salmonella* after chilling and freezing in liquid media. – J. Food Sci. 60: 509 – 512.

SMITH, R.E.H., ANNING, J., CLEMENT, P., COTA, G. (1988): Abundance and production of ice algae in Resolute Passage, Canadian Arctic. – Mar. Ecol. Prog. Ser. 48: 251 – 263.

SOHL, F., SPOHN, T., BREUE,R, D., NAGEL K. (2002): Implications from Galileo observation on the interior structure and chemistry of the Galilean satellites. - Icarus 157: 104 – 119.

SONOIKE, K., TERASHIMA, I., IWAKI, M., ITOH, S. (1995): Destruction of photosystem I iron-sulphur centers in leaves of *Cucurmis sativus* L. by weak illumination at chilling temperatures. – FEBS Lett. 362: 235 – 238.

SOUTH, G.R., WHITTICK, A. (1987): Introduction to phycology. –Blackwell Scientific Publications, Oxford London Edinburgh, Boston Palo Alto Melbourne, pp. 341.

STEINBERG, C.E.W., SCHÄFER, H., BEISKER, W. (1998): Do acid-tolerant cyanobacteria exist? – Acta Hydrochim. Hydrobiol. 26: 13 – 19.

STIBAL, M. (2001): Řasová společenstva sněhu: jejich ekofyziologické nároky & životní cykly [Algal communities of snow: their ecophysiological requirements & life cycles, Bc. thesis, in Czech]. Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic, pp. 32.

STIBAL, M. (2003): Ecological and physiological characteristics of snow Algae from Czech and Slovak mountains. – Czech Phycology 3: 141 – 152.

STIBAL, M. (2004): Comparison of life strategies in two green Algae from snow and soil of the Polar Regions or *Raphidonema nivale* in snow – a resident or a guest?[Mgr. thesis, in English]. Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic, pp. 28.

STIBOR, M., POTOCKÝ, M. (2000): Biotechnologické aplikace psychrofilních mikroorganismů [Biotechnological applications of psychrophilic microorganisms]. – Bioprospect 10 (2): 2 – 5.

STITT, M., HUBER, S., KERR, P. (1987): Control of photosynthetic sucrose formation. – In: HATCH, M.D., BOARDMAN, N.K. (eds.): Photosynthesis, Vol. 10 of the Biochemistry of plants, Academic Press, New York, pp. 220 – 275.

STONE, R. (1999a): Permafrost comes alive for Siberian researches. – Science 286: 36 – 37.

STONE, R. (1999b): Lake Vostok probe faces delays. – Science 286: 36 – 37.

STYRING, S., VIRGIN, I., EHRENBERG, A., ANDERSSON, B. (1990): Strong light photoinhibition of electron transport in photosystem. II. Impairment of the function of the first quinone acceptor, Q_A. – Bioch. Biophys. Acta 1015: 269 – 278.

SUKENIK, A., BENNET, J., FALKOWSKI, P. (1987): Light-saturated photosynthesis - limitation by electron transport or carbon fixation? – Biochim. Biophys. Acta 891: 205 – 215.

ŠABACKÁ, M. (2001): Adaptační změny ve vybraných klonech sinic [Adaptation changes in selected clones of Cyanobacteria, Bc. thesis, in Czech]. Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic, pp. 46.

ŠABACKÁ, M. (2004): Response of algal and cyanobacterial communities from Arctic and Antarctic wetland habitats to freezing and desiccation stress. [Mgr. thesis, in English]. – p., Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic, pp. 71.

ŠETLÍK, I., SEIDLOVÁ, F., ŠANTRŮČEK, J. (2000): Fyziologie rostlin [Plant physiology]. Scriptum, Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic, pp. 313.

ŠMIGÁŇ, P., GREKSÁK, M. (2000): Niekto to rád horúce [Someone loves it hot]. – Vesmír 79 (130): 327 – 329.

VAN DEN HOEK, C., MANN, D.G., JAHNS, J.M. (1995): Algae, an introduction to phycology. Cambridge University Press, Cambridge, pp. 623.

VAN THIEN, N., GARBARY, D.J. (1999): Life in rocks – endolithic Algae. – In SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht Boston London, p. 245 – 253.

VINCENT, W.F. (1988): Microbial ecosystem of Antarctica. – Cambridge University Press, Cambridge, pp. 304.

VISVIKI, I., PALLADINO, J. (2001): Growth and cytology of *Chlamydomonas acidophila* under acidic stress. – Bull. Environ. Contam. Toxicol. 66: 623 – 630.

VOET, D., VOET, J.G. (1995): Biochemie [Biochemistry]. Victoria Publishing, Praha, pp 1348.

VOROBYOVA, E., SOINA, V., GORLANKO, M., MINKOVSKAYA, N., ZALINOVA, N., MAMUKELASHVILI, A., GILICHINSKI, D., RIVIKOVA, E., VISHNIVETSKAYA (1997): The deep cold biosphere – facts and hypothesis. – FEMS Microbiology Reviews 20: 277 – 290.

WALLES, S., SRAHNA, M., DEVOS, N., GERDAY, C., LOPPES, R., MATAGNE, R.F. (1999): Protein adaptation to low temperatures: a comparative study of α-tubulin sequences in mesophilic and psychrophilic algae. – Extremophiles 3: 221 – 226.

WALSH, M.M., SECKBACH, J. (1999): The versatility of microorganisms. – In: SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht Boston London, p. 155 - 162.

WARD, D.M., CASTENHOLZ, R.W. (2000): Cyanobacteria in geothermal habitats. – In: WHITTON, B.A., POTTS, M. (eds.): Ecology of cyanobacteria: their diversity in time and space, Kluwer Academic Publishers, Dordrecht, p. 37 – 59.

WARD, P., BROWNLEE, D. (2004): Život a smrt planety Země [Life and death of planet Earth]. Dokořán a Argo, Prague, 263 pp.

WDCM (2003): www.wdcm.nig.ac.jp

WEBB, R., SHERMAN, L.A. (1994): The cyanobacterial heat-shock response and molecular chaperones. –In: BRYANT, D.A. (ed.): The molecular biology of Cyanobacteria, Kluwer Academic Publishers, p. 751 - 767.

WILLIAMS, W.E., GORTON, H.L., VOGELMANN, T.C. (2003): Surface gas-exchange process of snow algae. – PNAS 100: 562 – 566.

WITHMARH, J., CRAMER, W.A. (1979): Cytochrome *f* function in photosynthetic electron tranfer. – Biophys. J. 26: 223 – 234.

WORLD LAKE DATABASE (2003): www.ilec.or.jp/database/eur/deur23.html

XIONG, F., LEDERER, F., LUKAVSKÝ, J., NEDBAL, L. (1995): On the potential roles of sporopolenin and protein synthesis in the protection of freshwater Algae against elevated ultraviolet-B radiation. – In: Proceeding X. International Photosynthesis Congress, France, p. 194.

XIONG, F. LEDERER, F., LUKAVSKÝ, J., NEDBAL, L. (1996): Screening of freshwater Algae (Chlorophyta, Chromophyta) for the ultraviolet-B sensitivity of the photosynthetic aparatus. – J. Plant Phycol. 148: 42 – 48.

YARISH, C. (1976): Polymorphism of selected marine Chaetophoraceae (Chlorophyta). – Br. Phycol. J. 11: 29 – 38.

YARISH, Ch., Lee, K., EDWARDS, P. (1979): An improved apparatus for the culture of Algae under varying regimes of temperature and light intensity. – Botanica Marina 22: 395 – 397.

ZAPOMĚLOVÁ, E. (2004): Morfologická variabilita a růst vybraných kmenů sinic rodů *Anabaena* a *Aphanizomenon* v závislosti na podmínkách prostředí. [Morphological variability and growth of selected cyanobacterial strains of genera *Anabaena* and

Aphanizomenon in relation to environmental conditions, Mgr. thesis, in Czech]. Faculty of Biological Sciences, University of Southern Bohemia, České Budějovice, Czech Republic, p. 57.

ZAVARZIN, G.A. (2002): Microbial geochemical calcium cycle. – Microbiology 71: 1 – 17.

5. Enclosed papers

KVÍDEROVÁ J. & LUKAVSKÝ J. (2001): A new unit for crossed gradients of temperature and light. – In: ELSTER, J., SECKBACH, J., VINCENT, W.F., LHOTSKÝ O. (eds.): Algae and extreme environments: Ecology and physiology. – Nova Hedvigia Beih. 123: 541 - 550.

Nova Hedwigia, Beiheft 123, p. 541–550. December 2001
Algae and extreme environments

A new unit for crossed gradients of temperature and light

JANA KVÍDEROVÁ & JAROMÍR LUKAVSKÝ

with 5 figures

Abstract: Cultivation of algae and cyanobacteria in crossed gradients of temperature and light is a simple and an effective method for testing of their ecological, biochemical and ultrastructural variability when grown in extreme cultivation conditions, too. Although an apparatus is commercially available which provides a carefully controlled crossed gradients of temperature and light, there is little published data available describing the conditions which can be achieved. The paper provides measured temperature and irradiance across the cultivation area of the plate in different settings. The linearity of temperature gradient across the plate was found to have an $r = 0.995$ and the irradiance gradient to be described by a parabolic curve with $r^2 = 0.936$. The unit is equally suitable for growing algae in large or small Petri dishes. A method is also described which uses immunological plates with many small wells of volume of 0.25 ml to increase number of variants to hundreds or thousands providing accuracy comparable to larger cultures in Erlenmeyer flasks and cuvettes. The cultivation of giant colonies (large colonies originating from thousands of cells) on agar plates is also capable to increase the reliability of the method.

Key words: algae, cyanobacteria, cultivation, crossed gradients, temperature, light, Petri dishes, immunological plates, solid media, clonal colonies, giant colonies.

Introduction

Cultivation of algae in crossed gradients of temperature and light is a simple and effective procedure for testing growth constants, content of metabolites, survive, morphology, variability in ultrastructure and physiology etc. A unit to produce the crossed gradients of temperature and light for such studies was first described by HALLDAL & FRENCH (1958) the unit has been several times used, e.g. YARISH (1976), LUKAVSKÝ (1982), ALBERTANO et al. (1993). KAŠTOVSKÝ (p.c.) used the apparatus to test the influence of temperature to morphological variability of *Mastigocladus laminosus*. The unit has since been modified in our laboratory by step by step improvements to the design making it more suitable for studies on the culti-

Authors' address:
Academy of Sciences of Czech Republic, Institute of Botany Dukelská 135, CZ-379 82 Třeboň, Czech Republic. E-mail: kviderova@butbn.cas.cz, lukavsky@butbn.cas.cz

1438-9134/01/0123-541 $ 2.50

vation of algae in extreme conditions across gradients of temperature and light. The unit has also been used for testing of germination of seeds of the genus *Salix* etc. (JIČÍNSKÁ & KONČALOVÁ, p.c.), study of competition of phytoplankton assembly taken from the field (KOMÁRKOVÁ, p.c.) etc.

Aim of the study. Although the unit for crossed gradients is commercially available but there is little published information detailing use or describing parameters and settings required to produce particular experimental conditions. In this paper we present information on the environmental conditions which can be achieved with the aim of stimulating further development of the unit.

Material and methods

A new unit was developed and assembled with LABIO Co. Ltd. Prague, CZ (Fig. 1 A). **Temperatures** were measured with digital thermometer with thermistor sensor (type CE, PL) which is commercially available to be used in cars. The probe was adapted for laboratory use and was calibrated against a mercury in glass laboratory thermometer in a beaker of slowly cooling hot water ($r = 0.994$). **Light** was evaluated by LI-189 (LICOR, USA).

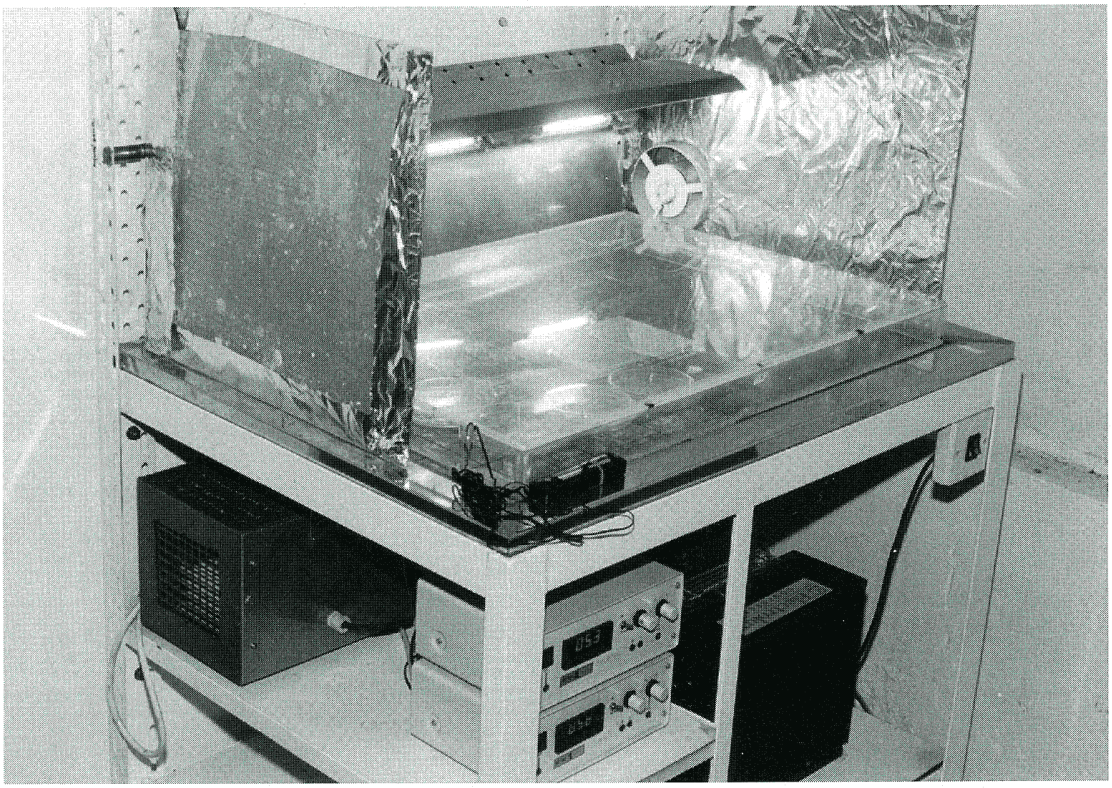

Fig. 1 A. A new unit for the crossed gradients of temperature and light.

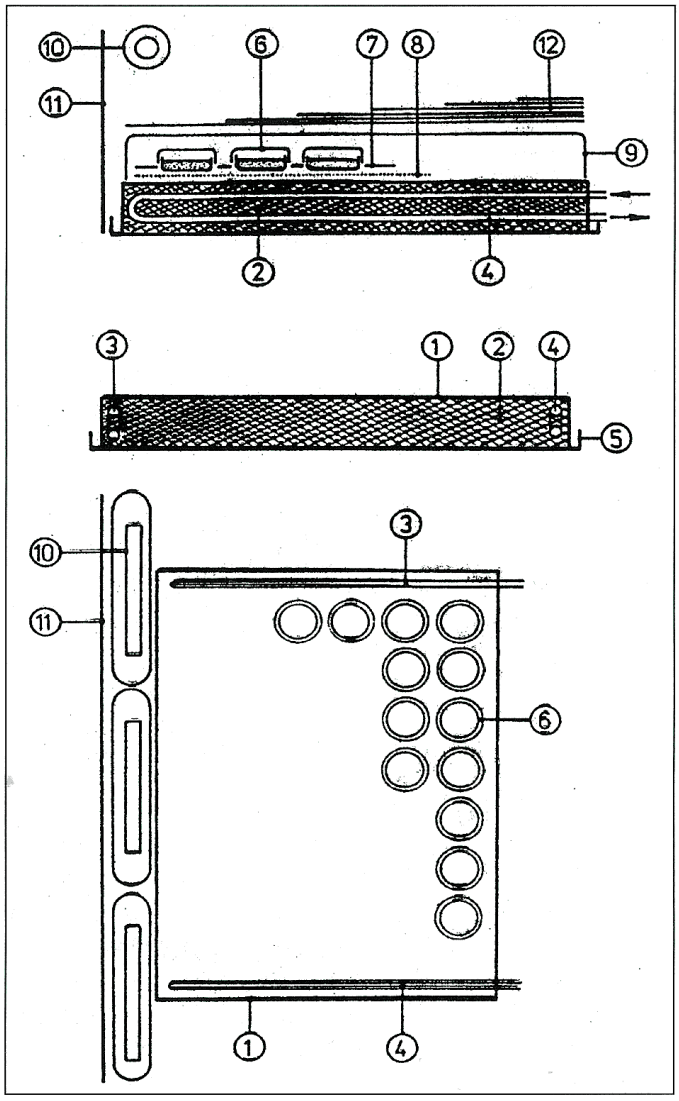

Fig. 1 B. The design of the unit according to LUKAVSKÝ (1982). **1** – surface of **2** – thick aluminium plate, **3,4** – heating and cooling channels, **5** – discharge trough, **6** – Petri dish, **7** – polyethylene foil with openings for dishes, **8** – gauze saturated with water, **9** – transparent lid, **10** – light source, **11** – mirror, **12** – determal glass and a strip filter (strips of tracing paper).

Description of the unit

The scheme of the original unit for crossed gradients used by LUKAVSKÝ (1982) is shown in Figure 1B. The new unit differs in the mode of heating and cooling of the plate and using of a new type of natrium lamps.

Temperature control

The temperature system ensures temperature setting from –16.5 °C to +100 °C. The system consists of aluminium plate 76 cm long by 65 cm wide by 4 cm thick. **Cooling** is achieved by expansion of compressed refrigerate gas directly into cooling channel in shorter right-hand side of the aluminium plate. The cooling is continuous and precise temperature control is

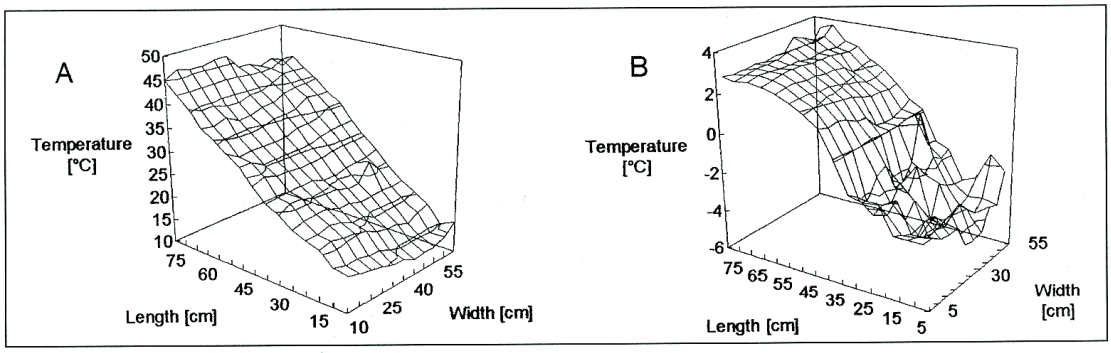

Fig. 2. The temperature gradient on the block. The temperature setting was for cooling +25 °C and heating +10 °C (**A**), and for cooling –16.5 °C and heating 0 °C (**B**).

achieved by means of a pair of electric heaters set into both shorter sides of the plate. Precise, stable temperature control is achieved by pulsing energy into heaters resulting in precise, linear and stable temperature gradient. Also heated side is controlled with pulse electric heater. In the previous unit the aluminium plate was cool or heated by circulating chilled or heated water through channels in the shorter opposite sides of the plate. The channels were joined with thermostatically controlled circulators (LUKAVSKÝ 1982).

Homogeneity of temperature over the block

The temperature gradient on the block is shown on Figure 2. The minimum temperature measured on the surface of the block was – 4.5 °C, the maximum + 45 °C. Measurements of the temperature along the gradient showed a high degree of linearity for temperatures above 10 °C with r = 0.995. For lower temperature around 0 °C the linearity found r = 0.955. Homogeneity of temperature along gradient can be expressed as coefficient of variation of a plane and was less than 10 %. Linearity can be evaluated as derivation from the straight line that is dependent on the slope of the gradient. Data showing the linearity of the gradient for different temperature regimes is shown in Figure 3.

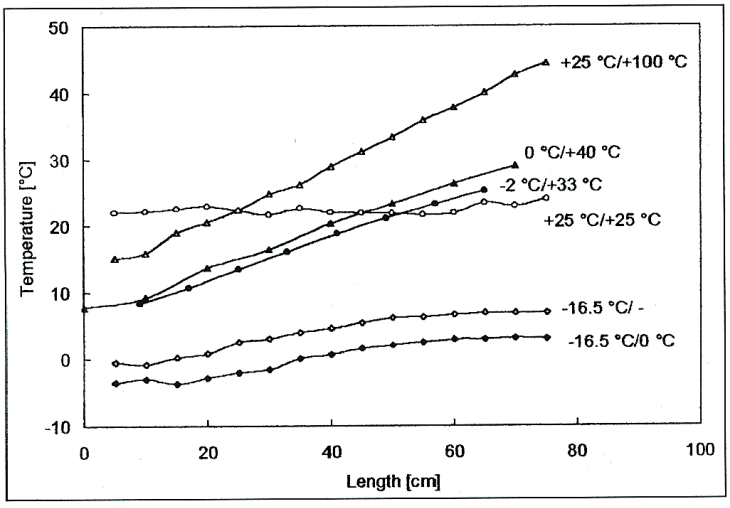

Fig. 3. Linearity of temperature gradient for different setting of cooling and heating (temperature of cooling/temperaure of heating; means not set).

Unit can be also set up to produce homogenous temperature over the entire surface of the plate. In this case the coefficient of variation is <3.15%. The difference of maximum and minimum temperature was 4.2°C. Linearity of temperature gradient is not essential, it does help with design of experiments and the predicting and calculating temperature in any point of the plate.

Irradiation unit

Sodium vapour lamps

The plate is illuminated by three pieces of sodium vapour lamps NAV TS 400 (OSRAM, D), arranged in a line. The lamps are very small and can be assembled into a linear compact unit. The system allowed range of different levels of irradiances up to 1400 $\mu mol.m^{-2}.s^{-1}$ (approximately equal to 250 $W.m^{-2}$). Lower irradiances were recorded along the shorter sides (a belt of approximately 10 cm) but it can be overcome by installing of reflection material along the sides or by excluding this area from experiments. More defined light gradient can be achieved by placing strips of filter material on the Perspex cover of the plate. These can be made by printing dots of different density on strips of tracing paper or transparent overhead foil. The production of heat is removed by fan and dethermal glass over the lid. Other types of light sources can be used including bulbs and fluorescent tubes according to the spectral composition and irradiance required. The linear light sources should be preferred.

Fluorescent tubes

Although the shape of this type of lamp make them ideal for this application, they have comparatively low irradiation output. We used two fluorescent tubes with no filters in our experiments with *Phaeodactylum tricornutum*. The level of photosynthetically active radiation (PhAR) ranged from 6.54 $\mu mol.m^{-2}.s^{-1}$ to 60.98 $\mu mol.m^{-2}.s^{-1}$. Homogeneity of light at a particular distance from the light source was characterized by r = 0.956 (KVÍDEROVÁ & LUKAVSKÝ, in prep.).

Higher levels of irradiance can be achieved using of fluorescent tubes of a new generation, such as OSRAM Dulux daylight 2G11, 55W/12-950 which are in a panel of 4 pcs are capable to emitt 500 $\mu mol.m^{-2}.s^{-1}$ (100 $W.m^{-2}$) in the first row.

Homogenous irradiance

Homogenous irradiance of the entire plate was not required in our experiments but could be obtained by replacing of irradiance unit with sodium lamps with an array of a horizontal panel of fluorescent tubes above the block. The irradiance from a compact panel of fluorescent tubes can reach about 140 $\mu mol.m^{-2}.s^{-1}$ PhAR and the coefficient of variation of about 5% (LUKAVSKÝ 1974).

Light gradient

Homogeneity of irradiance gradient is almost precise in the almost entire area of the plate (Fig. 4), the variation coefficient is <15% excluding the 10 cm border at both shorter sides. The gradient could be described as parabolic curved plane with $r^2 = 0.952$ with the height of the light source of 18 cm above the block. If the height of the lamps is increased to 45 cm,

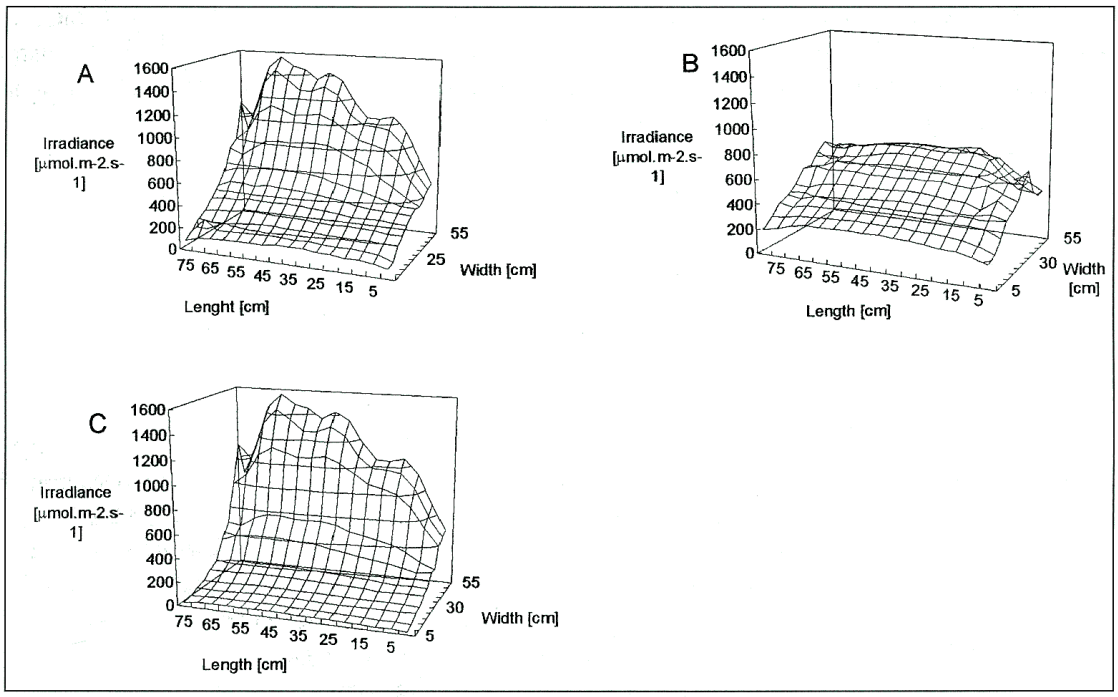

Fig. 4. The light gradient for different height of light source above the block – 18 cm (**A**) and 45 cm (**B**) and for strip filter (**C**).

the gradient becomes linear with r = 0.899. When the strip filter is introduced the gradient becomes rather exponential (Fig. 4).

Cultivation space

Algae can be cultivated in liquid media or on agar plates using various sized Petri dishes or in wells of immunological plates. Cultivation may be in suspension or colonies, both, clonal or giant ones. The whole cultivation area is covered by Perspex cover to reduce evaporation of the liquid medium or the desiccation of the agar plates. Enclosing the growing area with Perspex cover also allows the atmosphere to be enriched by CO_2 from a gas cylinder.

Petri dishes

The original unit of HALLDAL & FRENCH (1958) used a large, monolithic agar layer poured direct over the entire aluminium plate. This method was sensitive to contamination with a fungi or bacteria and may also be contaminated of nutrient solution by dissolved aluminium from the surface of the plate. In our studies we used a set of 35 glass Petri dishes with diameter of 9 cm. The dishes lied with lids up on the aluminium plate. To improve heat transfer between the dishes and the plate a layer of wet gauze was placed under the dishes covering entire area of the plate. To reduce evaporation, a sheet of polyethylene was placed over the gauze with

holes cut to accommodate the dishes, or immunological plates respectively. The difference in the temperatures between the top of the agar layer and the plate is 0.1 °C compared to 10 °C when the dishes were placed directly onto the aluminium plate (LUKAVSKÝ 1982). The agar plates can be inoculated by giant colonies (large colonies originating from thousands of cells in one point) or clonal colonies (a diluted inoculum is spread on the agar surface) or growth (a dense inoculum is spread on agar surface). More detailed body of information is available in SHREWSBURY (1931), LUKAVSKÝ (1975).

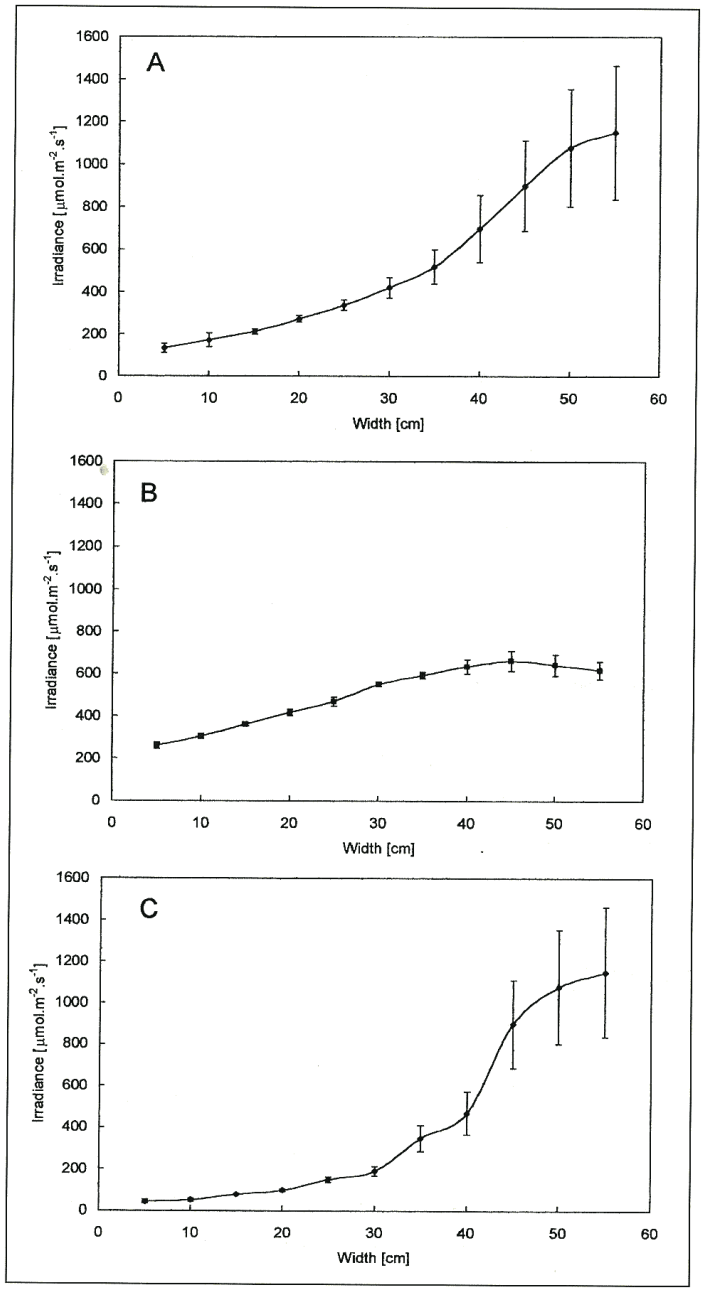

Fig. 5. The course of light gradient for different height of light source above the block – 18 cm (**A**) and 45 cm (**B**) and for strip filter (**C**).

Immunological plates

Immunological plates (9x12 cm, FB) with 96 wells are commonly used for algal bioassay for freshwater (BLAISE et al. 1986, LUKAVSKÝ 1983, 1992) but also for seawater (LUKAVSKÝ & SIMMER 2001). Growth is evaluated by measuring the increase of diameter of giant colonies on agar plates, or as optical density at 750 nm in suspension. Flat bottom plates allow in situ measurements of optical density also better contact with the aluminium plate. However, the majority of flat bottom plates have a raised edge around their base which can be removed or the space between the bottom and the aluminium plate can be filled with a heat conducting material, e.g. wet gauze. This reduces the difference of temperatures between the inside well and the aluminium plate to about 3°C. This constant error, which is also proportional to the temperature and irradiance, can be take into account for settings of temperature. The advantage of polystyrene immunological plates is also, that they are permeable to CO_2.

CO_2 supply

Carbon dioxide can be the factor limiting cell growth when yields approach 200 $mg.l^{-1}$ of dry weight of suspension (MILLER et al. 1978). Culture of algae in nutrient enriched media are also likely be limited by CO_2 levels in the atmosphere above the culture, unless it is enriched form a cylinder described above. A concentration of 2% v/v is enough to provide excess and meet the demands of most common algae. In our unit only temperature or light were found to limit algal growth.

Mixing of cell suspension

Mixing is not normally necessary, diffusion of CO_2 into colonies on agar late, as well as into such small volumes in wells of immunological plates is normally adequate but can be enhanced placing the aluminium plate on a shaker or tilting machine. When the shaker or the tilting machine is used, the algae can grow in larger volumes of suspension in Petri dishes or in Erlenmeyer flasks, too. The gradiented aluminium plate on tilting machine can also be used also for study of dynamics of chemical reactions etc.

Continuous cultivation

Batch cultures are the principle source of information about growth rates, yields, growth constants etc., however, there are disadvantages connected with this technique. Changing conditions within the culture vessels, e.g. the increased self-shadowing of cells, nutrients depletion and increment of metabolic extracellular products etc, can affect cell growth. The conditions within continuous culture vessel can stabilised optically (turbidistat) or by flow of nutrient (chemostat). Semi-continuous system, which rely on a periodic exchange of proportion of the culture volume, can provide an adequate continuous culture model. Microplates with well volumes of 0.2 ml can be operated in either chemostatic or turbidistatic regime, by regularly monitoring of OD750 and exchanges of a certain proportions of suspensions the total volume in each well with a fresh solution.

Nutrient gradient

In addition to creating gradients of temperature and light, gradients of nutrients, other substances, or pH to be tested can be also produced by method according to BRYSON & SZYBALSKI (1952). Two opposite slopes of agar are poured into a Petri dish. A basic slope is poured first, in an inclined position, using agar with no additions. Once this layer is set, the dish is returned to horizontal position and the second layer of agar containing the test substance is poured. Diffusion between the two layers produced a concentration gradient of the tested substance proportional to the ratio of the height of the thickness of both two layers and the length of the Petri dish. This procedure is commonly used in microbiology for testing and the selection of resistant strains to antibiotics. BENJAMIN & KLAINE (1995) used agar plates with gradients of copper for selection of resistant colonies of *Selenastrum capricornutum*.

Conclusions

This unit proved to be a valuable tool for testing the growth of algae, in a large scale, enabling a high degree of replication and in many combinations of different temperature and light regimes. The unit has been shown to be very efficient and to save time and money by enabling large number of independent combinations of temperature and light to be studied at the same time, e.g. 35 Petri dishes of diameter of 9 cm, or 20 immunological plates with a total of 1920 wells each with capacity of 0.25 ml. This study shown that the unit, which is commercially available now, is capable of producing precise gradients of both temperature and light suitable for examining the growth characteristics of algae. The specifications of the unit have now been defined. More detailed body of information is available at: sales@labio.cz.

Acknowledgements

We thank project of Ministry of Education of Czech Republic MSM 1231 00004 for financial support, Mr. K. HUBÁČEK and Mr. F. VONDRÁK for technical assistance. Labio Co. had to solve many problems to develop a new model of crossed gradient table. Dr. J. JOHANSEN kindly went through the manuscript.

References

ALBERTANO, P.; KOVÁČIK, L. & GARDAVSKÝ, A. (1994): Cross-gradient cultures of filamentous cyanophytes. – G. Bot. Ital. **127**: 855–856.

BENJAMIN, R.B. & KLAINE, S.J. (1995): Fitness trade-offs of *Selenastrum capricurnutum* strains selected for rapid growth on copper-spiked solidified nutrient medium. – Environ. Toxicol. Chem. **14**: 1789–1798.

BLAISE, C.; LEGAULT, R.; BERMINGHAM, N.; VAN COILLIE, R. & VASSEUR, P. (1986): A simple microplate algal assay technique for aquatic toxicity assessment. – Tox. Assessment: An Internat. Quart. **1**: 261–281.

BRYSON, V. & SZYBALSKI, W. (1952): Microbial selection II. – Science **116**: 45–51.

HALLDAL, P. & FRENCH, C.S. (1958): Algal growth in crossed gradients of light intensity and temperature. – Physiol. Plant. **33**: 249–252.

KVÍDEROVÁ, J. & LUKAVSKÝ, J. (in prep.): The cultivation of *Phaeodactylum tricornutum* in crossed gradients of temperature and light.

LUKAVSKÝ, J. (1974): Controlled cultivation of algae on agar plates. – Arch. Hydrobiol./Algolog. Studies **10**: 90–104.

LUKAVSKÝ, J. (1975): Analysis of growth rate of algae by cultivation on solid media. – Arch. Hydrobiol./ Algolog. Studies **14**: 105–136.

LUKAVSKÝ, J. (1982): Cultivation of chlorococcal algae in crossed gradients of temperature and light. – Arch. Hydrobiol./Algolog. Studies **29**: 517–528.

LUKAVSKÝ, J. (1983): The evaluation of algal growth potential by cultivation on solid media. – Water Res., Oxford **17**: 549–558.

LUKAVSKÝ, J. (1992): The evaluation of algal growth potential (AGP) and toxicity of water by miniaturised growth bioassay. – Water Res., Oxford **26**: 1409–1413.

LUKAVSKÝ, J. & SIMMER, J. (2001): Microprocedure for a standard marine algal bioassay (ISO 10253). – Arch. Hydrobiol./Algolog. Studies **101**: 137–147.

MILLER, W.E.; GREENE, J. & SHIROYAMA, T. (1978): The *Selenastrum capricornutum* PRINTZ algal assay bottle test. – EPA, Rep. No 600/9–78–818, p. 126, Oregon.

SHREWSBURY, J.F.D. (1931): Giant colony culture. – J. Pathol. Bacteriol. **34**: 283–285.

YARISH, CH. (1976): Polymorphism of selected marine Chaetophoraceae (Chlorophyta). – Br. phycol. J. **11**: 29–38.

YARISH, CH.; LEE, K. & EDWARDS, P. (1979): An improved apparatus for the culture of algae under varying regimes of temperature and light intensity. – Botanica Marina **22**: 395–397.

KVÍDEROVÁ, J. & LUKAVSKÝ, J. (2003): The cultivation of *Phaeodactylum tricornutum* in crossed gradients of temperature and light. - Algological Studies 110: 67 - 80.

Algological Studies **110**	67–80	Stuttgart, November 2003

The cultivation of *Phaeodactylum tricornutum* in crossed gradients of temperature and light

By JANA KVÍDEROVÁ and JAROMÍR LUKAVSKÝ

Institute of Botany, Academy of Sciences of Czech Republic, Třeboň
and University of South Bohemia, Faculty of Biological Sciences,
České Budějovice, Czech Republic

With 9 figures and 2 tables in the text

Abstract: *Phaeodactylum tricornutum* BOHLIN, is one of the most important species in the algal biotests. The cultivation of this species in the crossed gradients evaluated the dependence of the growth rates and morphology on particular combinations of environmental factors (temperature, light). The main advantage of this method is the simplicity and quickness of obtaining data from different conditions.

The results of the cultivation of *Phaeodactylum tricornutum*, in the crossed gradients, showed that maximal growth rates occurred at temperatures between 15 and 23 °C and at irradiance between 15 and 125 $\mu mol.m^{-2}.s^{-1}$). There were not observed differences in morphology of the alga cultivated at different combinations of growth conditions.

The conversion curves were calculated for three cultures of *Phaeodactylum tricornutum* (two old cultures and one young one) and for four different ways of measurements of optical density at 750 nm (in immunological plates, culture wells, Petri dishes and 20 ml cultivation flasks).

Key words: *Phaeodactylum tricornutum*, crossed gradients, cultivation, light, temperature, growth rate, growth optima, conversion curves.

Introduction

Algal bioassays and unialgal cultures represent an important tool for the elucidation of specific problems related to environmental pollution and eutrophication, but also cell morphology, cytology, taxonomy, life cycle and genetics. Unialgal cultures are applied in a number of ultrastructural, physiological, biochemical and molecular studies, due to their growth characteristics, e.g. high division rates, approximately equal size, ease of cultivation and synchronisation.

In ecology, they have been successfully used to evaluate the effects of environmental factors, such as light, temperature, pH, on growth rates. These studies allow understanding of the physiological behaviour of different algal species in nature. The unialgal cultures have been employed for evaluation of the trophic,

0342-1123/03/0149-067 $ 3.50

Algological Studies **110** = Arch. Hydrobiol. Suppl. **149**

growth potential of water and for determining of toxicity of organic and inorganic pollutants, heavy metals, etc. (CORDEIRO-MARINO et al. 1992, PUISEUX-DAO 1989).

Cultivation of algae in crossed gradients of temperature and light (HALLDAL & FRENCH 1958) is a suitable method for the testing of morphological variability, growth rates, contents of metabolites or photosynthetic pigments. Its advantage is a great number, e. g. 50, of combinations of temperature and light in one unit. The second advantage is that data are obtained from very wide spectrum of the conditions e. g. temperature from 0 to 50 °C, in the same time very simply and quickly (LUKAVSKÝ 1982).

Phaeodactylum tricornutum BOHLIN, is one of the most used algal species in the marine bioassays because of its easy cultivation. It is also a prospective alga in aquaculture as food for e. g. crustacean *Artemia*. It has been studied from many points of view; during the last ten years, we have found 168 articles published. Most of them (106) concerned metabolic processes, e. g. metabolism of phosphorus and nitrogen, and photosynthesis. Only 22 articles concerned cultivation of *Phaeodactylum tricornutum* and its use in the bioassays (e. g. YONGMANITCHAI & WARD 1992, GRIMA et al. 1996, CONTRERAS et al. 1998). It was observed that the alga changes its morphology depending on its ecological habitat. Three morphological forms are described - a triradiate, fusiform and oval one, according to marine littoral, planktonic and benthic habitats (ROUND et al. 1996).

The aim of this work was to find possible changes in growth rates and morphology of *Phaeodactylum tricornutum* in different temperatures and irradiances using the new unit for crossed gradients of temperature and light and to compare the found optima of temperature and irradiation to prove reliability of this method. The second aim of this work was to calculate conversion curves of different cultures of *Phaeodactylum tricornutum* for common use in laboratory.

Material and methods

Alga *Phaeodactylum tricornutum* BOHLIN, strain CAUGHT, was obtained from the CCAP-Provasoli Guillard Nat. Centre for Culture of Marine Phytoplankton, USA.

To obtain the **crossed gradients** of temperature and light, the new unit, assembled by LABIO Co.Ltd. (Czech Rep.) was used. The scheme of the crossed gradients is shown in Fig.1. The properties of the unit are described in detail in KVÍDEROVÁ & LUKAVSKÝ (2001).

The temperature gradient was set up to limiting values 8.4 °C and 25.3 °C in the first experiment and to 6.2 °C and 23.6 °C in the second one. The temperature was measured by digital thermometer Omega, USA. The temperature gradient was precise and stable (Fig.2). Linearity of the gradient was characterised with r = 0.995 (respectively r = 0.992 in the second experiment). Coefficient of variation was 0.71-6.28 % for individual columns (2.3 to 22.2 % in the second experiment).

The range of irradiance of photosynthetically active radiation (PhAR) was set up from 6.5 $\mu mol.m^{-2}.s^{-1}$ to 61 $\mu mol.m^{-2}.s^{-1}$ in the first experiment when two fluorescent tubes were used. In the second experiment, the irradiance ranged from 35 to 150 $\mu mol.m^{-2}.s^{-1}$ when three halogen incandescent lamps arranged in a row were used and light was cooled by passing through layer of water, stratified by vertical position of lights. The irradiance was meassured by Li 185B, LiCor (USA). The course of the light gradient was charac-

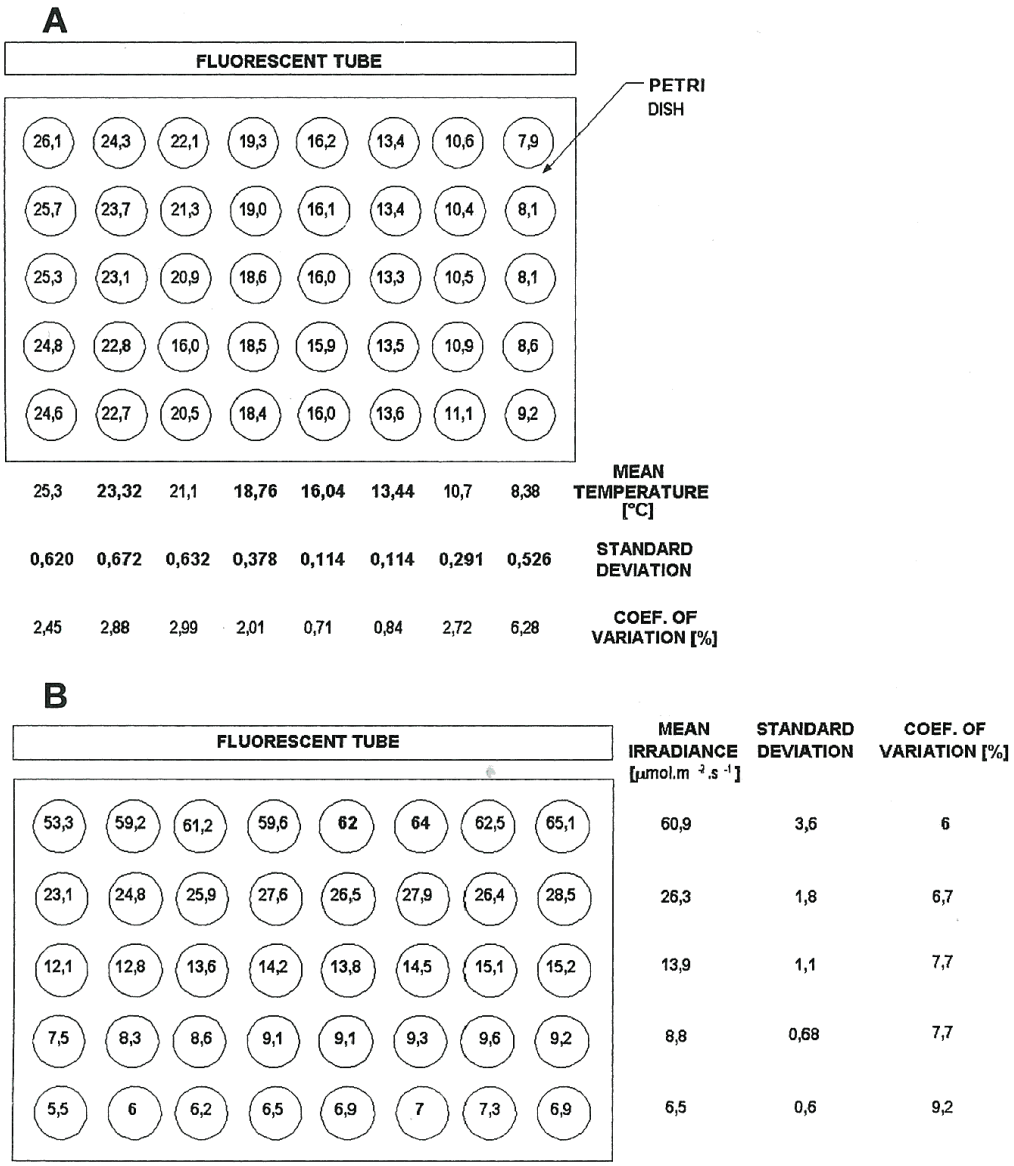

Fig. 1. Scheme of the crossed gradients of temperature (**A**) and irradiance (**B**). Measured values of temperature and irradiance are written in Petri dishes.

terised by parabolic curve and r = 0.956 for the fluorescent tubes, respectively 0.834 for halogen incandescent lamps. The variation coefficient was 6 to 9.2 % for individual rows in the first experiment and 12.8 to 20.7 % in the second one. The higher variation coefficient in the second experiment was caused by decreased irradiance at the sides (**F**ig. 3).

Phaeodactylum tricornutum was cultivated at 8 different temperatures in the first experiment and 7 in the second one, and in 5 different light intensities (see Fig. 1) in both for seven weeks, so 40, respectively 35, combinations of temperatures and light intensity were obtained. The alga grew in 15 ml of artificial sea water (after ISO 10253, 1995) in glass Petri dishes (diameter of 8 or 9 cm). The initial concentration (density) of cells in inoculum was set to 10 000 cells per ml. Optical density at 750 nm was measured by Titertek Uniscan II (Finland) every week. The measured absorbance values were converted to the number of cells per litre and dry matter (weight) per litre via conversion

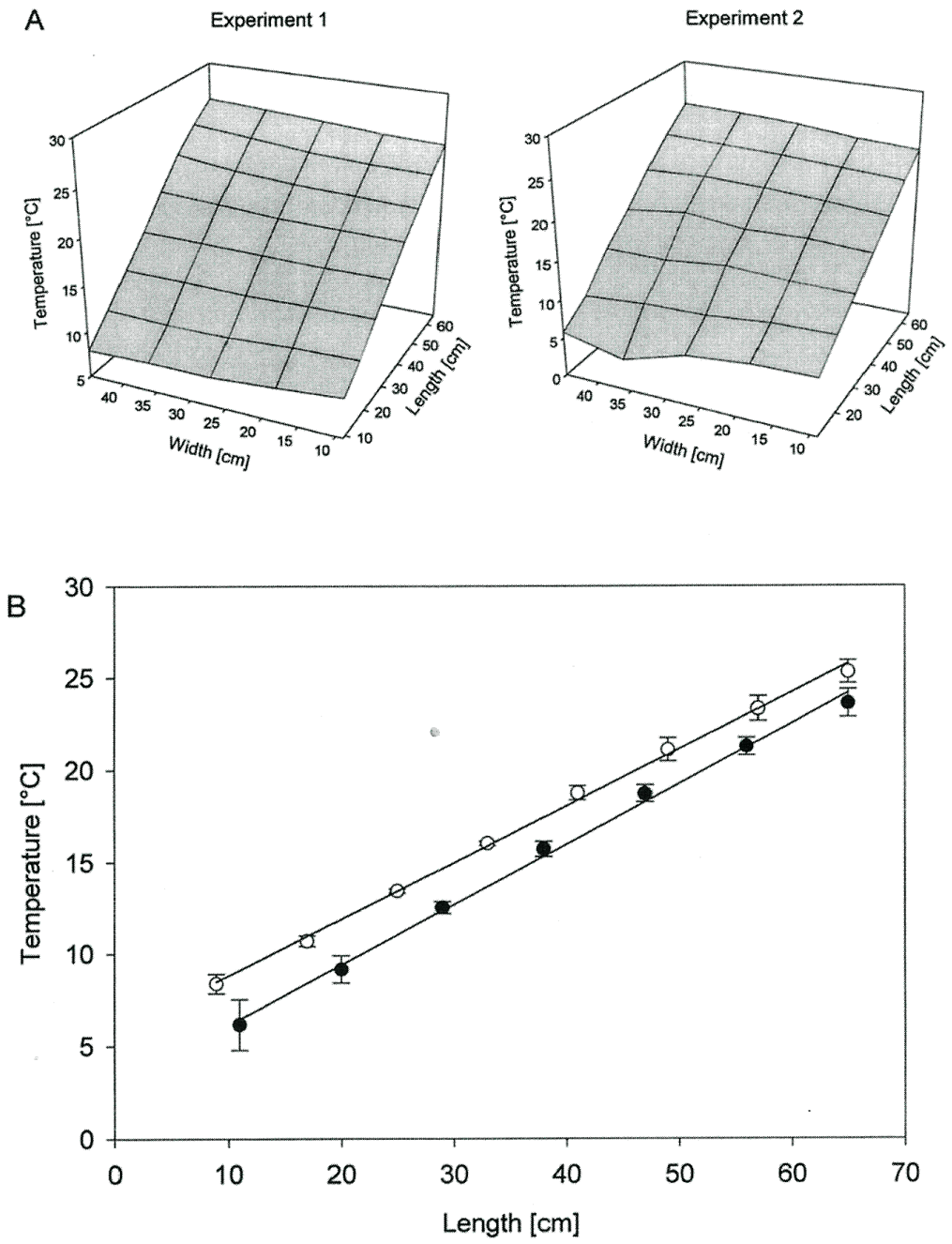

Fig. 2. The resulting gradients of temperature on the cultivation block (**A**) and the courses of the temperature gradients (**B**) in the first (o) and in the second (•) experiments. The error bars indicate standard deviation.

curves and equations. The microphotographs were taken by microscope Amplival, ZEISS (Germany) and digital camera system Olympus DP10 (Japan), in the end of cultivation. The samples from different conditions were thickened by centrifugation at 313 g-value/ 30 minutes. The used magnification of objective was 40x.

The **conversion curves** were calculated by computer software TCWIN. Three different cultures were used. The two old cultures were seven weeks old and were obtained

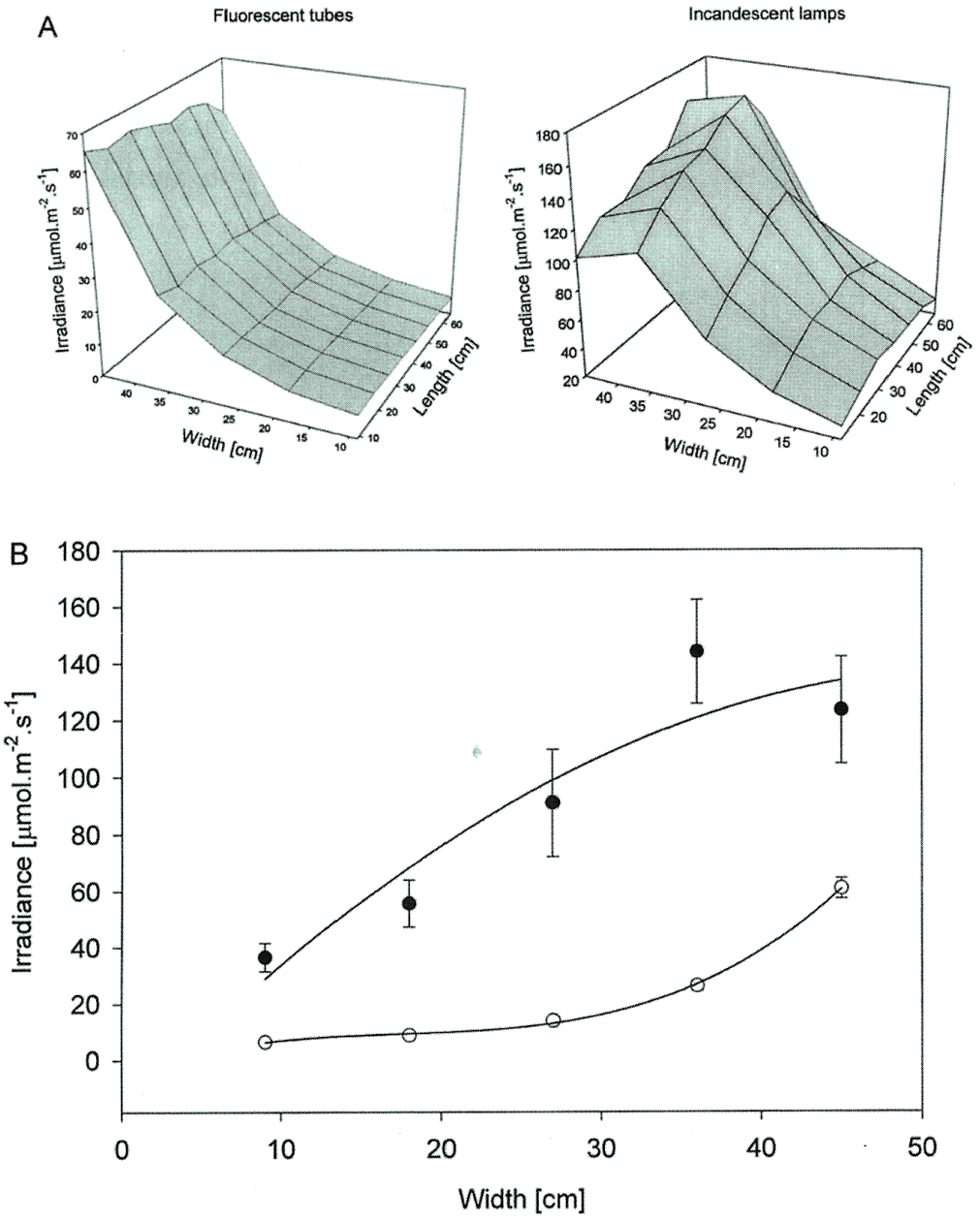

Fig. 3. The resulting gradients of irradiances on the cultivation block (**A**) for two fluorescent tubes (used in the first experiment) and for three incandescent lamps (used in the second experiment) and the courses of the light gradients (**B**) in the first (o) and in the second (•) experiments. The error bars indicate standard deviation.

from previous experiments. The young culture was 1 week old. A samples culture with a volume of approximately 1 l was concentrated by centrifugation at 153 g-value/40 min. The thickened sample of volume of 150 ml was diluted creating 0.3, 0.1, 0.03, 0.01, 0.003, 0.001, 0.0003 and 0.0001 solution, each of a volume of 100 ml. The optical density at 750 nm was measured for every solution by Titertek Uniscan II and iEMS Labsystems (Finland) for samples in immunological plates of well volume of 0.25 ml, cultivation wells of a volume of 3 ml, cultivation flasks of a volume of 20 ml and Petri

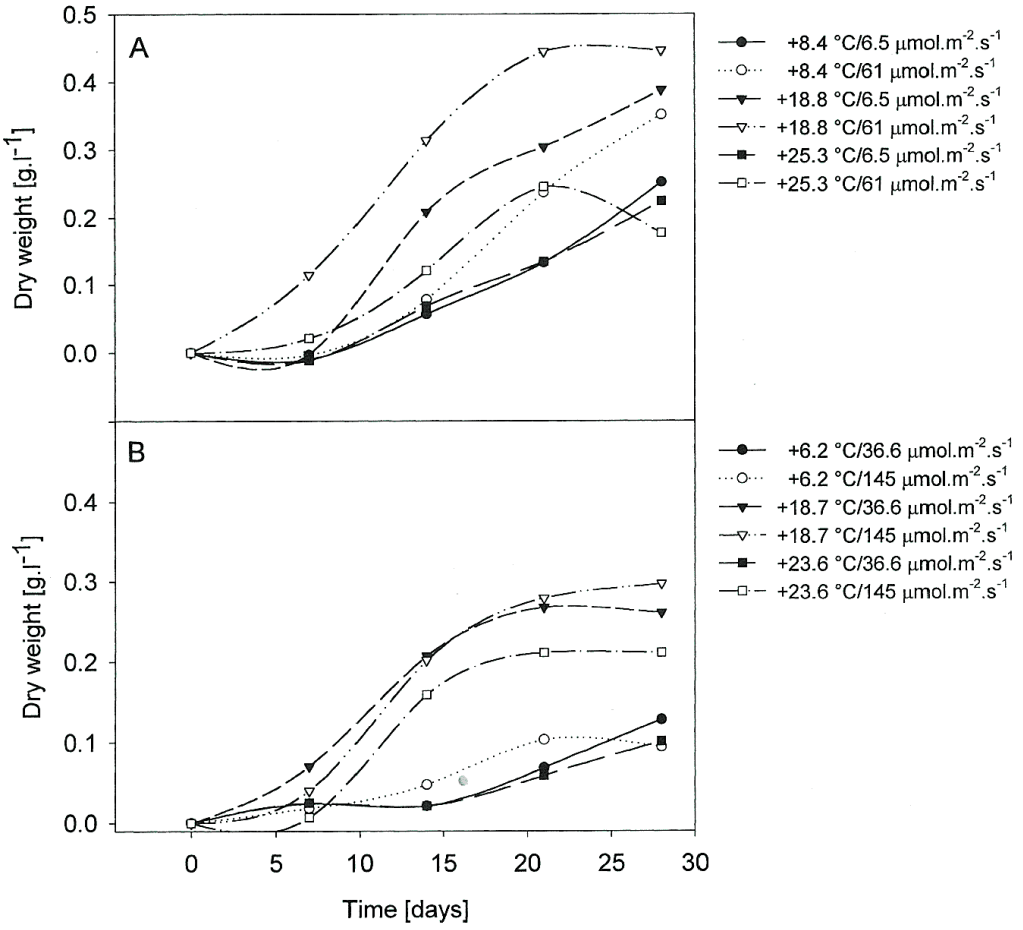

Fig. 4. Growth curves at combinations of maximal, optimal and minimal temperatures and maximal and minimal and irradiances in the first (**A**) and in the second (**B**) experiments.

dishes of diameters of 9 or 8 cm, and by Spekol 11 ZEISS (Germany), in cuvettes of lengths of 1 cm or 5 cm. Each measurement was repeated three times and average, standard deviation and variation coefficient were calculated. The number of cells per litre was counted in Bürker chamber. To calculate dry weight per litre, 50 ml of thickened sample was filtrated with GF/C Whatman (UK) and dried to constant weight at 110 °C.

Results and discussion

Algal growth and growth optima

Phaeodactylum tricornutum was cultivated in crossed gradients of temperature and light. We compared growth curves of the alga cultivated in combinations of maximal (61 or 145 μmol.m^{-2}.s^{-1}) and minimal (6.5 or 36.6 μmol.m^{-2}.s^{-1}) irradiances and maximal (25.3 or 23.6 °C), optimal (18.8 or 18.7 °C) and minimal (8.4 or 6.2 °C) temperatures in both experiments. The maximal and minimal val-

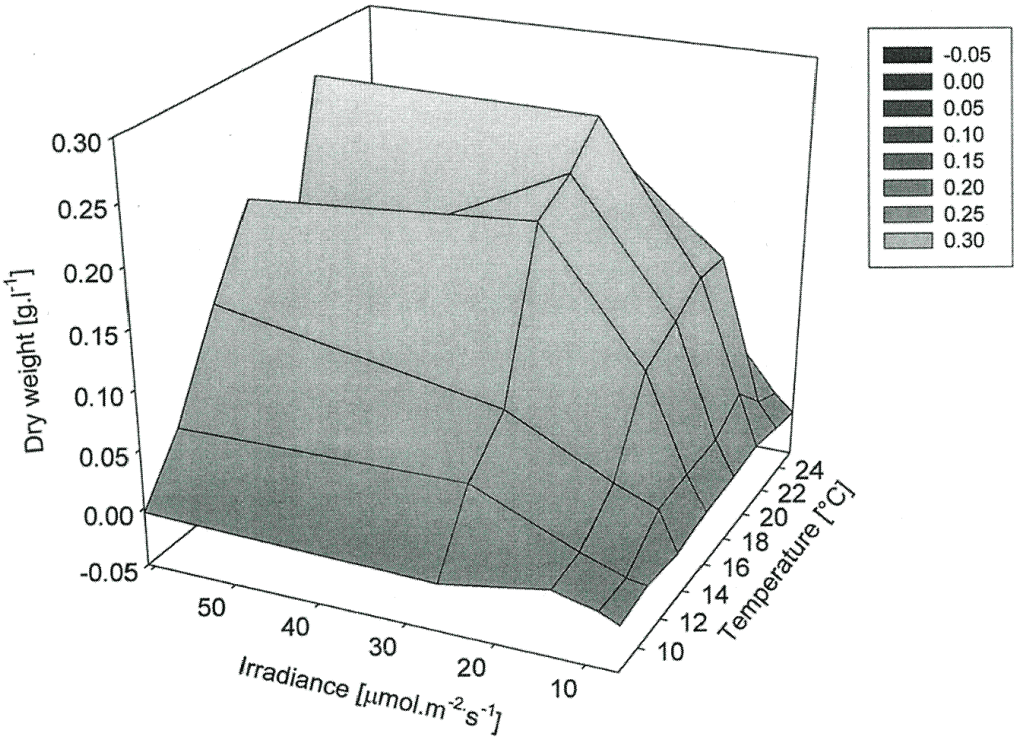

Fig. 5. Growth optima for *Phaeodactylum tricornutum.* This chart shows the increase of dry weight in the first week of cultivation in the first experiment.

ues were given by setting of the unit, the optimal temperature was estimated from our results. These curves are shown in Fig. 4.

The content of dry weight after one week of cultivation is shown in Fig. 5. The content was highest at approximately 19 °C and irradiances between 20 and 120 $\mu mol.m^{-2}.s^{-1}$ (the data from the second experiment are not shown) and was lower in marginal conditions. The irregularities are caused by errors during measurements.

We calculated the growth rates after four weeks of cultivation. Optimal conditions for growth are visible from Fig. 6 and are similar to the conditions found in dry weight measurements. The growth rates are maximal at temperatures between 15 and 23 °C. The optimal irradiance is between 15 and 125 $\mu mol.m^{-2}.s^{-1}$. The differences in growth rates were not significant. This is important for bioassay because the cultivation conditions have not to be so strictly controlled.

Our results prove reliability of determination of growth optima by the cultivation in the crossed gradients of temperature and light. In ISO 10253 (1995), the recommended temperature is 20 °C and recommended irradiance 60 to 120 $\mu mol.m^{-2}.s^{-1}$. These values of optimal temperature and irradiance are also consistent with SUDO et al. (1991) where the optimal temperature is 20 °C and optimum irradiance exceeds 44 $\mu mol.m^{-2}.s^{-1}$. According to KUDO et al. (2000), the optimal growth temperature is 20 °C. YONGANITCHAI & WARD (1991) found

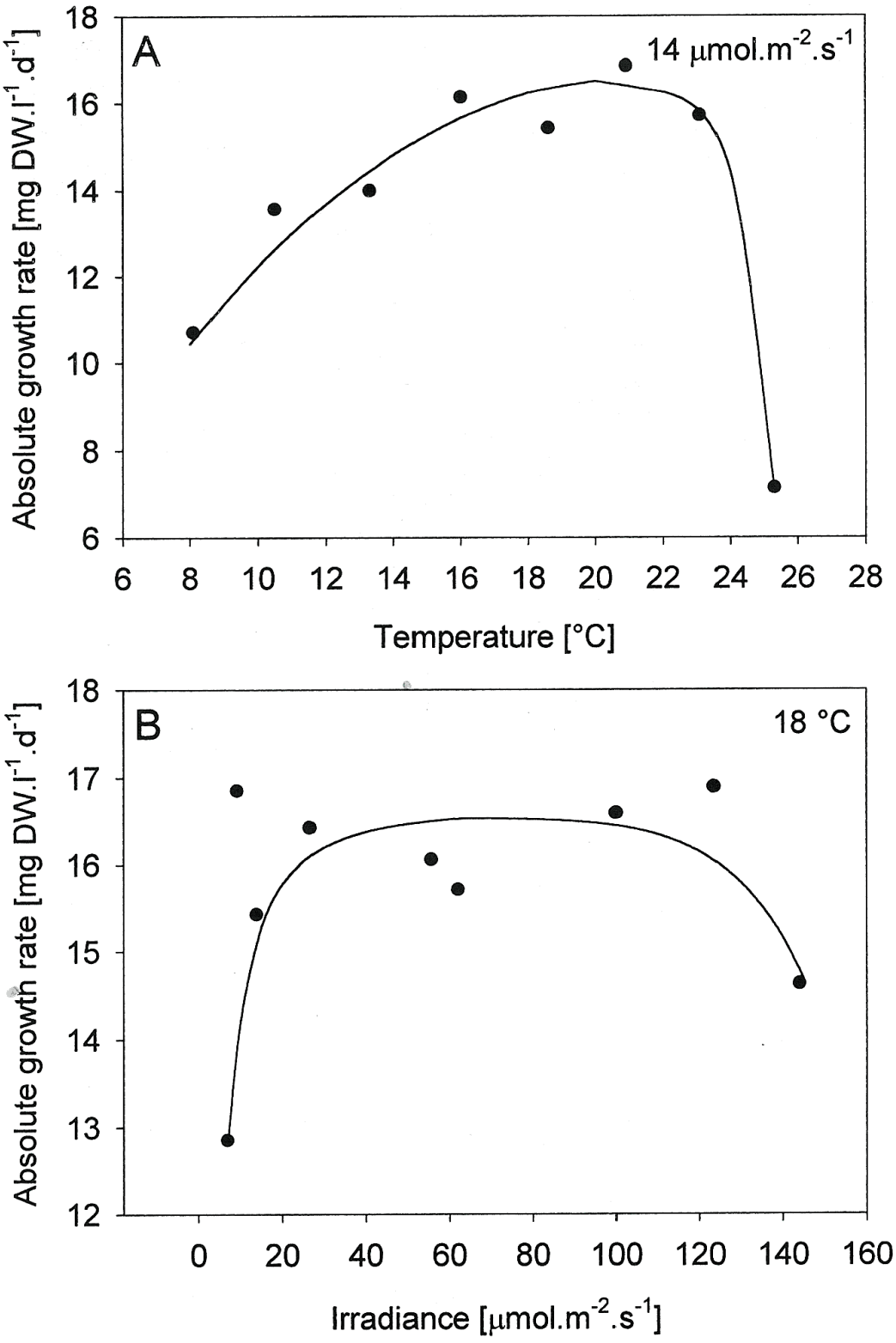

Fig. 6. Dependences of absolute growth rates (**A**) on temperature are irradiance of 14 µmol.m^{-2}.s^{-1} and (**B**) on irradiance at temperature of 18 °C, both after 4 weeks of cultivation.

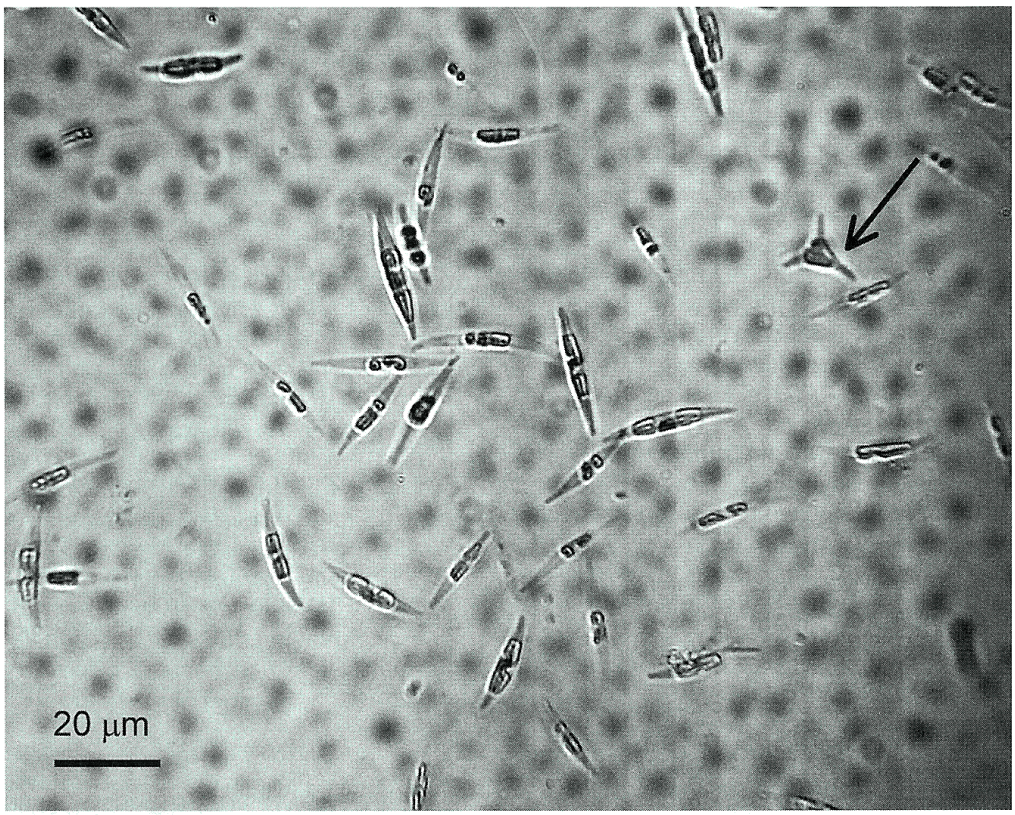

Fig. 7. Microphotography of *Phaeodactylum tricornutum*. This sample was cultivated at 18.8 °C and 6.5 µmol.m^{-2}.s^{-1}. The arrow shows the triradiate form.

the temperature optimum in range 21.05 to 23 °C. MIRON et al. (2000) recommend the optimal irradiance of 500 µmol.m^{-2}.s^{-1} in their bubble column and airlift photobioreactor.

Cell morphology

The photographs were taken at the end of the cultivation. The samples came from the same conditions from which the growth curves were obtained. Fig. 7 shows an example of photograph of *Phaeodactylum tricornutum.* No differences in morphology were observed, the fusiform cells prevailed. The triradiate forms were very rare (ca. 2%) and were observed only at 19 °C and 6.5 µmol.m^{-2}.s^{-1}.

This could be caused by the method of cultivation, not by conditions (light intensity, temperature). Three morphological forms of *Phaeodactylum tricornutum* are distinguished and the form depends on ecological environment of alga (see above). The fusiform cells are found in planktonic habitats (ROUND et al. 1996). Because of rapid changes in this environment, in comparison with another – littoral and benthic one, it is possible that planktonic forms are able to live in large range of studied condition.

Table 1. The conversion equations to dry weight [g . l^{-1}] for all measurements of OD_{750} of three cultures of *Phaeodactylum tricornutum*. r – coefficient of correlation

Basic conversion equation DW [g . l^{-1}] = $\frac{OD_{750} - a}{b}$

Culture	Parameters		r
	a	*b*	
Old culture, fluorescent tubes			
Immunological plates, 0.25 ml well, Uniskan	–0.005	1.293	0.999
Culture wells, 2.5 ml well	0.009	2.137	0.997
Culture wells, 3 ml well	–0.005	2.434	0.997
Petri dish, 8 cm diameter	0.016	0.763	0.998
Cultivation flask, 20 ml volume	0.059	2.959	0.995
Old culture, halogene bulbs			
Immunological plates,0.25 ml well, Uniskan	–0.057	1.714	0.934
Immunological plates, 0.25 ml, plate reader iEMS	0.057	1.531	0.999
Culture wells, 3 ml well	0.008	4.752	0.995
Petri dish, 9 cm diameter	–0.017	1.047	0.997
Cultivation flask, 20 ml volume	0.024	4.767	0.989
Specol, 1 cm cuvette	0.034	3.878	0.996
Specol, 5 cm cuvette	0.046	15.90	0.991
Young culture			
Immunological plates, 0.25 well, Uniskan	0.018	1.375	0.976
Culture wells, 3 ml	0.038	2.733	0.999
Petri dish, 9 cm diameter	0.001	0.599	0.997
Cultivation flask, 20 ml volume	0.024	3.058	0.998
Specol, 1 cm cuvette	0.017	2.345	0.999
Specol, 5 cm cuvette	0.046	8.320	0.995

Conversion curves

The conversion curves were calculated for each culture for samples in immunological plates of well volume of 0.25 ml, cultivation wells of a volume of 3 ml, cultivation flasks of a volume 20 ml, Petri dishes of a diameter of 9 or 8 cm and cuvettes of lengths of 1 cm or 5 cm. The basic equations are linear according to following form

$$\text{DW [g . l}^{-1}\text{]} = \frac{OD_{750} - a}{b} \quad (1)$$

respectively

$$\text{N [10}^9\text{ cells . l}^{-1}\text{]} = \frac{OD_{750} - a}{b} \quad (2)$$

with parameters *a* and *b* which are characteristic for each culture. The parameters and r-values for each curve are summarised in the Tables 1 and 2. The curves are shown in Figs 8 and 9. The parameters are different not only for young and old cultures but also for both old cultures, especially the dependence of OD_{750} on dry weight for the old culture grown under the incandescent lamps and the dependence of OD_{750} on the number of cells for the old culture grown under the fluo-

Table 2: The conversion equations to number of cells [10^9 cells . l^{-1}] for all measurements of OD_{750} of three cultures of *Phaeodacytlum tricornutum*. r – coefficient of correlation

Basic conversion equation N [10^9 cells . l^{-1}] = $\frac{OD_{750} - a}{b}$

Culture	Parameters		r
	a	*b*	
Old culture, fluorescent tubes			
Immunological plates 0.25 ml well, Uniskan	–0.005	0.081	0.999
Culture wells, 2.5 ml well	0.009	0.134	0.997
Culture wells, 3 ml well	–0.005	0.152	0.997
Petri dish, 8 cm diameter	0.016	0.048	0.998
Cultivation flask, 20 ml volume	0.059	0.185	0.995
Old culture, halogene bulbs			
Immunological plates, 0.25 ml well, Uniskan	–0.057	0.04	0.934
Immunological plates, 0.25 ml, plate reader iEMS	0.056	0.035	0.999
Culture wells, 3 ml well	0.008	0.11	0.995
Petri dish, 9 cm diameter	–0.017	0.024	0.997
Cultivation flask, 20 ml volume	0.024	0.11	0.989
Specol, 1 cm cuvette	0.034	0.09	0.996
Specol, 5 cm cuvette	0.045	0.344	0.991
Young culture			
Immunological plates, 0.25 well, Uniskan	0.018	0.049	0.976
Culture wells, 3 ml	0.037	0.098	0.999
Petri dish, 9 cm diameter	0.001	0.022	0.997
Cultivation flask, 20 ml volume	0.024	0.110	0.998
Specol, 1 cm cuvette	0.017	0.084	0.999
Specol, 5 cm cuvette	0.045	0.298	0.995

rescent tubes. Some of the curves are almost identical, e.g. the dependence of OD_{750} on dry weight for the old culture grown in the incandescent lamps and the young one. Other curves calculated in our laboratory have similar parameters (unpublished data). The differences can be caused by different cultivation conditions, but also by variability in dry weight determinations, cell counting and during measurements. We recommend calculating new conversion equations for every experiment, but consistent curves could be used.

Conclusions

The new unit for crossed gradients of temperature and light proved to be reliable and precise enough for testing growth characteristics of algae because our results are consistent with other publications. Growth optima for *Phaeodactylum tricornutum* BOHLIN strain CAUGHT are between 16 and 22 °C for temperature and 25–125 $\mu mol . m^{-2} . s^{-1}$ for irradiance. The peaks are broad enough, so the required cultivation conditions after ISO 10253 (1995) have not to be so strictly controlled, differences from the required temperature, about 3 °C, do not significantly affect growth rate.

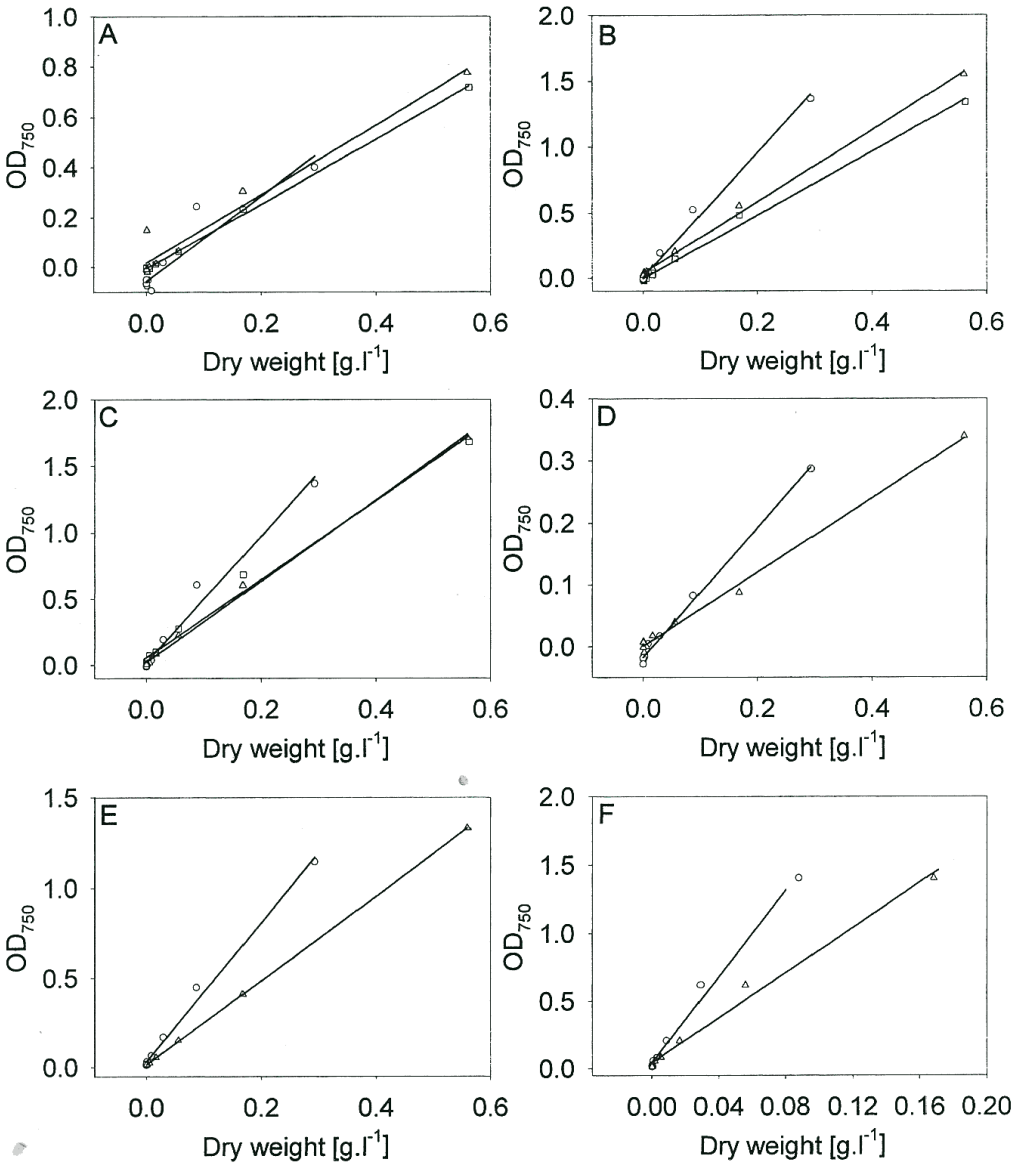

Fig. 8. Conversion curves from OD_{750} to dry weight in $g.1^{-1}$ for the old culture cultivated under fluorescent tubes (❑), old culture cultivated under incandescent bulbs (○) and young culture cultivated in laboratory in daylight (Δ) of *Phaeodactylum tricornutum.* The optical density at 750 nm (OD_{750}) was measured in immunological plates of 0.25 ml wells (**A**), culture wells of 3 ml wells (**B**), flat cultivation flasks of a volume of 20 ml (**C**) and Petri dishes of a diameter of 9 cm (**D**) by Uniskan II and in cuvettes of length of 1 cm (**E**) and 5 cm (**F**) by Specol 11.

We did not observe any changes in the morphology of the alga in given combinations of temperature and light. The fusiform cells prevailed, the triradiate cells were rare and occurred only at 18.6 °C and 6.1 $\mu mol.m^{-2}.s^{-1}$.

Some conversion curves were different for each culture. On the other hand, some curves are very similar and could be used. The differences were probably caused by variability in dry weight determination and cell counting.

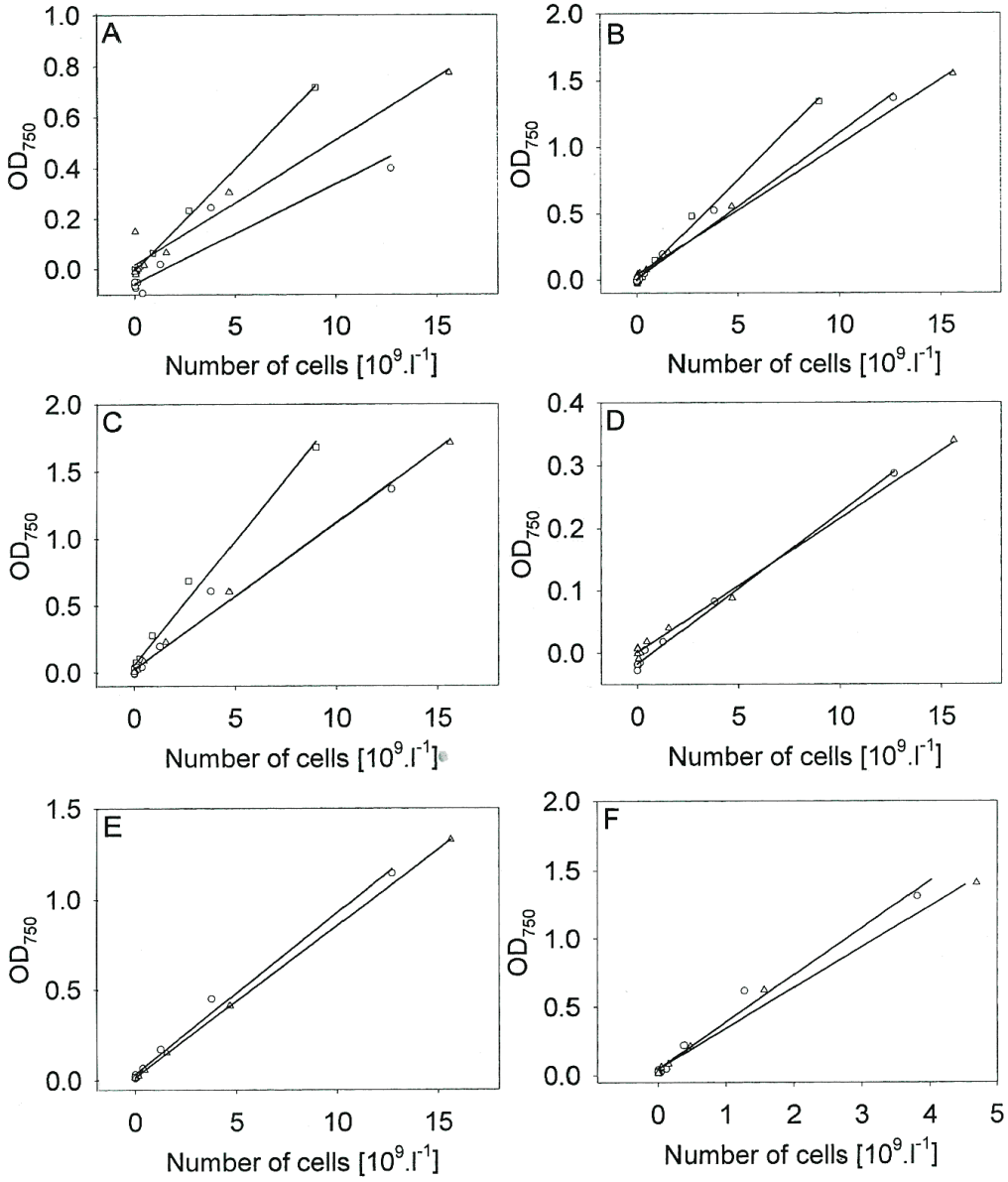

Fig. 9. Conversion curves from OD_{750} to number of cells in 10^9 cells per 1 for the old culture cultivated under fluorescent tubes (❑), old culture cultivated under incandescent bulbs (○) and young cultures cultivated in laboratory in daylight (Δ) of *Phaeodacytlum tricornutum*. The optical density at 750 nm (OD_{750}) was measured in immunological plates of 0,25 ml wells (**A**), culture wells of 3 ml wells (**B**), flat cultivation flasks of a volume of 20 ml (**C**) and Petri dishes of a diameter of 9 cm (**D**) by Uniskan II and in cuvettes of length of 1 cm (**E**) and 5 cm (**F**) by Specol 11.

Acknowledgements

The project has been supported by the ME419, MŠMT, and K 60 051 14 of Acad. Sci. Czech Republic. We thank Dr. Š. KUBÍN for assistance in light measurements.

References

CONTRERAS, A.; GARCIA, F.; MOLINA, E. & MERCHUK, J. C. (1998): Interaction Between CO_2 Mass Transfer, Light Availability, and Hydrodynamic Stress in the Growth of *Phaeodactylum tricornutum* in a Concentric Tube Airlift Photobioreactor. – Bioechnol. Bioengen. **60**: 317–325.

CORDEIRO-MARINO, M.; DE PAVIA AZEVEDO, M. T. P.; SANT' ANNA, C. L.; YAMAGUISHI, N. T. & PLASTINO, E. M. (eds.) (1992): Algae and environment: A general approach, p.65–82. – Socidade Brasileria de Ficologia, Sao Pãulo.

GRIMA, E. M.; PEREZ, J. A. S.; CAMACHO, F. G.; SEVILLA, J. M. F. & FERNANDEZ, F. G. A. (1996): Productivity Analysis of Outdoor Chemostat Culture in Tubular Airlift Photobioreactors – J. appl. Phycol. **18**: 369–380.

HALLDAL P. & FRENCH C. S. (1958): Algal growth in the crossed gradients of light and temperature. – Physiol. Plant. **11**: 401–420.

ISO 10253 (1995): Water quality – Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum.* – 8 pp.

KUDO, I.; MIYAMOTO, M.; NOIRI, Y. & MAITA, Y. (2000): Combined effect of temperature and iron on the growth and physiology of the marine diatom, *Phaeodactylum tricornutum* (Bacillariophyceae). – J. Phycol. **36**: 1096–1102.

KVÍDEROVÁ, J. & LUKAVSKÝ, J. (2001): A new unit for crossed gradients of temperature and light. – In: ELSTER, J.; SECKBACH, J.; VINCENT, W. F. & LHOTSKÝ, O. (eds): Algae and extreme environments. – Nova Hedwigia, Beih., **123**: 539–548.

LUKAVSKÝ, J. (1982): Cultivation of chlorococcal algae in crossed gradients of temperature and light. – Arch. Hydrobiol./Algological studies **29**: 517–528.

MIRON, A. S.; CAMACHO, F. G.; A. C.; GRIMA, E. M. & CHRISTI, Y. (2000): Bubble column and airlift photobioreactors for algal culture. – AICHE Journal **46**: 1872–1887.

PUISEUX-DAO (1989): Phytoplancton model in ecotoxicology. – In: BOUDOU & RIBEYRE (eds.): Aquatic ecotoxicology: Fundamental concepts and methodologies. Vol II., p. 163–186.

ROUND, E. F.; CRAWFORD, R. M. & MANN, D. G. (1996): The diatoms: Biology and morphology of the genera, p. 747. – Cambridge University Press.

SUDO, Y.; OKAVARA, H. & OSHIMA, E. (1991): Growth rate of *Phaeodactylum tricornutum.* – Kugaku Kogaku Ronbunshu **17**: 484–490.

YONGMANITCHAI, W. & WARD, O. P. (1991): Growth and omega-3-fatty acid production by *Phaeodactylum tricornutum* under different culture conditions. – Appl. Environm. Microbiol. **57**:419–425.

YONGMANITCHAI, W. & WARD, O. P. (1992): Growth characteristics and continuous culture of *Phaeodactylum tricornutum.* – J. Amer. Oil Chem. Soc. **69**: 542–548.

Manuscript received January 8, 2002, accepted June 10, 2002.

Abbreviations and units: OD_{750} = optical density at 750 nm. PhAR= photosynthetically active radiation, ca 400–700 nm. 1 $W.m^{-2}$ = 227 lx = 4,6 $\mu mol.m^{-2}.s^{-1}$

The authors' addresses:

JANA KVÍDEROVÁ,
JAROMÍR LUKAVSKÝ,
Academy of Sciences of the Czech Republic,
Institute of Botany, Dep. Plant Ecology,
Dukelská 135,
CZ-379 82 Třeboň, Czech Republic.
E-mail: kviderova@butbn.cas.cz
E-mail: lukavsky@butbn.cas.cz

KVÍDEROVÁ J. (submitted): The ecological characteristics of *Stichococcus* (Chlorophyta) strains isolated from polar and temperate regions. Algological Studies.

The comparison of ecological characteristics of *Stichococcus* (Chlorophyta) strains isolated from polar and temperate regions

Jana Kvíderová[1,2] and Jaromír Lukavský[1,2]

[1]Institue of Botany, Academy of Sciences of the Czech Republic
and

[2]Faculty of Biological Sciences, University of South Bohemia

Abstract

Ecological characteristics, i.e. growth optima, upper and lower growth limits, of six *Stichococcus* strains were evaluated. Three strains originated from Svalbard, the Arctic, the next three ones from temperate regions. The ecological characteristics were evaluated by cultivation in the unit with crossed gradients of temperature (5 to 28 °C) and light (170 to 1150 μmol.m^{-2}.s^{-1}). We studied growth in ten temperatures and five irradiances so 50 different combinations were obtained and compared. The strains originating from "cold" localities had significantly lower temperature limit and optimum of growth, but no statistically significant difference was found in the upper growth limits. The differences in irradiance optima were not observed. The correlations between ecological characteristics and locality microenvironments were significant for low and optimum temperature only.

Key words: Chlorophyta, *Stichococcus*, Polar Region, temperate, crossed gradients, light, temperature, growth rate, optimum, limit.

Introduction

The growth of algae is limited by many environmental factors but temperature and light belong to the most important ones (RAI et GAUR 2001). Every strain has adapted to the microenvironment in the original location, so the optimum growth temperature and light should be close to the original ones, and strains originating from different localities should have individual ecological requirements. However, the microclimate of the original locality often differs from prevailing conditions and the ecological demands of the strain cannot be the same as the prevailing conditions (e.g. NIENOW et al. 1988a,b).

The studies comparing ecological requirements for temperature and light of algae isolated from various geographic localities are rare (e.g. WIENCKE et al. 1993, PAKKER et al. 1996), and the temperature and light requirements of polar and temperate freshwater or soil algae have not been compared yet, although the dependencies of growth of individual algal species on temperature and light were described (e.g. SEABURG et al. 1981, TANG et al. 1997, TANG et al. 1999, NADEAU et CASTENHOLZ 2000). The temperature range is narrow (ca 10 °C) in stable conditions, e.g. in a marine diatom *Nitzschia seriata* (syn. *Pseudonitzschia* seriata, SMITH et al 1994, STAPLEFORD et SMITH 1996) but can reach even 20 °C in localities characteristic by a large temperature variation, e.g. in polar soils (ELSTER et al. 1999). Generally, a polar strain should have the temperature optima lower then a temperate one, even if the algae are subjected to low temperatures in both regions.

According to their reactions to temperature, generally three types of organisms are distinguished. The psychrophils grow at temperatures near 0 °C, their growth optimum is lower then 15 °C and they die at temperatures above 20 °C (WALSH et SECKBACH 1999). On contrary, the psychrotrophs also survive at low temperatures, but their growth optimum is higher then 15 °C and their upper growth limit can exceed even 40 °C. The mesophils do not survive at low temperatures and growth optima and the upper growth limits are similar to the psychrotrophs (ELSTER 1999). If we want to sort the polar and temperate micro-organisms into one of these three categories, it is necessary to know their lower and upper growth limits and growth optima, i.e. their ecological characteristics.

Light, the only source of energy for algae, is another limiting factor. Although the Polar Regions receive lower annual average irradiance the summarised summer daily input averages are comparable with temperate and even tropic regions (FOGG 1998). The irradiance optima depend on the locality, deep-sea macroalgae or bottom cyanobacterial mats, adapted to low irradiances at the bottom will have lower optima then algae or mats on the surface, adapted to high light up to 1500 $\mu mol.m^{-2}.s^{-1}$ (HAWES et SCHWARZ 1999, NIENOW et al. 1988a).

In this study, we wanted to evaluate and to compare the lower limit, optimum and upper limit of growth in gradients of temperature and light of temperate and polar strains, and to determine if the selected polar strains are psychrophilic or psychrotrophic. Further, we want to confirm how the ecological requirements correlate with the geographic position and the microclimate of the original locality.

For our experiments, we decided to use polar and temperate strains of the cosmopolite genus *Stichococcus* (Fig. 1). This genus belongs to primitive trichal green algae but its further

taxonomic position is not clear (VAN DEN HOEK et al. 1995, LEE 1995) because molecular taxonomy studies of genus *Stichococcus* are rare. Recent results propose that *Stichococcus* belongs to Trebouxiophyceae (KATANA et al. 2001). According to WDCM (2003), more 100 strains have been isolated from a wide spectrum of localities, from tropical strains isolated in Florida to strains of polar soils in Arctica and Antarctica. Their habitats include periphyton, plankton, and soil localities, so this genus is supposed to have a large ecological and physiological plasticity and could be suitable for study of mechanisms of adaptation-acclimatisation.

For ecological characteristics evaluation, the unit for crossed gradients of temperature and light allows simple and effective testing of growth constants. The unit was first described by HALLDAL et FRENCH (1958) and has been improved several times (e.g. YARISH 1976, LUKAVSKÝ 1982, ALBERTANO et al. 1993). In our laboratory, the unit was modified for studies in extreme conditions across gradients of temperature and light (KVÍDEROVÁ et LUKAVSKÝ 2001) and has been used for ecological characteristics evaluations of polar cyanobacteria and periphyton in winter temperate river (ELSTER, p.c.).

Material and methods

Stichococcus strains were obtained from the Culture Collections of Algal Laboratory (CCALA), Třeboň, Czech Republic. The strains and localities and their environmental conditions where they were isolated from are summarised in Table 1. The strains were precultivated in Z medium according to ZENDER in STAUB (1961) at 20 °C and 400 $\mu mol.m^{-2}.s^{-1}$. The medium was used in all experiments. The localities were divided into polar and temperate ones by their geographic position and into warm and cold by their microenvironment temperatures (Table 2).

The ecological characteristics were studied in unit for crossed gradients of temperature and light (KVÍDEROVÁ et LUKAVSKÝ 2001). The temperature ranged 5.4 to 28.4 °C; linearity of the resulting temperature gradient was characterised by $r^2 = 0.96$ and was divided into ten zones. The irradiance ranged 170 to 1150 $\mu mol.m^{-2}.s^{-1}$ (= 40 to 250 $W.m^{-2}$) from Osram Dulux L 55W/12-950, Italy; the irradiance gradient was fitted by quadratic curve ($r^2 = 0.99$) and five values were used in further evaluations. The algae were cultivated in 25 (5x5 arranged) polystyrene microplates (FB, 96 wells) in suspension of volume of 0.2 ml and the initial cell density was set up to 10^5 cells/ml. During the cultivation, the CO_2 was supplied

twice per day to concentration ca 2 % v/v. The experiment lasted 14 days and after the cultivation, the microplates were scanned.

The absorbance (light scattering) at 750 nm, $A_{750,}$ was measured daily by iEMS Plate Reader, LabSystems, Ltd., Finland. The measured values were converted to number of cells, *N* (cells.ml^{-1}) and dry weight, *DW* (mg.ml^{-1}) according to the individual conversion curves and equations. The growth rate μ (d^{-1}) was calculated as the slope of linear regression of dependence of *N* on time.

For optima estimations, temperature and irradiance of ten maximal μ were taken and means and standard deviation of both factors were calculated; the optimum range was estimated as a range in which the growth rates are higher then 95 % of the maximum observed μ .

The statistical significance of the differences in growth limits and optima of the strains were evaluated by ANOVA, the differences between polar and temperate strains, and between "cold" and "warm" ones by t-test, The correlations between minimum, average and maximum temperatures and irradiances and optimum and growth limits by correlation analysis. The results were considered significant when $p < 0.05$.

Results

The dependence of μ on temperature and irradiance in the crossed gradients is shown in Fig. 2, the optima, optimum ranges in Fig. 3. The values of optima and both limits are summarised in Table 3. The temperature optima of individual strains differed significantly ($p = 0.002$). No significant differences in temperature dependence of clusters of polar and temperate strains were observed but when the microclimate of the original locality was considered, we found significantly lower temperature limit ($p < 0.01$) and optimum ($p < 0.04$) in "cold" strains. The upper temperature limit was also lower in "cold" strains but the differences were not significant. On the contrary, we did not find significant difference in irradiance requirements between polar and temperate strains and even in "cold" and "warm" ones.

We found positive correlations between the optima and growth limits and lower, average and upper temperatures and significant positive correlations between upper and lower growth limit and minimum irradiance (Table 4). The other correlations between growth limits or optima and minimum, average and maximum irradiances are also positive, but not statistically significant.

Discussion

In general, the ecological characteristics of algae reflect adaptations to environmental conditions in their original locality. On contrary, these ecological characteristics could be influenced by a long-term adaptation to culture collection conditions, i.e. 15 °C and 45 $\mu mol.m^{-2}.s^{-1}$, and to pre-cultivation (20 °C, 400 $\mu mol.m^{-2}.s^{-1}$). This acclimation, i.e. adaptation to culture collection conditions (ELSTER 1999), does not occur in all strains. The experimental strains kept their ecological characteristics almost unchanged for more then one year (KVÍDEROVÁ, unpublished data) and the growth characteristics are consistent with other observations with newly isolated strains (e.g. STIBAL 2003). Moreover, the strong positive statistically significant correlations between growth characteristics and temperatures indicate that the strains kept their growth characteristics adapted to the original conditions. However, the acclimation process cannot be entirely excluded due to long-term keeping in the culture collection conditions (PRÁT 1970).

The temperate strains have their temperature optima higher than polar soil ones, with exception of the *S. exiguus* KOMÁREK 1962/1. The temperature optimum lies between 20 and 25 °C reflecting the water temperature in original location (HAFNERSEE 2003). However in the Lake Ammersee (Germany), the maximum cell density of the autotrophic picoplankton that included *Stichococcus minutissimus* appeared in at temperature range 9 to 20 °C (CHANG 1998). The lower growth temperatures can be caused by lower water temperatures in the Lake Ammersee ranging 3.2 to 21 °C during the year (WORLD LAKE DATABASE 2003) then in Haffnersee (HAFNERSEE 2003). The species also could be out-competed by other algae or cyanobacteria that grow faster in higher temperatures. In this case, the conditions of maximum abundance in the lake do not correspond to optimum growth conditions estimated from the laboratory experiments.

The temperate strain *S. exiguus* KOMÁREK 1962/1 was isolated from the snow detritus in the High Tatra Mountains, so the adaptation to lower temperatures should be expected. The similar temperature ranges of snow algae were found by other authors, e.g. HOHAM (1975) recorded the growth temperature optima and growth temperature ranges of snow algae, for growth of *Stichococcus bacillaris* the temperature ranged 0 to 35 °C, but the growth optimum was not further defined. Another *Stichococcus bacillaris* strain, isolated from snow in the Labský Důl, Krkonoše, Czech Republic (Giant Mountains), grows in range 5 to 20 °C, and the optimum temperature is 20 °C (STIBAL 2003).

The growth limits of the experimental polar soil strains, *S. bacillaris* ELSTER 1998/28 and *S. exiguus* ELSTER 1998/31 are similar because originate from the same locality. The growth

limits and optima of both polar soil strains, and the temperate snow *S. exiguus* KOMÁREK 1962/1 correspond to the definition of a psychrotrophic organism, i.e. an organism living in temperatures near 0 °C, with growth optimum temperature above 15 °C, and surviving in temperatures above 20 °C (ELSTER 1999, WALSH et SECKBACH 1999). The polar soil strains have to cope with broad range of temperatures and the maximum ones can exceed even 20 °C in sunny days (ELSTER 1999), so the ability to survive in high temperature and broad temperature ranges should be an advantage in competition with other algae and cyanobacteria. On the other hand, the polar planktic strain *S.* sp. KOVÁČIK 1988/9 is probably mesophilic, i.e. dying in temperatures near 0 °C (ELSTER 1999, WALSH et SECKBACH 1999). The strain is probably adapted to the temperatures in warm Troll springs that are stable and range 9.5 to 25.9 °C in individual springs (BANKS et al. 1998).

The irradiance requirement was not a significant factor for distinguishing polar and temperate strains, or "cold" and "warm" ones respectively. The average values of irradiances are lower in the Polar Regions but are still comparable in all localities. The average daily irradiance reach approximately 1 500 $\mu mol.m^{-2}.s^{-1}$ in the temperate region and approximately 1 000 $\mu mol.m^{-2}.s^{-1}$ in the Polar Regions at the surface (during vegetative season). The actual values at the noon should be higher, up to 2 300 $\mu mol.m^{-2}.s^{-1}$ (e.g. FRIEDMANN 1988, ØRBÆK et al. (1999), BISCHOF et al. 2002). Unfortunately, the present experimental setting of the unit for crossed gradients does not allow to reach irradiances then higher 1 300 $\mu mol.m^{-2}.s^{-1}$ where any differences in upper limits could be observed.

At the marginal temperatures, the observed irradiance optimum decreased in all strains. In the low temperatures, the rate of electron transfer is lower and the photoinhibition occur at lower irradiances. On the other hand at the high temperatures, the temperature-sensitive enzymes of photosynthetic reactions are damaged and the rate of photosynthesis decreases, although the rate of electron transport is faster. In both cases, the decrease in photosynthesis leads to decrease in growth rate (FALKOWSKI & RAVEN 1997).

The correlations between growth characteristics and irradiances were also positive, but not statistically significant with exception of two cases (Tab. 4). There are two reasons: The photosynthetic apparatus of cells must be ready to react to sudden changes in irradiance and these acclimatization-adaptation processes include short-time changes lasting seconds to hours (FALKOWSKI et RAVEN 1997). On the other hand, the irradiance values used in calculation are only approximate estimates of the real ones from other observations (ØRBÆK

et al. 1999, BISCHOF et al. 2002) because it is not possible to obtain actual values from the given locality.

Conclusion

The strains *S. bacillaris* Elster 1998/28, *S. exiguus* Elster 1998/31 and *S. exiguus* Komárek 1962/1 isolated from "cold" environments, i.e. polar soil and snow detritus, are psychrotrophic, i.e. tolerating low temperature.

The ecological characteristics of strains *S. bacillaris* Hindák 1984/15, *S. mirabilis* Pringsheim/Praha Ac. A 146 and *S.* sp. Kováčik 1988/9 correspond to the definition of a mesophilic microorganism. The temperature growth limits and optima reflect the microclimate temperature in their original localities.

The irradiance requirements were not crucial in distinguishing polar and temperate strains, and in "cold"and "warm" ones. The irradiance gradient used in the experiment also did not reached maximum irradiances encountered in both ecosystems, so the difference in reaction to the high light could not be determined.

Acknowledgements

The work was supported from grant Academy of Sciences of the Czech Republic K6005114 , MŠMT 123100004 and Czech – Japan Contract No. 2/2001. We thank Dr. J. Simmer and Mrs. M. Kovářová for statistical analyses proposals and Mrs. M. Kašpárková and Mrs. V. Titlová, CCALA technicians, for preparation of the cultures.

References

ALBERTANO, P., KOVÁČIK, L., et GARDAVSKÝ, A. (1994): Cross-gradient cultures of filamentous cyanophytes. – Giorn. Bot. Ital. 127: 855 – 856.

BANKS, D., SLETTEN, R.S., HALDORSEN, S., DALE, B., HEIM, M., et SWENSEN, B. (1998): The thermal springs of Böckfjord, Svalbard: Occurrence and major ion hydrochenistry. – Goethermics 27: 445 – 467.

BISCHOF, K., HANELT, D., AGUILERA, J., KARSTEN, U., VÖGELE, B., SAWALL, T. et WIENCKE, C. (2002): Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. – Marine Biology 140: 1097 – 1106.

CCALA (2003): www.butbn.cas.cz/ccala/ccala.html

CHANG, T. (1998): Occurence of picoplantonic algae in Lake Ammersee, Bavaria, Germany. – Biologia 55: 425 – 431.

ELSTER, J. (1999): Algal versatility in various extreme environments. – In: SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht, p. 215 – 227.

ELSTER, J., LUKEŠOVÁ, A., SVOBODA, J., KOPECKÝ, J., et KANDA, H. (1999): Diversity and abundance of soil algae in the polar desert, Sverdrup Pass, central Ellesmere Island. – Polar Records 35: 231 – 254.

FALKOWSKI, P.G., et RAVEN, J.A. (1997): Aquatic photosynthesis. – Blackwell Science, 375 pp.

FOGG, G.E. (1998): Biology of polar habitats. – Oxford University Press, Oxford, New York, Tokio, 274 pp.

HAFNERSEE (2003): www.umwelt.ktn.gv.at/seenbericht2001/hafnersee/mitte.html

HALLDAL P., et FRENCH, C.S. (1958): Algal growth in the crossed gradients of light and temperature. – Physiol. Plant. 11: 401 – 420.

HAWES, I. et SCHWARZ, A. (1999): Photosynthesis in an extreme shade environment: Benthic microbial mats from Lake Hoare, a permanently ice-covered Antarctic lake. – J. Phycol. 35: 448 – 459.

HOHAM, R. W. (1975): Optimum temperatures and temperature ranges for growth of snow algae. – Arctic and Alpine Research 7: 13 – 24.

KATANA, A., KWIATOWSKI, J., SPALIK, K., ZAKRYŚ, B., SZALACHA, E., et SZYMAŃSKA, H. (2001): Phylogenetic position of *Koliella* (Chlorophyta) as inferred from nuclear and chloroplast small subunit rDNA. - J. Phycol. 37: 443 - 451.

KOMÁREK, J., HINDÁK , F., et JAVORNICKÝ, P. (1973): Ecology of green kryophilic algae from Belanské Tatry Mountains (Czechoslovakia). – Arch. Hydrobiol./Suppl. 41 Algological Studies 9: 427 – 449.

KVÍDEROVÁ, J., et LUKAVSKÝ, J. (2001): The unit for crossed gradients of temperature and light.- In: ELSTER, J., SECKBACH, J., VINCENT, W.F., et LHOTSKÝ O. (eds.): Algae and extreme environments: Ecology and physiology. - Nova Hedvigia Beih. 123: 541 - 550.

LEE, R.E. (1995): Phycology. – Cambridge University Press, Cambridge, 2nd edition, pp. 645.

LUKAVSKÝ, J. (1982): Cultivation of chlorococcal algae in crossed gradients of temperature and light. – Arch. Hydrobiol. /Algological studies 29: 517 – 528.

NADEAU, T., CASTENHOLZ, R.W. (2000): Characterization of psychrophilic oscillatorians (Cyanobacteria) from Antarctic meltwater ponds. – J. Phycol. 36: 914 – 923.

NIENOW, J.A., MCKAY, C.P., et FRIEDMANN, E.I. (1988a): The cryptoendolithic microbial environment in the Ross Desert of Antarctica: Mathematical models of the thermal regime. – Microb. Ecol. 16: 253-270.

NIENOW, J.A., MCKAY, C.P., et FRIEDMANN, E.I. (1988b): The cryptoendolithic microbial environment in the Ross Desert of Antarctica: Light in the photosynthetically active region. – Microb. Ecol. 16: 271 – 289.

ØRBÆK, J.B., HISDAL, V., et SVAASAND, L.E. (1999): Radiation climate variability in Svalbard: surface and satellite observation. – Polar Research 18: 127 – 134.

PAKKER, H., BREEMAN, A.M., VAN REINE, W.F.P., VAN OPPEN, M.J.H., VAN DEN HOEK, C. (1996): Temperature response of tropical to warm-temperate Atlantic seaweeds. 1. Absence of ecotypic differentiation in amphi-Atlantic tropical Canary Islands species. – Europ. Journ. of Phycology 31: 123 – 132.

PRÁT, S. (1970): Problémy sbírek kultur [Problems of culture collections]. – Biologické listy 35 (2): 81 – 89.

RAI, L.C., et GAUR, J.P. (eds.) (2001): Algal adaptation to environmental stresses. Physiological, biochemical and molecular mechanisms. – Springer-Verlag Berlin Heilderberg, 421pp.

SEABURG, K.G., PARKER, B.C., WHARTON, JR., R.A., et SIMMONS, Jr., G.M. (1981): Temperature-growth responses of algal isolates from Antarctic oases. – J. Phycol. 17: 353 – 360.

SMITH, R.E.H., STAPLEFORD, L.C., et RIDINGS R.S. (1994): The acclimated response of growth, photosynthesis, composition, and carbon balance to temperature in the psychrophilic ice diatom *Nitzschia seriata*. – J. Phycol. 30: 8 – 16.

STAPLEFORD, L.C., et SMITH, R.E.H. (1996): The interactive effects of temperature and silicon limitation on the psychrophilic ice diatom *Pseudonitzschia seriata*. – Polar Biol. 16: 589 – 594.

STAUB, R. (1961): Ernahrungsphysiologisch - autökolgische Untersuchungen an der planktonische Blaualgae *Oscillatoria rubescens* DC. - Schweiz. Z. Hydrol. 23: 82-198.

STIBAL, M. (2003): Ecological and physiological characteristics of snow algae from Czech and Slovak mountains. - Czech Phycology (Olomouc) 3: 141 - 152.

TANG, E.P.Y., TREMBLAY, R., et VINCENT, W.F. (1997): Cyanobacterial dominance of polar freshwater ecosystems: are high-latitude mat-formers adapted to low temperature? – J. Phycol. 33: 171 – 181.

TANG, E.P.Y., et VINCENT, W.F. (1999): Strategies of thermal adaptation by high-latitude cyanobacteria. – New Phytol. 142: 315 – 323.

VAN DEN HOEK, C., MANN, D.G., et JAHNS, H.M. (1995): Algae: An introduction to phycology. - Cambridge University Press, Cambridge, pp 623.

WALSH, M.M., et SECKBACH, J. (1999): The versatility of microorganisms. – In: SECKBACH, J. (ed.): Enigmatic microorganisms and life in extreme environments, Kluwer Academic Publishers, Dordrecht, p 155 – 162.

WDCM (2003): www.wdcm.nig.ac.jp

WIENCKE, C., RAHMEL, J., KARSTEN, U., WEYKAM, G., et KIRST, G.O. (1993): Photosynthesis of marine macroalgae from Antarctica – light and temperature requirements. – Bot. Acta 106 (1): 78 – 87.

WORLD LAKE DATABASE (2003): www.ilec.or.jp/database/eur/deur23.html

YARISH, C. (1976): Polymorphism of selected marine Chaetophoraceae (Chlorophyta). – Br. Phycol. J. 11: 29 – 38.

Authors' addresses:

JANA KVÍDEROVÁ
JAROMÍR LUKAVSKÝ

Institue of Botany
Academy of Sciences of the Czech Republic
Dukelská 135
CZ - 379 82 Třeboň
Czech Republic,
e-mail: kviderova@butbn.cas.cz
e-mail: lukavsky@butbn.cas.cz

Table 1. *Stichococcus* strains used in experiments and estimated conditions in original localities:

Strain	Locality	Ref	Temperature [°C]			Irradiance[1] [µmol.m^{-2}.s^{-1}]		
			Min	Max	Avg	Min	Max	Avg
Temperate strains								
S. bacillaris HINDÁK 1984/15	Hafnersee Lake , Austria	[2]	5	24	14.5	280	3000	1650
S. exiguus KOMÁREK 1962/1	snow detritus, High Tatra Mts., Slovakia	[3]	0	10	5	3	3000	650
S. mirabilis PRINGSHEIM/Praha Ac. A146	freshwater, Central Europe		5	25	15	280	3000	1650
Polar strauns								
S. bacillaris ELSTER 1998/28	soil, Ny Alesund, Svalbard	[4,6,7]	0	8	4	5	1300	650
S. exiguous ELSTER 1998/31	soil, Ny Alesund, Svalbard	[4,6,7]	0	8	4	5	1300	650
S. sp. KOVÁČIK 1988/9	periphyton, Troll Springs, Svalbard	[5,6,7]	9.5	25.6	17.8	200	1300	750

Ref …number of reference, Min…minimum, Max…maximum, Avg...average, arithmetic mean

[1]Average incoming surface radiation calculated from total daily radiation estimated from solar constant and latitude (50°for temperate regions

[2]HAFNERSEE (2003), [3]KOMÁREK et al. (1973), [4]ELSTER et al. (1999), [5]BANKS et al. (1998), [6]ØRBÆK et al. (1999), [7]BISCHOF et al. (2002)

Table 2. The types of the localities fot the statistical evaluation.

	Type	
	Geographic position	Microclimate[1]
Hafnersee Lake	temperate	warm
High Tatra Mountains	temperate	cold
Ny Alesund	polar	cold
Troll Springs	polar	warm

[1]The microclimates were determined according to average temperatures.

Table 3. Ecological characteristics of individual *Stichococcus* strains evaluated by cultivation.

Strain	Temperature [°C]			Irradiance[1] [μmol.m^{-2}.s^{-1}]		
	Lower growth limit	Optimum	Uppew growth limit	Lower growth limit	Optimum	Upper growth limit
Temperate strains						
S. bacillaris HINDÁK 1984/15	8.2	21.4 ± 2.1	>28.4	< 170	802 ± 266	>1150
S. exiguus KOMÁREK 1962/1	< 5.4	18.6 ± 1.5	26.9	< 170	575 ± 825	>1150
S. mirabilis PRINGSHEIM/Praha Ac. A146	6.4	20.4 ± 3.3	28.4	< 170	660 ± 299	>1150
Polar strauns						
S. bacillaris ELSTER 1998/28	5.4	17.2 ± 2.8	26.9	150	670 ± 408	>1150
S. exiguous ELSTER 1998/31	< 5.4	17.5 ± 2.5	26.9	< 1705	625 ± 362	>1150
S. sp. KOVÁČIK 1988/9	12.0	21.8 ± 2.8	28.4	< 170	708 ± 182	>1150

Table 4. Correlations between ecological characteristics and locality environments. The value is the table indicates the correlation coefficient, the values in parentheses indicates the p-value).

		Locality environment					
		Temperature			Irradiance		
		Min	Avg	Max	Min	Avg	Max
Growth	Lower limit	0.93** (0.007)	0.81* (0.050)	0.74 (0.094)	0.90 (0.014)	0.58 (0.109)	0.98 (0.001)
	Optimum	0.93** (0.008)	0.97** (0.002)	0.96* (0.002)	0.72 (0.109)	0.61 (0.20)	0.80 (0.052)
	Upper limit	0.91* (0.012)	0.99** (<0.001)	0.99** (<0.001)	0.17 (0.753)	0.80 (0.880)	0.45 (0.371)

Min…minimum, Max…maximum, Avg...average, arithmetic mean
The statistically significant correlations of $p < 0.05$ are marked by *, of $p < 0.01$ by **.

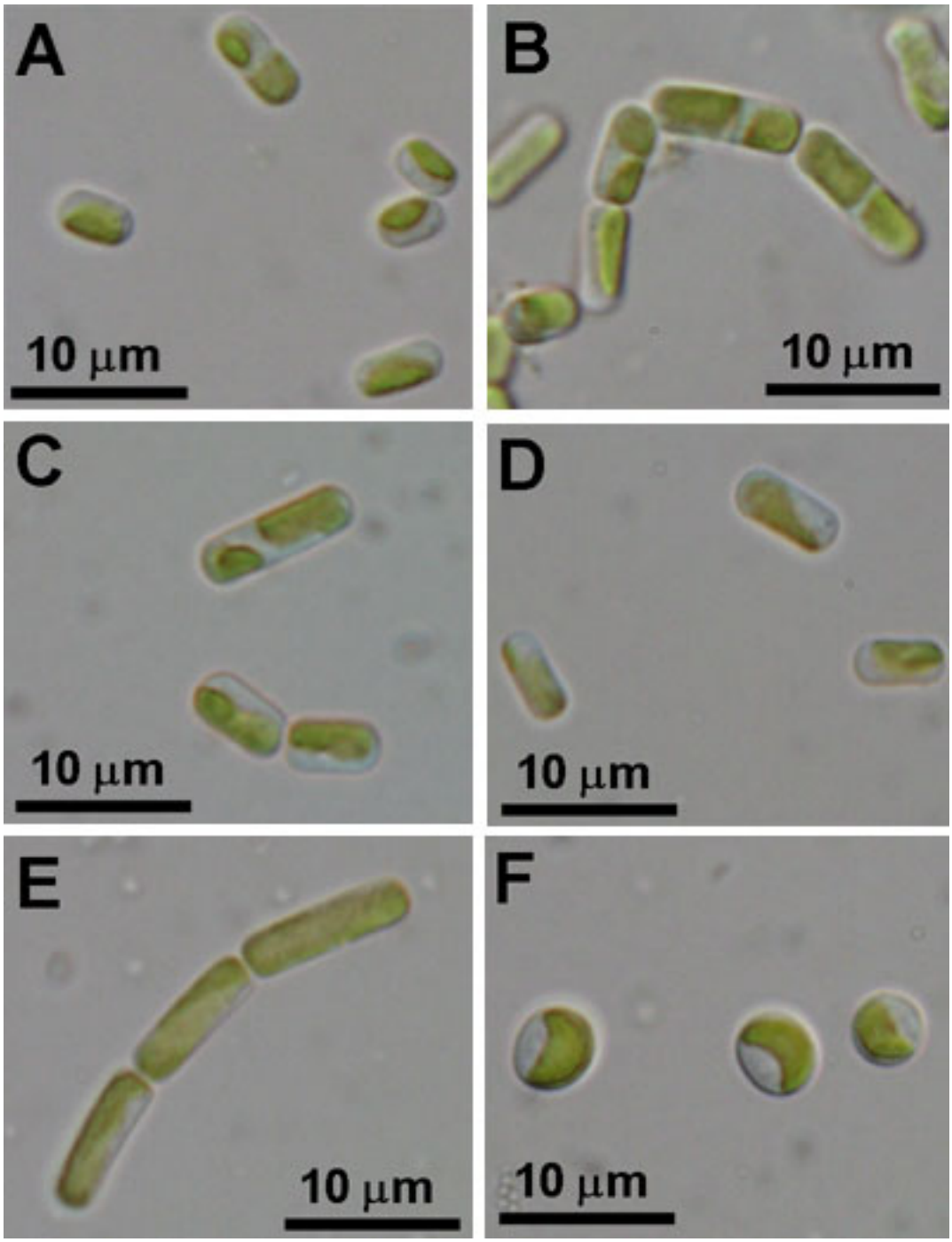

Fig. 1: Experimental *Stichococcus* strains in pre-cultivation conditions: **(A)** *Stichococcus bacillaris* Hindák 1988/15, **(B)** *Stichococcus exiguus* Komárek 1962/1, **(C)** *Stichococcus mirabilis* Pringsheim/Praha Ac. A146, **(D)** *Stichococcus bacillaris* Elster 1998/28/51, **(E)** *Stichococcus exiguus* Elster 1998/31 and **(F)** *Stichococchus* sp. Kováčik 1988/9. The microphotographs were taken by Olympus DC 10 camera, objective magnification 100x, with immersion oil.

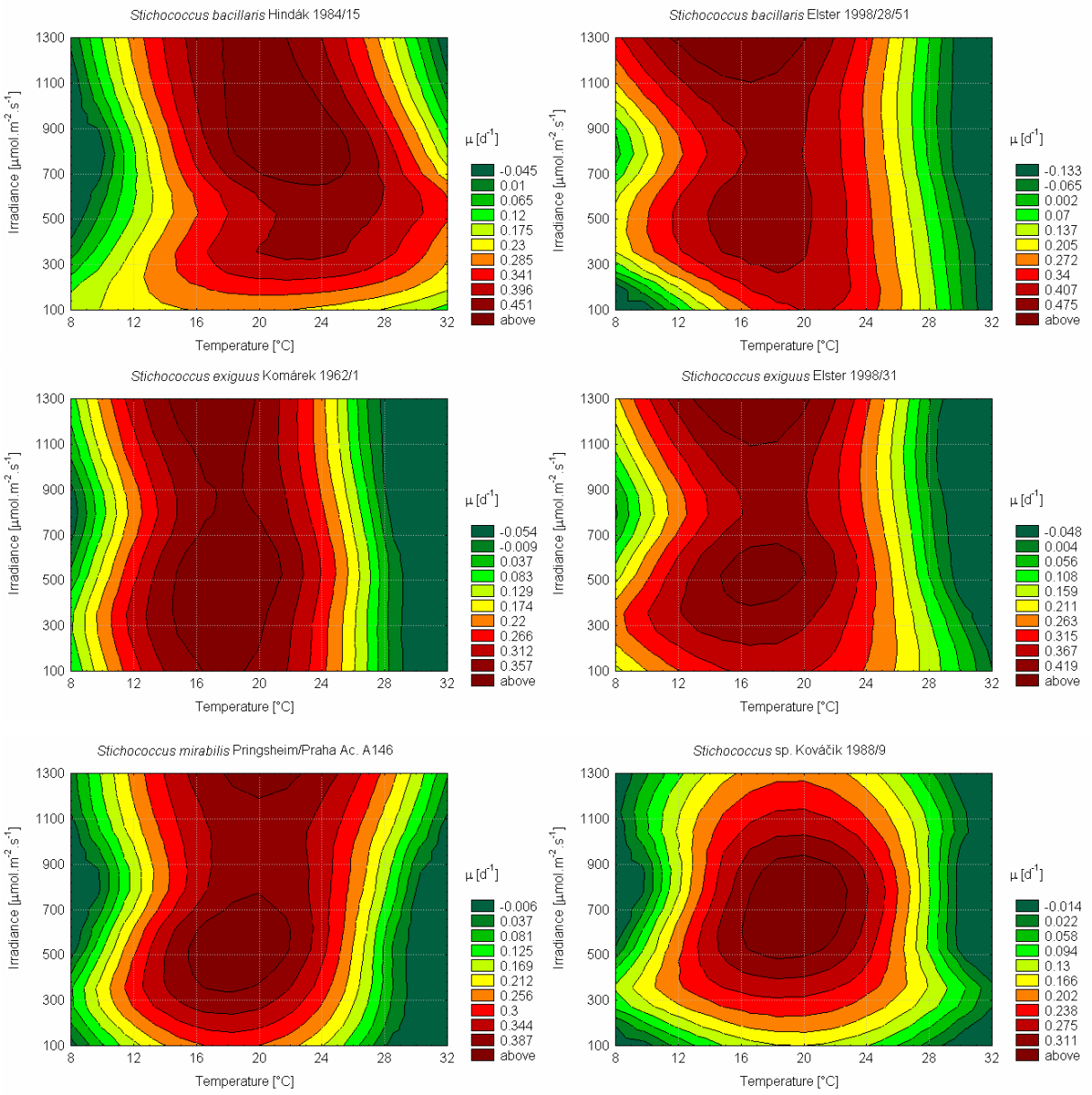

Fig. 2: Growth rates of individual *Stichococcus* strains in crossed gradients of temperature and irradiance. The data were smoothed by last squares method.

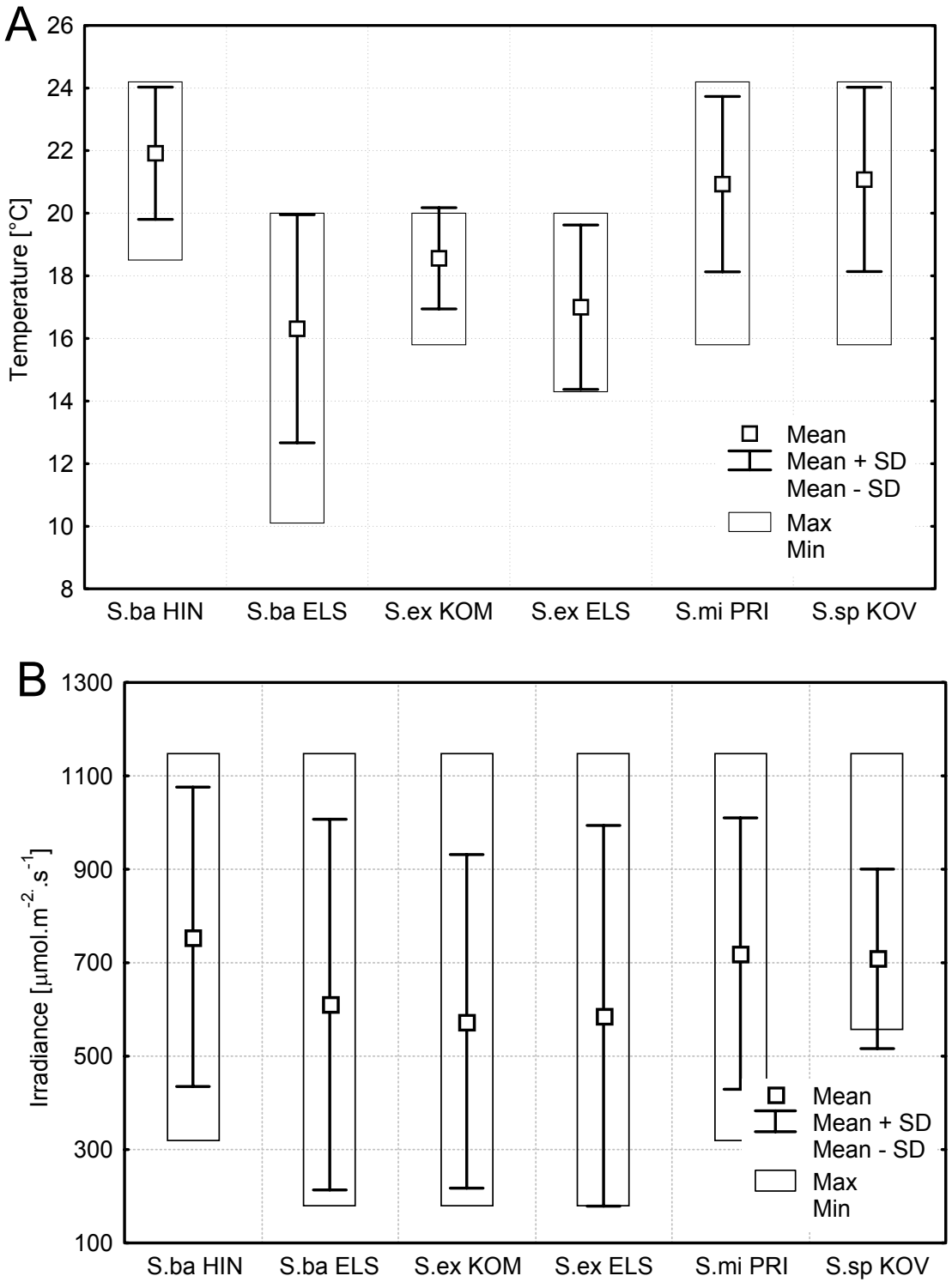

Fig 3. Temperature **(A)** and irradiance **(B)** growth optima calculated from top ten growth rates. **S.ba HIN** = *Stichococcus bacillaris* Hindák 1984/15, **S.ba ELS** = *Stichococcus bacillaris* Elster 1998/28/51, **S.ex KOM** = *Stichococcus exiguus* Komárek 1962/1, **S.ex ELS** = *Stichococcus exiguus* Elster 1998/31, **S.mi PRI** = *Stichococcus mirabilis* Pringsheim/Praha Ac. A146 and **S.sp KOV** = *Stichococcus* sp. Kováčik 1988/9.

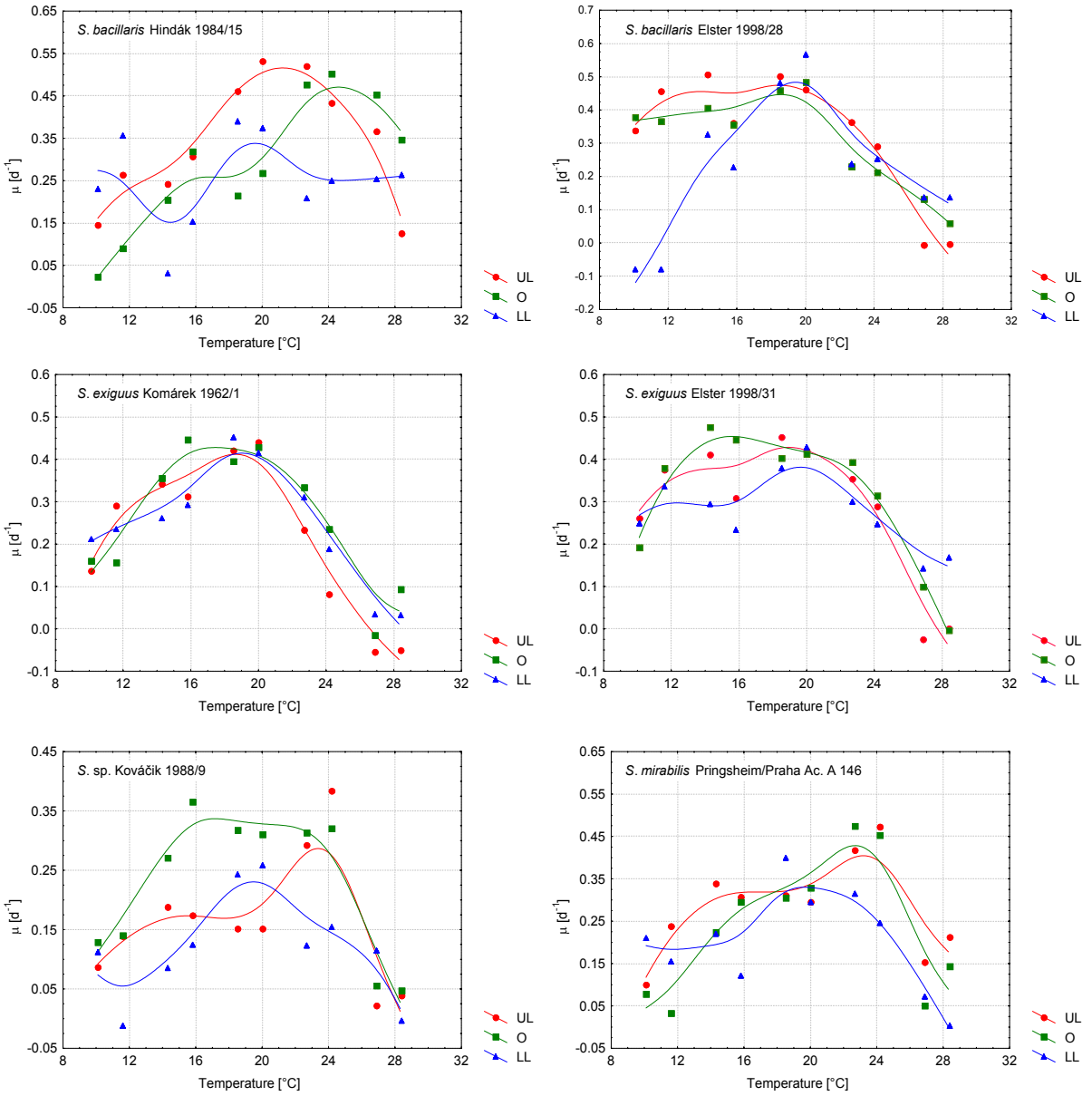

Fig. 4: Temperature optima and growth limits of the experimental *Stichococcus* strains at irradiances corresonding to the upper growth limit **(UL)**, growth optimum **(O)** and lower growth growth limit **(LL)** according to Table 3. The curves were fitted by method of least squares.

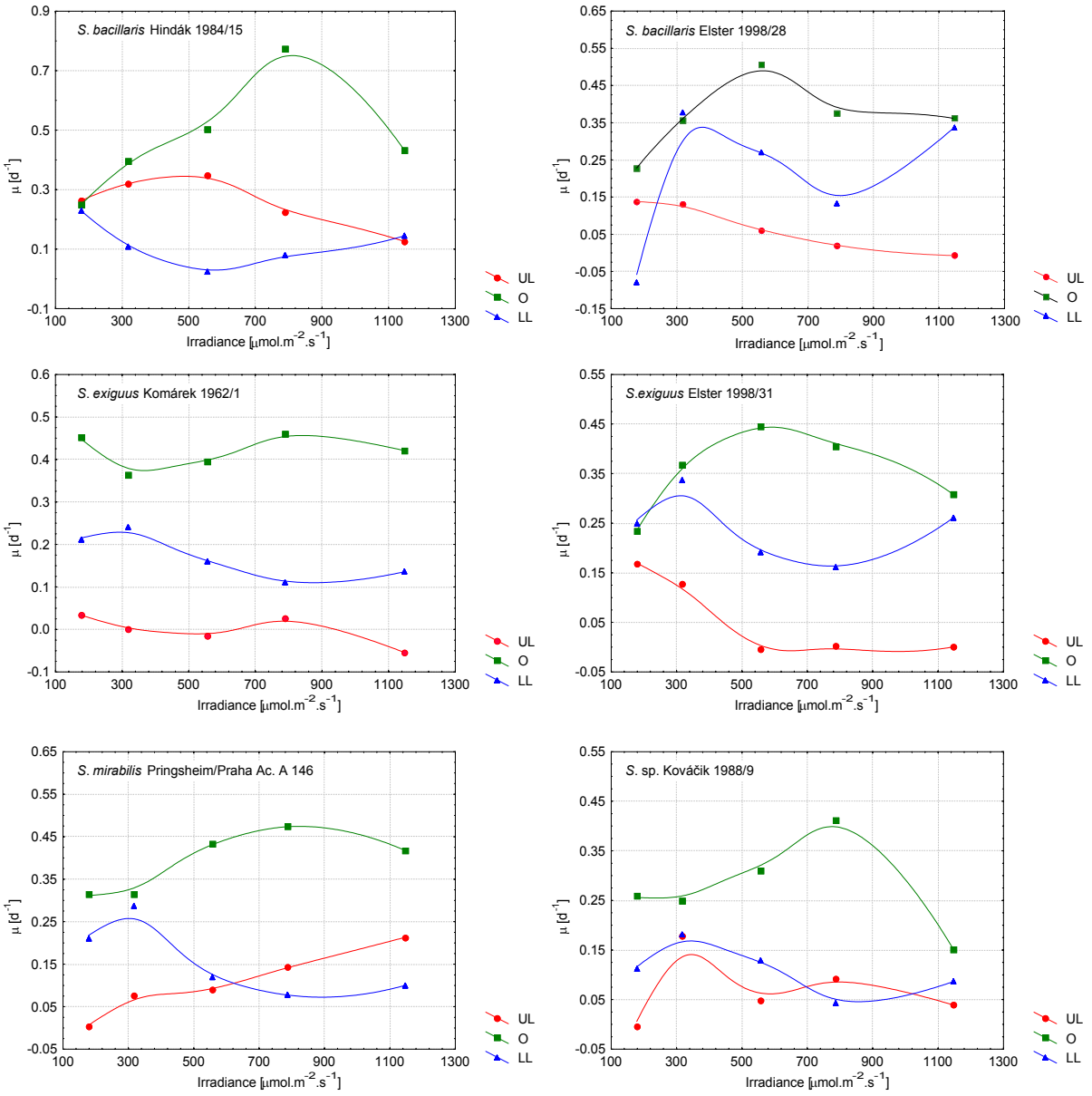

Fig. 5: Irradiance optima and growth limits of the experimental *Stichococcus* strains at temperatures corresonding to the upper growth limit **(UL)**, growth optimum **(O)** and lower growth growth limit **(LL)** according to Table 3. The curves were fitted by method of least squares.

6. List of all publications, conference presentations and lectures

Publications

Elster, J., **Kvíderová, J**., Lukavský , J. (2000): Book reviews: Seckbach, J. ed (1999): Enigmatic microorganisms and life in extreme environments. – Kluver Acad.Press, 687pp. – Arch.Hydrobiol./Algological Studies 100: 199-200.

Kvíderová J., Lukavský J. (2000): Unit for crossed gradients of temperature and light. - Book of abstracts, Algae and extreme environments, September 11 – 16, 2000, Třeboň, p. 92.

Kvíderová, J. (2001): Modelling of gradients of temperature and light -Book of Abstracts, IXth days of Plant Physiology, September 17 – 21, 2001, České Budějovice.

Kvíderová, J., Lukavský, J. (2001): A new unit for crossed gradients of temperature and light. – In: Elster, J., Seckbach, J., Vincent, W.F., Lhotský, O. (eds.): Algae and extreme environments. N.Hedwigia, beih. 123, p.539-548.

Kvíderová, J., Lukavský, J. (2001): Gradienty – základní charakter prostředí a možnosti jejich modelování v laboratoři [Gradients - basic character of the environment and the possibilities of their simulation in the laboratory]. – Czech Phycology 1: 77-85.

Kvíderová, J. (2002): Book reviews: Hemminga, M.A., Duarte, C.M. (2000): Seagrass ecology. –Cambridge University Press, 306pp. – Algological Studies 104: 197 – 198.

Kvíderová J. (2002): Zpracování a archivace dat z biotestu [Computerized processing and archiving of data in a bioassay]. - Czech Phycology 2: 125 – 128.

Kvíderová, J., Lukavský, J. (2003): The cultivation of *Phaeodactylum tricornutum* in crossed gradients of temperature and light. - Algological Studies 110: 67 – 80.

Švadlenková, M., Lukavský, J., **Kvíderová, J.** (2003): Radionuklides ^{137}CS and ^{60}CO uptake By freshwater and marine microalgae *Chlorella, Navicula, Phaeodactylum*. - NATO Adv. Res. Workshop„Equidosimetry for radiecology and ecotoxicology“.

Kvíderová, J. (2004): *Haematococcus pluvialis.* – Třeboňský svět, submitted.

Kvíderová, J. (2004): The ecological characteristics of *Stichococcus* (Chlorophyta) strains isolated from polar and temperate regions. Book of abstracts, COBRA Partner Review Meeting, March 24 – 26, 2004, Třeboň.

Kvíderová, J., Lukavský, J. (submitted): The ecological characteristics of *Stichococcus* (Chlorophyta) strains isolated from polar and temperate regions. – Algological Studies.

Kvíderová, J. (submitted): *Haematococcus pluvialis.* – Třeboňský svět.

Kvíderová, J., Stibal, M., Nedbalová, L., Kaštovská, K. (submitted): The first record of the P-E curve of the snow algae *in situ*. – Czech Phycology.

Henley, W.J**., Kvíderová, J.** (in prep.): The effect of streptomycin on growth and photosynthesthis of two chlorophycean algae.

Kvíderová J. (in prep.): Semi-continuous cultivation of *Chlorella kesslerii* LARG/1 in microplates.

Kvíderová, J. (in prep.): Diurnal changes of photochemical activities in *Chlamydomonas moewusii*: a preliminary study using the variable fluorescence.

Conference participations

Algae and extreme environments, September 11 – 16, 2000 - poster: Unit for crossed gradients of temperature and light

40th conference of the Algological Section of the Czech Botanical Society, November 6 – 9, 2000, Rožmberk nad Vltavou – lecture: Gradienty – základní charakter prostředí a možnosti jejich modelování v laboratoři [Gradients – basic character of the environment and possibilities of their simulation in the laboratory]

IXth Days of Plant Physiology, September 17 – 21 2001, České Budějovice – poster: Modelling of gradients of temperature and light

41st conference of the Algological Section of the Czech Botanical Society, November 5 – 8, 2001, Rožmberk nad Vltavou – lecture: Počítačové zpracování a archivace dat z biotestu [Computerized processing and archiving of data in a bioassay]

Meeting of PhD. students in plant physiology, Nové Hrady, March 27 – 28, 2002 - lecture: The comparison of temperate and polar strains of genus *Stichococcus* (Chlorophyta)

42nd conference of the Czech Algological Society, October 7 - 10, 2002, Nové Hrady – lecture: Vliv streptomycinu na růst a fotosyntézu dvou zelených řas [Influence of streptomycin on growth and photosynthesis of two green algae]

43rd conference of the Czech Algological Society, September 22 – 25, 2003, Rožmberk nad Vltavou – lecture: Denní změny fluorescenčních charakteristik *Chlamydomonas moewusii*: předběžná studie [Diurnal changes of fluorescence characteristics in *Chlamydomonas moewusii*: a preliminary study]

COBRA Partner Review Meeting, March 24 – 26, 2004, Třeboň – lecture: The ecological characteristics of *Stichococcus* (Chlorophyta) strains isolated from polar and temperate regions

44th conference of the Czech Algological Society, September 20 – 23, 2004, Rožmberk nad Vltavou – lecture: První záznam P-E křivky sněžných řas in situ [The first record of the P-E curve of the snow algae *in situ*]

Workshop on Changes of the Polar Ecosystem, November 4 – 5, 2004, Josefův Důl, Jizerské hory – lecture: Ekologické charakteristiky kmenů rodu *Stichococcus* izolovaných z polárních a temperátních oblastí [The ecological characteristics of *Stichococcus* (Chlorophyta) strains isolated from polar and temperate regions]

Invited lectures

Astrobiologie ve Sluneční soustavě [Astrobiology in the Solar System]. Department of Botany, Faculty of Biological Sciences, University of South Bohemia, April 7, 2004

7. Curriculum vitae

Personal data

Date and place of birth: March 24, 1976, Rokycany

Present adress: Institute of Botany, AS CR
Dukelská 135
379 82 Třeboň
Czech Republic
Phone: +420 384 721 156
E-mail: kviderova@butbn.cas.cz

Fields of scientific interests

Ecophysiology of cyanobacteria and algae, ecology and physiology of cyanobacteria and algae in extreme environments, experimental phycology, astrobiology (exobiology)

Education

1999-present PhD. study in physiology and developmental biology (interrupted 2003-2004)
Faculty of Biological Sciences, University of South Bohemia
PhD. thesis: Adaptation of algae to extreme environments

1997- 1999 Mgr. in plant physiology, Faculty of Biological Sciences, University of South Bohemia České Budějovice
Mgr. thesis: The influence of sucrose on photosystem 2 in the course of water stress

1994-1997 Bc., Faculty of Biological Sciences, University of South Bohemia, České Budějovice
Bc. thesis: *In toto* staining as a model of penetration of compounds into plant tissue

Employment

2000-present Section of Plant Ecology, Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň

Scholarships

Oklahoma State Univerity (Dr. W.J. Henley, Department of Botany), June 14 – September 11 2002.

Grant participations

EU project - COBRA (The conservation of a vital European scientific and bacterial resource: microalgae and cyanobacteria) No. QLRI-CT-2001-01645.

GA ČR 206/01/1113, project 30-46 Active movement of phytoflagellates: a cost and benefit study.

NSF research project 9973203 LExEn: Responce of photosynthetic microbes of the Salt Plains National Wildlife Refuge to dynamic extreme conditions

Ecological and physiological adaptation of algae to low temperature and low irradiance. Czech-Japan Project - Kontakt ME 576

GA ČR 206/04/0967 Reproduction biology and autecology of quillworts *Isoëtes echinospora* and *I. lacustris* in Šumava lakes and *ex situ* cultures.

No work is over if the "paperwork" is outstanding.