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Master Thesis

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Response of Algal and Cyanobacterial Communities from Arctic and Antarctic Wetland Habitats to Freezing and Desiccation Stress

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Annotation: Freezing and desiccation tolerance of pure strains and natural samples of algae and cyanobacteria isolated from various Antarctic wetland habitats was assessed. Furthermore, the ability of algae and cyanobacteria from similar Arctic wetlands to survive conditions on barren ice was tested in situ.

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

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Contents

1. Introduction

Hydroterrestrial habitats (wetlands) represent a unique mosaic of unstable extreme environments (Elster 2002). They are widely distributed in deglaciated areas of the Arctic and Antarctic and provide a major source for microbial activity (Vincent et al. 1993b). They group together all types of habitats where liquid water is available for a few weeks to months during the summer season (Elster 2002). The main difference between aquatic and wetlands is that wetlands freeze completely during the winter deep lakes (Hawes et al. 1992).

The most abundant types of wetland habitats are saturated mineral soils, saturated ornithogenous soils, seepages and true wetlands.

Mineral soils occur both in the Arctic and Antarctica. Microbial communities, usually dominated by cyanobacteria, develop on the soil surface saturated by liquid water during the summer (Vincent et al. 1993b) or in the active layer of the soil (Bölter et al. 1994).

Ornithogenous soils are specific for maritime Antarctica, where large rookeries of penguins occur, and are strongly eutrofised by animal faeces. Presence of green alga *Prasiola crispa* in the adjacent bird colonies is a typical feature of these soils (Vincent et al. 1993b).

Seepages represent a unique environment of deglaciated areas of maritime Antarctica (Komárek and Komárek 2003). They develop submersed in shallow waters and cover the flat depression on the soil surface. The special structure of the community and the species composition is unique and different from other wetland habitats (Vincent et al. 1993a,b; Komárek and Komárek 1999).

As true wetlands, shallow streams and pools are meant. They develop both in the Arctic and Antarctic and are inhabited by various types of algal and cyanobacterial communities (Vincent et al. 1993b).

Algae and cyanobacteria, mainly from the group Oscillatoriales, are one of the dominant components of the polar wetlands (Broady 1996; Tang et al. 1997a, Vézina and Vincent 1997). Microorganisms living in wetlands have two life strategies how to produce macroscopically visible growths: algal mats and crust, and mucilaginous clusters and jelly biomass floating in waters (Elster 2002).

Due to lack of whole-year monitoring (Arnold et al. 2003), very little is known about the annual cycle of communities inhabiting the polar wetland habitats. In spring and summer, deglaciated areas of the Arctic and Antarctic are saturated by liquid water from melting permafrost, snowfields and glaciers and the wetland communities start to develop. Temperatures heavily fluctuate during the day, may be high up to 8°C (Elster and Benson 2004), but rarely fall far below 0°C (Davey et al. 1992). In autumn, temperatures are stable around 0°C and numerous day-to-day freeze-thaw cycles occur (Davey et al. 1992). This overnight freezing is restricted to the vege-

tation surface and to only a few degrees below zero, temperatures that are not sufficiently severe to cause freezing damage to even the most susceptible Arctic or Antarctic organisms (Block 1990; Hawes 1990). Nevertheless, this mild period of sublethal temperatures may be of importance in promoting cold-hardiness of organisms before the decline to lower winter temperatures. In the winter, the temperatures fall far well below 0°C (Davey et al. 1992), the water level drops and the mats are completely desiccated or frozen in the shallow wetlands. At any rate, no liquid water is available until the spring comes. The length of each season depends on geographical position of site, primarily the latitude.

It is clear that the winter freezing is the major event and may be the only one that can be expected to cause significant mortality in the communities (Hawes 1990; Davey et al. 1992). Studies based on field or laboratory experiments showed that some cyanobacteria (*Phormidium*, *Nostoc*) and algae (*Prasiola*, *Zygnema*) are able to tolerate prolonged desiccation (Davey 1989; Hawes et al. 1992; Jacob et al. 1992; Qiu et al. 2003) or freezing (Holm-Hansen 1963; Davey 1989; Hawes 1990) It is obvious that there should be differences in overwintering strategies between algal and cyanobacterial species (Hawes 1990), and between species inhabiting different habitats (Becker 1982; Davey 1989; Hawes et al. 1992; Jacob et al. 1992).

As a result of freeze-thaw action, microbial mats must have the ability to tolerate freeze-over and resume growth after rehydration. Because of changes in water chemistry (i.e. pH, salinity, precipitations of solutes) during freezing and thawing, they must be also able to tolerate chemical extremes (Cockell et al. 2000), and additionally high radiation fluxes. High solar radiation flux may be particularly injurious to cyanobacteria, because of very short distances between the cell surface and genome (Garcia-Pichel 1994; Rozema et al. 1999). To avoid the damage caused by freezing or desiccation the algal cells release soluble sugars, particularly trehalose and sucrose (Crowe et al. 1984; Hawes 1990; Leslie et al. 1995). In addition, ice-active substances, which significantly lower the freezing point of water, have been found in cyanobacterial mats, isolated from the Polar Regions (Raymond and Fritsen 2000).

What happens to the overwintering communities while desiccated on the soil surface or trapped in frozen water bodies is not very clear. Nevertheless, the Polar Regions (especially the continental and arid parts) typically present strong katabatic winds during the winter that mobilizes most of the material throughout and deposits it on the ice and snow surface (Paerl and Priscu 1998; Priscu and Christner, in press). Present-day studies, based on analysis of particulate organic carbon and DNA sequence distribution (Burkins et al. 2000, 2001; Fritsen et al. 2000, Gordon et al 2000; Brambilla et al. 2001; Christner et al. 2003a) provide strong evidence that microbes from wetland habitats are widely distributed throughout the McMurdo Dry Valleys in Antarctica via aeollian processes. Thus, they provide the biological seed for cryoconite holes on the glacier surface (Christner et al. 2003a) or permanently frozen lakes (Gordon et al. 2000; Brambilla et al. 2001). They may also be washed away into

the drainage of the glacier and potentially colonize subglacial systems (Skidmore et al. 2000; Grasby et al. 2003; Kaštovská et al., unpublished data). Even though, the microbes found in ice habitats are very similar to types inhabiting adjacent wetlands. However, the species diversity in ice is much lower; hence not all wetland algae or cyanobacteria are capable of colonizing the ice. This is not surprising considering that environmental conditions on or inside ice are much harsher and less hospitable for life: temperatures below zero, no or very little water available, excessive light on the surface or dark in the subglacial systems, low nutrient content, high pressure, strongly acidic or saline solution (Price 2000; Vincent et al. 2004). It is suggested that these icy habitats acted as a biotic refuge (Hoffman and Schrag 2000; Vincent and Howard-Williams 2001; Vincent et al. 2000) during two major glacial periods in the Earth's history (Kirschvink et al. 2000).

The first aim of this study was to assess freezing and desiccation tolerance of cyanobacterial and algal strains and natural samples from various types of wetland communities from continental and maritime Antarctica and to analyse this data using multiregression methods and to try to find out significant trends, that enable some species to survive and.

The second aim of this study was to simulate the winter conditions, when algal and cyanobacterial mats are deposited on the barren ice substratum by wind or washed inside the drainage system of the glaciers. Communities of algae and cyanobacteria from Arctic wetland habitats were directly spattered on snow, firn or ice or in subglacial caves. Changes in community structure were evaluated after one week, two weeks and one month. Whether and which cyanobacteria and algae can survive a month exposure to ice, firn or snow ice substratum was tested.

2. Review

2.1. Earth's Icy Biosphere

Since the Polar Regions are very vulnerable early detectors of environmental changes because snow and ice cover variation markedly affect all ecological variables (Quayle et al. 2002), intensive global climatologic and ecological studies have been focused on them recently (Hansell et al. 1998; Johannessen et al. 1999; Aanes et al. 2002, Yili-Tuomi et al. 2003). Additionally, in last years, life have been found in the most unlikely, extremely cold and frozen habitats in the McMurdo Dry Valley, Antarctica, valley described by Captain Robert Falcon Scott as the "Valley of Dead" (Scott 1905). These new findings completely changed what was thought of as insurmountable physical and chemical barriers of life and it has been suggested that life could be more common than previously thought (Rothschild and Mancinelli 2002).

Earth's biosphere is undoubtedly cold with 14% being Polar and 90% (by volume) cold ocean (<5 °C). More than 70% of Earth's freshwater occurs as ice (Priscu et al. 2002) and large portion of the soil ecosystem (~20%) exists as permafrost (Rivkina et al. 2000).

Paleoclimatic records for the past 500,000 years have shown that the surface temperature on Earth has fluctuated drastically, with four major glaciations occurring during this period (Pettit et al. 1999). Strong evidence also showing that the Earth was completely ice-covered during two major intervals of glacial activity in the Paleoproterozoic (0,8 Ga) and Neoproterozoic (0,6 Ga) periods (Kirschvink et al. 2000). According to this so-called "Snowball Earth Hypothesis", the Earth would have been completely ice covered for 10 million years or more, with ice thickness exceeding 1 km. Only the deepest ocean would have contained liquid water (Hoffman et al 1998; Shrag and Hoffman 2001, 2002).

One of the primary criticisms of the Snowball Earth Hypothesis is that the thick ice cover over the world ocean would cut off the supply of sunlight to organisms in the seawater below and thereby eliminate photosynthesis and all life associated with photosynthetic carbon production. Others have conducted that global-scale freezing would extinguish all surface life (Williams et al. 1998). Therefore, some scientists (Hoffman and Schrag 2000; Vincent and Howard-Williams 2001; Vincent et al. 2000) suggest that photosynthetic cyanobacteria and bacteria, similar to those found in the permanent ice covers of contemporary polar systems, may have acted as an icy biotic refuge during this period, until post-snowball melting introduced conditions suitable for activity in terrestrial and marine habitats. The resultant high concentration of microbes in these icy envi-

ronments would favour intense chemical and biological interactions between species, which would force the development of symbiotic associations, and eventually eukaryotic development through evolutionary time (Vincent et al. 2000).

It is generally thought that life on Earth evolved in hot environments (Pederson 1997; Huber et al. 2000). However, according to the Snowball Earth Hypothesis, it is highly probable that ice-bound habitats also provided opportunities for microbial evolution, including the radiation of the eukaryotic cell type at the onset of the Neoproterozoic (Knoll 1994; Hoffman and Schrag 2000)

2.2. Microbial Life in the Polar Regions

Even though both South and North Polar Regions seem to have similar environmental conditions, mainly cold climate, there are many differences determined by the fact that Antarctica is an isolated continent surrounded by ocean, whilst the Arctic is actually a sea-basin surrounded by three continents. It can be assumed that the circumpolar Arctic is, in terms of biological transport, much more accessible than the Antarctic. Antarctica has not always been cold: a variety of evidence indicates that seawater of high Southern latitude cooled more or less steadily since the Tertiary (Clarke 1991).

Antarctica, the fifth largest continent (Kanda and Komárková 1997), covered in ice up to 4 km deep (Block 1994), along with wide Arctic Region present indisputably an extremely harsh environment for terrestrial and freshwater plants with very low temperatures, frequent and rapid fluctuations from freezing to thawing, severe winds, low humidity, and long periods of light and darkness. The vegetation of these areas is limited to forms, which have developed certain adaptation mechanisms in order to survive under these conditions. The origin of the present polar flora is not yet clear, but it seems likely that the majority of the species are postglacial immigrants with optimal adaptation to the prevailing conditions, especially to long periods of sub-zero temperatures (Becker 1982).

Similar to high temperature counterparts, polar and frozen ecosystems are dominated by microorganisms (Priscu and Christner, in press). Cyanobacteria and algae are widespread in all polar terrestrial environments, including extremes, and frequently produce visible biomass. Their combined biomass represents a sizeable pool of global fixed carbon, influencing mineral cycling and energy flow, and affects the mineral and biological development of polar soils (Elster 2002; Cockell et al 2003).

Nitrogen fixation, mainly by cyanobacteria, is a major source of nitrogen in Arctic environments, contributing up to 82% of the total annual ecosystem nitrogen input and

20% of total annual uptake by plants (Chapin and Bledsoe 1992; Solheim et al. 1996; Solheim et al. 2002).

2.2.1. Colonization of Pristine Areas in the Polar Regions

The role of cyanobacteria and algae as pioneer colonizers of new substrata is well known from classical studies of the succession on new volcanic island (Doctres van Leeuwen 1936). Even though their role in the primary colonization cannot be generalized for all types of habitats, sometimes lichens, mosses or liverworts occur first (Brock 1973), in the latitudes of the Polar Regions the pioneer colonization of barren soils after glacier retreat by bacteria, cyanobacteria and algae is of special importance. Microorganisms bind the soil prior to the growth of mosses and lichens on the substratum. Since nitrogen is often limiting during initial community development, it is N-fixing cyanobacteria, which first occur (Ohtonen et al. 1999). It may be hypothesized that the earliest colonizers of the substratum are subjected to the greatest extremes of environmental stress, being unprotected by the greater water retention and buffering of temperature change of more advanced communities. Therefore, it is these organisms, which may possess the greatest resistance to environmental stress (Davey 1988 and citations therein).

As already mentioned above, cyanobacteria and algae have been very successful in occupying all polar terrestrial environments. It can be generally said: the more extreme environment the more important role play the simplest organisms, such as cyanobacteria. Prokaryotes are distinguished from eukaryotic life forms by being structurally simple, but functionally (metabolically) diverse (Stanier 1976 in Paerl et al. 2000).

In mild-temperature nutrient-rich habitats, where liquid water is available for most of the summer season (maritime Antarctica, coastal areas of high Arctic), communities of mosses, lichens, and liverworts or, in the case of Arctic, higher plants develop and are important primary producers. Nevertheless, as soon as the substrate does not provide enough nutrients and the temperatures often drops below zero and thereby liquid water is seldom accessible, cyanobacteria play a key role even in the most extreme environments.

Cyanobacterial incredible resistance to various extreme conditions is probably connected with their evolution that is marked by a range of environmental extremes over geological time scales. These include geochemical (volcanism, tectonics) events, their impacts on global temperature and irradiance (including UV), heating and desiccation and vast changes in nutrient availability and ionic composition, i.e. salinity (Paerl et al. 2000).

CO_2 and N_2 fixation were the “linchpin” processes of cyanobacterial colonization, proliferation and diversification (Paerl et al. 2000). The ability of some cyanobacteria to fix

atmospheric N_2, a physiological trait shared only with some other prokaryotes, confers a distinct advantage over eukaryotic microalgae under N-limited conditions (Fogg 1982). Furthemore, the ability to migrate vertically by buoyancy alteration (gas vesicles) or gliding provides access to subsurface nutrient-rich waters and sediments. Surface-dwelling cyanobacterial bloom or mat species capture maximal irradiance for supporting photosynthesis and energy needs (Paerl et al. 2000). Production of photoprotective accessory carotenoids and other pigments enables cyanobacterial bloom or mat taxa to persist near the highly illuminated surfaces of either planktonic or benthic systems (Paerl et al. 1983; Garcia-Pichel 1994).

In general, an extreme environment may be viewed as one that is more selective for prokaryotic growth than for eukaryotic growth (Paerl et al. 2000).

Cyanobacteria play an important role as the only primary producers of desert areas of continental Antarctica. They have been found growing beneath the rock surface (endolithicly) in the McMurdo Dry Valleys (Friedmann 1971, 1980, 1982; Friedmann and Ocampo 1976; Golubic et al. 1981), where is more favourable climate for growth than on the surface of rocks or soils (Friedmann 1982).

2.2.2. Permanently Frozen Ecosystems as Analogues for Cold Extraterrestrial Life

When life was recently found in habitats such as cold and saline lakes (Franzmann et al. 1997; Priscu et al. 1999a; Takacs et al. 2001), permanent lake ice (Abyzov et al. 1998; Priscu et al. 1998; Fritsen and Priscu 1998; Psenner et al. 1999; Mitskewich et al. 2001; Poglazova et al. 2001), glacial ice (Christner et al. 2000; Christner et al. 2001; Skidmore et al. 2000) and polar snow (Carpenter et al. 2000), it was not surprising that the most conspicuous elements of these environments were prokaryotic organism, bacteria and cyanobacteria. According to these new discoveries and with potential future discoveries of life in Lake Vostok, a subglacial lake with liquid water, situated almost 4.000 km under the ice (Siegert et al. 1996, 2001; Jouzel et al. 1999; Karl et al. 1999; Priscu et al. 1999b; Christner et al. 2001), some scientists admitted that so far generally accepted limits, where life still could exist, must be changed. If life can be found in ecosystems completely frozen for many million years, it is at least possible, that it could be found existing under similar conditions outside of the Planet Earth. It seems to become fundamental law that, wherever microbial life can survive, it will be found to exist (Gold 1992) or in other words where there is liquid water on Earth, virtually no matter what the physical conditions, there is life (Rothschild and Mancinelli 2002). Planet Mars and Jovian Moon Europa provide the most similar conditions such those found under the ice of continental Antarctica (Priscu et al. 1998; Priscu et al. 1999a,b; Paerl and Priscu 1998;

Wynn-Williams and Edwards 2000; Baker 2001; Hiscox 2001; Cavicchioli 2002; Thomas and Dieckmann 2002).

Evidence from Martian orbiter laser altimeter images has revealed that water exists at the poles and below the surface of Mars (Malin and Carr 1999; Boynton et al. 2002) and studies of Martian meteorites have inferred that prokaryotes were once present (Thomas-Keprta et al. 2002).

During high obliquity, increases in the temperature and atmospheric pressure at the northern pole of Mars (McKay and Stoker 1989; Malin and Carr 1999) could result in the discharge of liquid water that might create environments with ecological niches similar to those inhabited by microorganisms in terrestrial polar and glacial regions. Periodic effluxes of hydrothermal heat to the surface could move microorganisms from the Martian subterranean where conditions may be more favourable for extant life (McKay 2001). The annual partial melting of the ice caps might than provide conditions compatible with active life or at least provide water in which these microorganisms may be preserved by subsequent freezing (McKay and Stoker 1989; Clifford et al. 2000).

Surface ice on Europa appears to exist in contact with subsurface liquid water (Greenburg et al. 1998; Kivelson et al. 2000). Geothermal heating and the tidal forces generated by orbiting Jupiter are thought to maintain a 50-100 km deep liquid ocean on Europa with perhaps twice the volume of the Earth's ocean (Carr et al. 1998; Chyba and Phillips 2001) but beneath an ice shell at least 3-4 km thick (Turtle and Pierazzo 2001). Cold temperatures (<128 K; Orton et al. 1996) combined with intense levels of radiation would appear to preclude the existence of life on the surface, and the zone of habitability (i.e., where liquid water is stable) may be present only kilometres below the surface appears strikingly similar to terrestrial polar ice floes, suggesting that the outer shell of ice is periodically exchanged with the underlying ocean. The ridges in the crust and the apparent rafting of dislocated pieces implies that subterranean liquid water flows up through stress-induced tidal cracks, which may then offer provisional habitats at shallow depth for photosynthesis or other forms of metabolisms (Gaidos and Nimmo 2000; Greenberg et al. 2000).

2.3. Polar Hydroterrestrial Environments

Although polar hydroterrestrial (wetland) environments does not provide such extreme conditions as the icy environments, and it is highly unlikely that similar habitats will be found on Mars or Europa or anywhere else in the Universe, they represent a very important component of the polar ecosystems. In addition, very recent studies have brought strong evidence that organisms, largely cyanobacteria, inhabiting polar wetland environments represent a source of organic material for the icy ecosystems. This topic will be

further discussed more in detail.

Hydroterrestrial habitats represent a unique mosaic of unstable extreme environments where a large variation in algal species composition and biomass between various types of habitat is evident (Elster 2002). They are widely distributed around the margins of the Arctic and Antarctic and provide a major source for microbial activity (Vincent et al. 1993b).

Wetlands will be proposed here, to group together all types of habitats where liquid water is available for few weeks to months during the summer season – shallow lakes and pools, wetlands, wetted soils, saturated mineral soils, irrigated rock faces, wet walls, wet slope seepages, springs and salt marshes (Elster 2002). The flowing waters are derived from melting snow-banks or glacier ice. They range from percolating flows that intermittently recharge the "wetland communities" on flushed soils and rock surfaces to well-defined meltwater streams in perennial channels to rivers. These three types of habitat form a continuum of flowing-water environments, and often each physically grades into the next (Vincent et al. 1993b).

The main difference between hydroterrestrial (wetland) and limnetic (lake) ecosystems is that deep lakes may contain liquid water year round and offer a relatively benign refuge to organisms, while wetlands offer less protection and may expose communities to desiccation on several time scales (Hawes et al. 1992). The discharge signature of Antarctic streams is marked by a high level of variability at all time scales (interannual, seasonal, day-to-day and over the 24-hour cycle). Small variations in the energy balance during snow and ice melt are translated into major fluctuations in size and flow characteristics of the stream environment (Vincent et al. 1993b).

Arctic and Antarctic freshwater environments are often characterised by low nutrient concentrations, although some Antarctic ponds can be highly enriched in phosphate and ammonium. Differences in nutrient content can be explained by the drainage basin characteristics, extent of rock weathering and input of allochthonous material, for example from catchment vegetation. The latter input would be of much importance in the Arctic than in the Antarctic (Vézina and Vincent 1997).

2.3.1 Microorganisms in the Polar Wetland Habitats

Cyanobacteria and algae are the most abundant components of polar wetland habitats. Earlier observations (McLean 1918) concluded that there was a predominance of cyanobacteria in the Antarctic benthic and soil ecosystems relative to the Arctic. However, more recent studies (e.g. Vézina and Vincent 1997) questioned it and have shown that the abundance and diversity of cyanobacteria is similar in both Polar Regions. The dominance of oscillatorian cyanobacteria, a group that is also widely distributed in Ar-

chean and Proterozoic fossil records (Schopf 1993), in benthic microbial communities is a feature of most freshwater ecosystems in the Antarctic (e.g. Broady 1996) and in the Arctic (Tang et al. 1997a; Vézina and Vincent 1997). Their dominance is attributed to both a high tolerance for the extreme environment and a paucity of predators and competing species that are excluded by the inhibiting effects of low temperatures and potential freezing (Nadeau and Castenholz 2000).

Algae living in wetland habitats have two life strategies how to produce macroscopically visible algal growths: algal mats and crust, and mucilaginous clusters and jelly biomass floating in waters. Similarities have been found in life-strategy and ecological role in the Arctic and the Antarctic wetland habitats (Elster 2002).

2.3.1.1. Microbial Mats

Mats and crusts are up to several centimetres thick cohesive, skin-like, mucilaginous films, floccules and aggregated structures produced mainly by prokaryotic microorganisms. Whereas some mats are products of single cyanobacterial species, others are complex of differentiated microbial communities (Howard-Williams et al. 1986; Vincent et al. 1993a; Davey and Clarke 1991). Prokaryotic microbial mats are not specific organisation structures of polar wetland habitats, they represent a pioneer and often the only biota inhabiting various types of extreme aquatic and terrestrial environments (Paerl et al. 2000).

Microbial mats exemplify functionally integrated, self-sustaining, laminated microbial consortial systems. They contain the essential biocomplexity for carrying out life-sustaining processes under the most extreme environmental conditions that still harbour life (Paerl et al. 2000). Present-day microbial mats are believed to be direct descendants of the first extant biological communities on Earth (Schopf and Walter 1982), which are believed to have had a key role in the oxidation of the primitive oceans and atmosphere (Hoehler et al. 2001).

Most mats have a similar pigment organisation. The carotenoid-rich surface layer serves as a protection against damage from excessive light. Under this layer, a chlorophyll-*a* maximum enriched. Sometimes deep living trichomes can migrate to the surface (Vincent and Howard-Williams 1986; Vincent 1988; Howard-Williams and Vincent 1989; Davey and Clarke 1991; Hawes et al. 1992; Vincent et al. 1993a; Bebout and Garcia-Pichel 1995; Elster and Svoboda 1996; Elster et al. 1997). Benthic mats are composed mainly of *Phormidium* and *Nostoc*, together with *Leptolyngbya* trichomes. Interestingly, these cyanobacterial species occurs across habitats that have highly divergent environmental extremes (Priscu and Christner, in press). In localities where percolating water flows and desiccation occurs, *Gloeocapsa, Schizothrix, Dichothrix* and *Scytonema*,

(again) together with *Leptolyngbya* trichomes, occur (Vincent and Howard-Williams 1986; Elster et al. 1997, 1999; Wynn-Williams 1990).

2.3.1.2. Mucilaginous Clusters and Jelly Biomass

Mucilaginous clusters are attached to stones (epilithic) and to submersed mosses or vascular plants (epiphytic). Both prokaryotic and eukaryotic microorganisms produce jelly biomass, however, eukaryotic is more often visible (Elster 2002). The most frequent components are *Tribonema, Hydrurus, Microspora, Prasiola, Klebsormidium, Zygnema, Mougeotia, Spirogyra* or pennate diatoms, such as *Tabellaria, Hannaea, Fragilaria, Synedra, Eunotia, Pinnularia* etc. (e.g. Vincent and Howard-Williams 1986).

2.3.2. Types of Hydroterrestrial Habitats

As already mentioned above, there are many various types of hydroterrestrial habitats in the Polar Regions. For the purpose of this study, four important types will be described in detail:

2.3.2.1. Saturated Mineral Soils

The presence of permafrost limits the subsurface system to the active layer (1-3 cm; Bölter et al. 1994) and restricts the water migration to deeper soil. In an active layer which, in milder localities, is frequently saturated with water, and where, besides the wet soil, various shallow temporary freshwater ecosystems (lotic and lentic wetlands) arise, favourable conditions exist for mass algal microflora development (Elster 2002). Soils experience greater diurnal temperature fluctuations than aquatic habitats (Johansen and Shubert 2001).

Surface mats and colonies of algae, usually dominated by cyanobacteria, are often extensive on soils that are saturated for much of the summer and over which shallow films of meltwater may percolate (Vincent et al. 1993b).

Algal crusts on soil surface consist of water-stable, surface soil aggregates held together by algae, fungi, lichens, and mosses (Elster 2002). Soils can differ markedly in moisture from extreme aridity to water saturation (Elster 2002). In soils of central Antarctica, cyanobacteria and algae predominate, in milder and moister maritime Antarctica, bryophytes and lichens are more common (Smith 1984). Arctic soil microflora is more species-rich and more abundant than that of Antarctica. In addition, green algae are probably more diverse than cyanobacteria in the Arctic (Elster et al. 1999).

Nitrate is probably the limiting nutrient in Antarctic fellfields (Davey and Rothery 1992; Arnold et al. 2003).

2.3.2.2. Saturated Ornithogenous Soils

This type of habitat is characteristic for Antarctica, where close to seashore large penguin colonies and pinipeds occur. These soils are strongly eutrofised by animal faeces. In the Arctic, where bird nest on steep rocks, and so the nutrient input to soils is much lower, ornithogenous soils develop in lower intensities. Algae living in these environments must be adapted to high mechanical disturbance caused by tread downing.

The foliose macroscopic alga *Prasiola crispa* is ubiquitous on water-flushed soils adjacent to bird colonies all around the Antarctic. Abundant microscopic associate are *Navicula muticopsis* and *Oscillatoria* sp. (Vincent et al. 1993b).

2.3.2.3. Seepages

Freshwater seepages, a unique environment of deglaciated areas in the maritime Antarctica, represent a special habitat, supplied by water from melting permafrost, snowfields and glaciers during the austral summer season. They are shallow (typically less than 10 cm deep) and cover flat depressions on the soil surface. The water here is stagnant or slowly streaming (Komárek and Komárek 2003). The special structure of the community and the species composition is very unique and distinctly different from adjacent creeks and pools (Vincent et al. 1993a,b; Komárek and Komárek 1999).

They develop usually submersed in shallow water, but in the later growth period the water level drops and the mats are exposed to the air. Dominant species in prominent layers of the mat are always the same, mostly cyanobacteria, endemic for this type of habitat.

2.3.2.4. True wetlands

This type of habitat is developed both in the Arctic and Antarctic. As true wetlands, shallow streams and pools are thought. They are supported by water from glaciers and snowfields. Five broad groups of phototropic communities grow in perennial streams: mats, crusts and films of cyanobacteria; tufts and mats of green algae, notably *Zygnema*, *Mougeotia*, *Spirogyra* and *Binuclearia*; uniseriate filaments and multiseriate ribbons of the green alga *Prasiola*; diatom-dominated communities associated with sandy substrates at low stream slope angles; and a diverse assemblage of species epiphytic on stream mosses (Vincent et al. 1993b).

Most common cyanobacterial communities are composed entirely of Oscillatoriales similar to those occurring on saturated mineral soils. The second type of cyanobacterial mat is composed primarily of *Nostoc commune*.

2.3.3. Annual Cycle of Wetland Habitats

The annual cycle of development and destruction of the mat communities is probably similar in the above-mentioned wetland habitats. Most of the observations have been limited to some part of the year and only few studies (Davey 1991; Davey et al. 1992; Hawes et al. 1992, 1999; Arnold et al. 2003) have been focused on some whole-year monitoring. The development of mats starts in spring, when vast deglaciated areas of the Arctic and Antarctic are saturated by liquid water from melting permafrost, snow fields and melting glacier.

Three main periods within the annual temperature cycle are evident. Firstly spring/summer, when day temperatures may be high, although with much diurnal variations, temperature rarely fall far below 0°C (Davey et al. 1992). Secondly autumn, when temperatures are stable around 0°C and numerous day-to-day freeze-thaw cycles occur (Davey et al. 1992). This overnight freezing is restricted to the vegetation surface and to only a few degrees below zero, temperatures that are not sufficiently severe to cause freezing damage to even the most susceptible Arctic or Antarctic organisms (Block 1990; Hawes 1990). Nevertheless, this mild period of sublethal temperatures may be of importance in promoting cold-hardiness of organisms before the decline to lower winter temperatures. Thirdly, in winter the temperatures fall well below 0°C (Davey et al. 1992), the water level drops and the mats are completely desiccated or frozen in the shallow ponds. At any rate, no liquid water is available until the spring comes. It is clear that the winter freezing is the major event and may be the only one that can be expected to cause significant mortality in the communities (Hawes 1990; Davey et al. 1992). The duration of winter-freezing period is dependent on the geographical position (primarily the latitude) of each locality. While in the ponds of McMurdo Ice Shelf, the temperature rise above zero for less than a month each year (Hawes et al. 1999), the mats from Signy Island, South Orkney Islands, are exposed to winter freezing only for two or three months every year (Davey et al. 1992).

2.3.4. Airborne Dispersal of Mats

What happens to the overwintering communities, while desiccated on the soil surface or trapped in frozen water bodies is not very clear. Nevertheless, the Polar Regions (especially the continental and arid parts) are typical of presence of strong and persistent

down-valley katabatic winds during the winter, that mobilizes most of the material throughout and deposits it on the ice and snow surface (Paerl and Priscu 1998; Priscu and Christner, in press). When dried mats are subject to wind erosion, they may act as significant sources of propagules for airborne dispersal (Ellis-Evans and Walton 1990; Wynn-Williams 1991; Marshall and Chalmers 1997). For the first time, pieces of algal mat of *Oscillatoria* with *Nostoc* were observed moving upward through the ice by Wilson (1965). On reaching the surface of the ice the algal mat dried and blown away, presumably to colonize other lakes and ponds. Wilson used a thermodynamic model to show that the upward movement was a function of gas-induced buoyancy coupled with absorption of solar radiation by the mat itself, which melts the overlying ice. Using this mechanism the algal mat could move through as much as 2 m of clear ice (Wilson 1965).

The hypothesis that dried mats dispersed by wind comprise a propagules source for colonization of other similar wetland habitats (Howard-Williams et al. 1990) has been more or less accepted since the viable propagules of algae and cyanobacteria were found in airborne material (Hawes et al. 1992).

On the other hand whether the same communities can act as primary source of organic material for habitats such as cryconite holes on the surface of the glacier, permanently frozen lakes, subglacial lakes etc. is not generally accepted. Organisms inhabiting these icy environments are exposed to far more severe conditions than those inhabiting the wetland ones: temperature below zero, no or very little water available, excessive light on the surface or dark in the subglacial systems, low nutrient content, high pressure, strongly acidic or saline solution (Price 2000; Vincent 2004). Therefore it seems to be highly unlikely that cyanobacteria or algae originally from wetlands could be able to survive or even reproduce on or inside the ice after deposited there via aeollian processes. Nevertheless, analysis of particulate organic carbon (POC) and DNA sequence distribution (Burkins et al. 2000, 2001; Fritsen et al. 2000, Gordon et al 2000; Brambilla et al. 2001) provides strong evidence that microbes are distributed widely throughout the McMurdo Dry Valleys via strong katabatic winds. In addition DNA hybridization studies between cyanobacteria and other prokaryotes found in the permanent lake ice in the McMurdo Dry Valleys have revealed that cyanobacterial mats in ephemeral streams provide the biological seed for the lake ice cyanobacteria (Gordon et al. 1996, 2000; Priscu et al. 1998). It is clear that lakes and streams colonized by cyanobacteria are the "life support system" of Antarctic polar deserts, providing organic carbon to the surrounding areas and seeding numerous habitats with microbes (Priscu and Christner, in press).

Parker et al. (1982) suggested that cyanobacterial mats lift off the bottom of the larger permanently ice covered lakes and freeze onto the underside of the lake ice. Annual freezing of new ice to the bottom of the lake ice together with ablation at the surface, eventually brings the mats to the surface where aeollian processes distribute them to the

surrounding environment. Parker et al. (1982) further proposed that this mechanism reduces organic carbon within the lakes leading to oligotrophy over time.

Based on results from a phylogenetic survey of a cryoconite hole in Antarctica (Christner et al. 2003a), these ecosystems are also inhabited by species very similar to those in adjacent microbial mat and lake communities. Cryoconite holes formed as windblown particulates accumulate on the surface of a glacier, are warmed by the sun, and melt into the ice producing a cylindrical basin of liquid water (Priscu and Christner, in press). They occur globally in the Arctic (Gerdel and Drouet 1960; DeSmet and Van Rompu 1994; Grøngaard et al. 1999; Säwström et al. 2002; Mueller et al. 2001), Antarctic (Wharton et al. 1981, 1985; Christner et al. 2003a; Mueller and Pollard 2004; Tranter et al. 2004), and alpine glaciers (Kohshima 1989; Takeuchi et al. 2000). Every cryoconite hole is unique, and therefore may support a novel and discrete ecosystem (Priscu and Christner, in press). Although these environments become completely frozen during the winter, upon summer warming and glacial melting, the surviving members of these communities might serve in reverse to ensure the re-seeding of surrounding environments (Priscu and Christner, in press).

Studies indicate that the topography, local and global environmental conditions, and proximity of ecosystems contributing biological particles to a particular air mass influence the concentration and diversity or airborne microorganisms (Lighthart and Shaffer 1995; Giorgio et al. 1996; Fuzzi et al. 1997; Marshall and Chalmers 1997).

Aerosolized microorganisms can travel large distances on atmospheric currents, often in a viable, but dormant state (Priscu and Christner, in press). Remarkably, some air conditions actually provide a medium for growth, and microbial metabolism has been detected in fog particles (Fuzzi et al. 1997) and super-cooled clouds (Sattler et al. 2001). For an airborne microorganism deposited in glacial ice to retain viability, the stress associated with desiccation, solar irradiation, freezing, an extend period of no growth, and subsequent thawing must not result in a lethal level of unrepairable cellular damage. Many of these organisms have thick walls or polysaccharide capsules, and resist repeated cycles of freezing and thawing (Priscu and Christner, in press).

When viable cells are transported on the glacier surface, they might be washed away inside the drainage system of the glacier and remain preserved here for hundreds or thousands years (Abyzov 1993; Christner 2002; Christner et al. 2003b).

2.3.5. Extreme Environmental Conditions in the Polar Wetland Communities

The organisms living in these habitats are subjected to rigorous physical conditions and experience extreme physiological stress for much of the year (Davey 1989).

However, microorganisms isolated from polar wetland communities are mostly only

psychrotolerant, not truly psychrophilic (Roos and Vincent 1998; Quesada et al. 1999). It means that they are able to grow at or near the freezing point of water, but have temperature optima above 15°C and maximum temperature for growth above 20°C (Nadeau and Castenholz 2000). It may well be that polar nonmarine waters that are neither diurnally nor seasonally physically stable, are more receptive to broadly tolerant organisms than to those with a narrow niche (e.g. Vincent and James 1996). Based on growth parameters (Vincent and James 1996; Tang et al. 1997a) and phylogenetic analysis (Nadeau et al. 2001), oscillatorian cyanobacteria in Antarctic meltwater ecosystems, as well as other microalgal species, likely originated from more temperate organisms.

The lack of cyanobacterial and algal psychrophiles in the polar wetland habitats (and therefore in the majority of other polar environments, including icy environments) may be related to selection factors other than temperature (e.g. freeze-thaw tolerance, tolerances of high fluxes of solar radiation) that dictate which organism survive and later potentially colonize new habitats (Tang et al. 1997a; Vincent et al. 1997; Vincent 2000).

Obviously, the communities of polar wetland habitats have to be somehow adapted to desiccation-rehydration changes or salinity stress, freeze-thaw cycles, and bright, continuous mid summer solar radiation including UV-B (Howard-Williams and Vincent 1989; Vincent and Howard-Williams 1989; Howard-Williams et al. 1989; Davey 1989; Vincent et al. 1993a,b; Vincent and Roy 1993; Vincent and Quesada 1994; Wynn-Williams 1994; Tang et al. 1997a).

2.3.5.1. The Effect of UV

To reduce the damage caused by UV, the microbial mats are highly structured in terms of their light-capturing and light-screening characteristics. The surface layer of the mats is optimised for filtering out high energy blue and UV wavelengths, while the bottom layer is optimised for harvesting longer wavelength PAR (Quesada et al. 1999).

Cyanobacteria are concurrently vulnerable to ultraviolet (UV) damage (Wynn-Williams et al. 2002), because of short distance between the cell surface and genome (Garcia-Pichel 1994; Rozema et al. 1999). To avoid the damage caused by UV radiation, microorganisms can produce screening compounds (Karentz et al. 1991; Rozema et al. 2002). Scytonemin, for example, is a cyanobacterial compound produced in response to UV stress (Garcia-Pichel et al. 1992). The surface scytonemin-rich layers of mats are highly effective in filtering out of UV region (Quesada et al. 1999). Carotenoids can perform the function of quenching excited oxygen states and they are found in microbial mats in the Antarctic (Vincent et al. 1993a; George et al. 2001) and the Arctic (Quesada et al. 1999)

UV radiation is also functioning as a primary cue for avoidance of damaging solar ra-

diation in the *Oscillatoria* sp. population, and suggests that UV is involved in the migratory behaviour of motile cyanobacteria in microbial mats worldwide (Nadeau et al 1999).

Different cyanobacterial species have differing photochemical sensitivity to UV-B irradiation, which may confer a subtle advantage to the UV-B tolerant species over the less tolerant type during a period of high UV-B irradiance. *Phormidium*-dominated mats show great tolerance to elevated UV-B (George et al. 2001).

2.3.5.2. Nitrogen Limitation

Nitrogen availability is a key nutritional factor controlling microbial production in Antarctic freshwater and soil habitats. Since there are no sources of biologically available N entering these ecosystems, nitrogen fixation may be a major source of "new" N supporting primary and secondary production (Olson et al. 1998).

Davey and Rohtery (1992) suggested that microalgal communities from maritime Antarctica are during the summer nitrogen limited. This hypothesis was also supported by study of Arnold et al. (2003).

Ponds of McMurdo Ice Shelf are usually nitrogen limited and N_2-fixation, conducted by heterocytous cyanobacteria is enormously important there (Fernández-Valiente et al. 2001). Permanently frozen lakes of the McMurdo Dry Valleys area are also nitrogen limited, however, inhabited mostly by non-heterocytous cyanobacteria. Even though, experimental studies proved that nitrogen fixation is a key process in these environments and that non-heterocytous cyanobacteria were able to fix atmospheric N (Paerl and Priscu 1998).

2.3.5.3. Effect of Freezing and Desiccation

Water act as and important buffer to temperature change by its large heat storage capacity (Elster 2002).

Algae which colonize the shallow ephemeral meltwater streams of Antarctica are exposed to prolonged winter freezing and might be expected to show some degree of resistance to, or tolerance of, freezing in Antarctic algae compared to temperate algal species (Holm-Hansen 1963).

Ponds in continental Antarctica freeze from the top down, hence there is a period in autumn as temperature decline when they are ice covered, but still contain liquid water. Associated with the decline in temperature there is likely to be a decrease in irradiance, due to both declining incident radiation and the effect of the ice and any snow cover, possibly an increase in concentration of dissolved salts, caused by exclusion during ice formation, and depletion of dissolved oxygen (Hawes et al. 1999).

There are two types of freeze-thaw cycle; numerous diurnal cycling to 0° to –4°C during summer (Chambers 1966; Walton 1977) and annual freeze to –15° to –20° for long periods (Hawes 1990). Based on experimental data, summer freezing has little deleterious effect, while winter conditions must result in very extensive mortality (Hawes 1990).

Many mats with *Phormidium* freeze at the end of summer and remain as dry crust exposed to the winter ambient temperatures, which can be as low as –50°C. These mats then rehydrate during the next spring and may act as an innoculum for summer mat growth (Vincent and Howard-Williams 1986).

During exposure, the mats are subject to other stresses in addition to low water potential – salt stress – can be more injurious than simple water stress (e.g. De Winder et al. 1989) and high radiation fluxes. High solar radiation flux may be particularly injurious to cyanobacteria while they are partially hydrated (Deming-Adams et al. 1990)

Continued activity during wintertime would ensure a positive selection process for cold adaptation rather than only a destructive one against freeze-intolerant organisms (Deming 2002).

2.4. Effect of Freezing and Desiccation on Cyanobacterial and Algal Cells

It has been suggested that both extracellular freezing and desiccation act in a similar manner, that is through the deprivation of free water to the plant tissues and increased osmotic stress (Burke et al. 1976)

Dry tolerance is determined mostly by the ability to restore photosynthesis and respiration after rehydration. Furthermore a decrease of photosynthesis under desiccation is caused by interruption of electron transport in the chloroplasts (Wiltens et al. 1978). A similar event in mitochondria may be the reason for the reduction of respiration rates (Jacob et al. 1992). Once consequence of desiccation might be oxidative stress, i.e., the accumultion of reactive oxygen species (ROS), which damage cellular structure. It has been shown that the formation of superoxide radical increases under water stress in a number of species and there was an increase in oxidative damage in thylakoids of water-stressed leaves (Tambussi et al. 2000). Upon reintroduction of water, the metabolic recovery of desiccated-cells depends on protective mechanism set in place during drying and the repairing mechanism taking place during and following rehydration. The photosynthetic apparatus is very sensitive and liable to injury, and needs to be maintained or quickly repaired upon rehydration (Qiu et al. 2003). Ascorbate is the main component against oxidative stress in higher plants, however, its occurrence in cyanobacteria is

speculative (Asada 2000; Qiu et al. 2003) and it is believed that superoxide dismutase (SOD) play the important repairing role (Qiu et al. 2003).

It has been discovered that spores, microscopic animals, mosses and plant seeds accumulate large amounts of soluble carbohydrates, particularly trehalose, sucrose and oligosacharides (Wendell 1984). Such sugars are able to substitute water molecules during dehydration; therefore they stabilize the structures and functions of macromolecules, membranes, and cellular organization (Crowe et al. 1984).

Freezing damage resulted in the release of soluble sugars from the algal cells (Hawes 1990). The similarity between extent of solute loss and depression of photosynthesis supports the view that the plasma membrane is a primary location for freeze-thaw damage (Steponkus 1984). Antifreeze agents, such as polyols were found in *Phormidium* (Tearle 1987).

It is generally believed that there are two major physical or physico-chemical threats that the bacterium needs to respond to in freezing temperature regimes. The first is ice formation within the cell, which might lead to cell lysis because of the volume increase on expansion of water as ice is formed. The second is the increased salinity outside the cell as ice formation leads to the separating out of pure water (as ice) and a corresponding increase in salt concentration leading to an osmotic gradient across the cell membrane. It is thought that a general mechanism to counteract both of these phenomena would be an increase of solute concentration inside of the bacterial cell. The generally accepted adaptative response is an increase in the intracellular amounts of certain metabolites, especially glycine betaine, glycerol, manitol, and sorbitol, both as cryoprotectants and as osmolytes (Mindock et al 2001 and citations therein).

Nowadays, the research is focused mainly on organisms from the Polar Regions. It is thought that they might produce some special chemical compounds to avoid the freezing damage on their cells. Indeed, extracts of several Antarctic photosynthetic organisms contain macromolecules that modify the shape of growing ice crystals (Raymond and Fritsen 2000).

These ice-active substances (IASs) have been found in cyanobacterial mats, lichens, eukaryotic green algae and mosses but not in cyanobacteria or mosses from temperate climates. Their ability to affect the shape of ice and their preferential retention by centrifuged ice indicate that they are ice-binding molecules. Little is known about their chemical nature, although it is obvious that they have a glykoprotein component (Raymond and Fritsen 2001). The IASs do not significantly lower the freezing point at their natural concentrations. In each of these properties, they strongly resemble other IASs that have been found associated with Antarctic sea ice diatoms (Raymond 2000). The role of these proteins is unclear but they may mitigate physical damage by ice (Knight et al. 1995; Thomashow 1998). In view of the absence of freezing point depressing activity of the

IASs, the finding that they are strong inhibitors of recrystallization raises the possibility that they protect against mechanical damage to cell membranes that otherwise might occur during recrystallization. In terrestrial environments, where extreme subzero temperatures are possible, such a mechanism would appear to be more useful than freezing point depressing mechanisms, which typically provide only a few degrees of protection. Althought the IASs are effective at preventing ice recrystallization, other functions cannot be ruled out. For example, as membrane damage is thought to result primarily from the severe dehydration that occurs during freezing (Thomashow 1999), ice binding proteins might help to prevent damage, at least during short-term freezing episodes, by retarding the accretion of extracellular ice (Raymond and Fritsen 2001).

3. Materials and methods

3.1. Sample collection, culturing and isolate maintenance

Algal and cyanobacterial mats from several types of continental and maritime Antarctic wetland habitats were collected by Josef Elster at the end of summer season in March 2002. Localities from continental Antarctica were situated on Trump Island and near Rothera and Palmer research stations and therefore named after them. Likewise, the localities from maritime Antarctica were named after adjacent research station Jubany and Signy. Map of the Antarctic region with marked localities is displayed on Fig 1. All collected samples are listed in Appendix 1. Monthly mean air temperatures for the last twenty years of the studied areas, measured on adjacent research stations, are shown in Fig 2.

Samples of mats were transported to laboratory in frozen stage. Small quantities of mat samples were placed into tubes containing liquid BG-11 culture medium (Rippka et al. 1979) or plates containing the same medium solidified with 2% agar or plates half-filled with liquid medium. The communities were all incubated in dim light (ca 50 $\mu mol.m^{-2}.s^{-1}$) at low temperature (12°C), and subsequently used for further characterization and experiments. Aliquots of the samples were also used to obtain unialgal cultures of dominant and subdominant species.

Isolation was performed on agar plates with BG-11 medium. The plates were streaked with a small amount of field material or with liquid samples pre-cultivated in tubes containing BG-11 medium. After few days of cultivation at 12 or 18°C, visible colonies of diverse algal-cyanobacterial species were observed and separately transferred to sterile agar tubes. Since that, pure strains (unialgal with low bacterial contamination) have been grown at 6°C and 30 $\mu mol.m^{-2}.s^{-1}$.

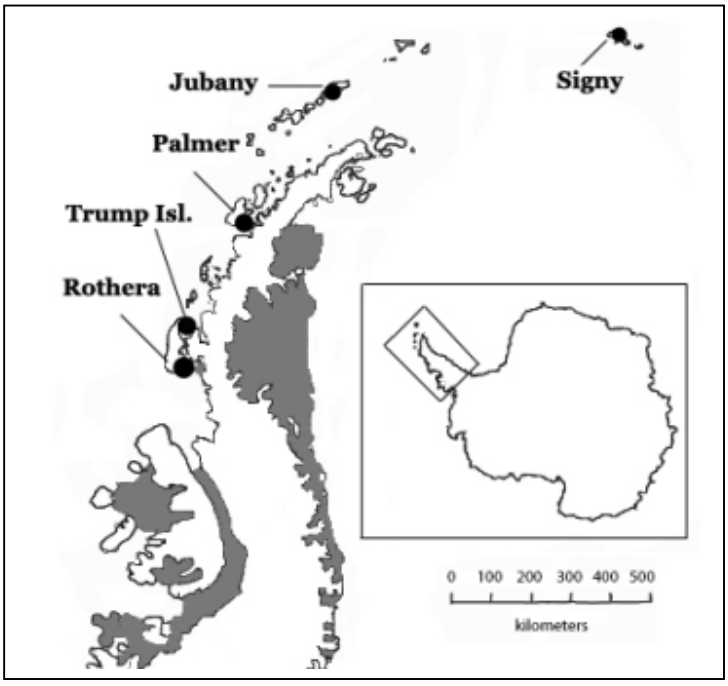


Fig 1 Map of Antarctic localities

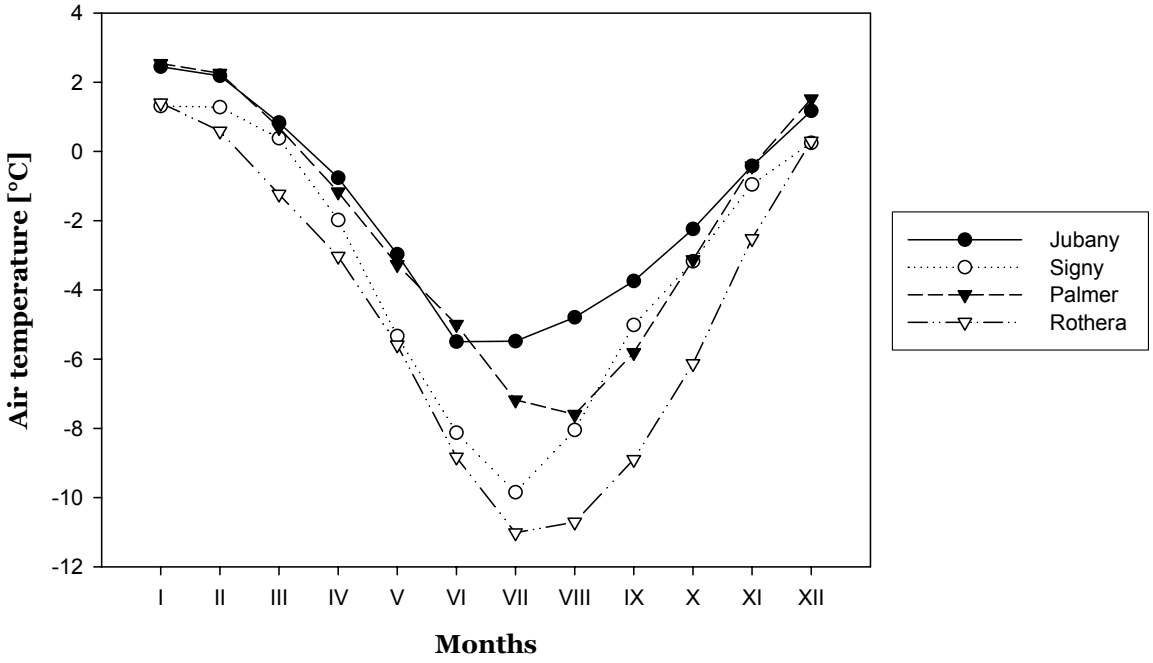


Fig 2 Monthly mean air temperatures for last twenty years, measured on four research stations close to the locations where the samples were collected. Decadal-length composite West Antarctic air temperature records (Shuman and Stearns 2002) were used for creating this graph.

3.2. Strains Determination

Isolated strains were regularly observed in light microscope Olympus CX 40, so as to detect all life forms and life-stages. Pictures of strains were taken using the digital camera Olympus DP 10 and subsequently processed with Adobe Photoshop 7.0. A wide range of literature was used for identifications of strains. Together with original descriptions made on material collected by members of first scientific British expeditions to Antarctica from the beginning of the 20th century (West and West 1911; Fritsch 1912, 1917), more modern studies were used for determining isolated strains into species. Both classical diagnostic keys, made for identification of cyanobacteria and algae of the whole world (Geitler 1932; Ettl and Gärtner 1995) and studies focused on determination of algae and cyanobacteria from specific Antarctic sites (Broady 1979, 1996; Broady and Ohtani 1990; Broady and Kibblewhitte 1991; Mataloni and Tesolín 1997; Broady and Weinstein 1998; Kawecka 1998; Komárek 1999; Cavacini 2001; Mataloni and Pose 2001; Mataloni and Tell 2002) were used.

The determination of strains belonging to the genus *Phormidium* into species demands a detailed morphological study, preferably with combination of molecular biology methods. Therefore, *Phormidium* strains were carefully described and impor-

tant quantitave features (cells width, length, attenuation of apical cells) were measured using Olympus DP soft. In all cases more than 100 individual trichomes of each strain was measured. For statistical analysis of measured data, ANOVA/MANOVA - Statistica 5.5. was used. Results of morphological evaluation were compared with West and West (1911); Fritsch (1917); Broady et al. (1984); Broady and Kibblewhitte (1991) and Komárek (1999).

3.3. Freezing and Desiccation Experiments

Viability after freezing and desiccation was tested in both isolated algal/cyanobacterial strains and natural samples.

Dense suspension of algae in 2 ml cryovial tubes was placed in a freezer (Planer Kryo 10 programmable freezer, Planer UK) and exposed to several freezing regimes (Table 1) simulating natural and subnatural freeze-thaw cycles. Two aliquots were desiccated for a week in a desiccator at 0°C and 20°C, respectively.

Viability evaluation was based on controlled cultivation of algal colonies on agar plates (Lukavský 1975). After exposure to described regimes, cryovials with algae were either quickly melted in lukewarm water bath for ca 2-5min (if frozen), or resuspended in ca 0.5 mL of liquid medium (if desiccated), whereupon kept in the dark for twenty-four hours so as to prevent light injury. After that the inoculum of 0.1 mL was uniformly spread by a glass rod on an agar plate with the BG11 nutrient solution solidified with 2% agar. Agar plates were maintained in identical unit and conditions as pre-cultivation for ca 5 days and evaluated under the light microscope as

$V = (N_C \times 100)/(N_C + N_D)$ for coccoid species,

where V is the viability in %, N_C the number of colonies and N_D the number of dead cells.

$V = N_L/(N_L \times N_D) \times 100$ for filamentous species,

where V is the viability in %, N_L the number of living cells in trichome and N_D the number of dead cells in trichome.

The resultant viability was converted to the viability of reference sample, sample that was inoculated on sterile agar plate but not exposed to any of the freezing and desiccation regimes.

In natural samples qualitative changes in community structures were assessed both after 5 days of cultivation at 6°C and after another 10 days in 12°C.

Table 1 Used regimes of freezing and desiccation. T_{start} = starting temperature of given phase, T_{final} = final temperature of given phase

		frozen -4°C	**frozen -40°C**	**frozen -100°C**	**direct in N_2**	**desiccated 0°C**	**desiccated +20°C**
Process:		freezing	freezing	freezing	freezing	desiccation	desiccation
Starting temperature:		20°C	20°C	20°C	20°C	20°C	20°C
Final temperature:		-4°C	-40°C	-100°C	-196°C	0°C	20°C
phase 1:	T_{start}	20°C	20°C	20°C	20°C	20°C	20°C
	T_{final}	0°C	0°C	0°C	-196°C	0°C	20°C
	rate	-4°C/min	-4°C/min	-4°C/min			
	time	5 min	5 min	5 min	60 min	1 week	1 week
phase 2:	T_{start}	0°C	0°C	0°C	-196°C		
	T_{final}	-4°C	-40°C	-40°C	20°C		
	rate	-5°C/min	-5°C/min	-5°C/min			
	time	1 min	8 min	8 min	5 min		
phase 3:	T_{start}	-4°C	-40°C	-40°C			
	T_{final}	-4°C	-40°C	-100°C			
	rate	0°C/min	0°C/min	-12°C/min			
	time	5 min	5 min	5 min			
phase 4:	T_{start}	-4°C	-40°C	-100°C			
	T_{final}	40°C	40°C	-100°C			
	rate			0°C/min			
	time	5 min	5 min	5 min			
phase 5:	T_{start}			-100°C			
	T_{final}			40°C			
	rate						
	time			5 min			
times repeated:		3	3	3	1	1	1

3.4. Surviving of Algal and Cyanobacterial Communities on Various Ice Substrata

In addition to laboratory experiments, the ability of algal/cyanobacterial communities to survive freezing and desiccation was tested in situ. The fieldwork took place during August and September 2003 and was located in Wedel Jarlsberg Land, southwest Spitsbergen, Svalbard (Fig 3).

Samples of the commonest cyanobacteria and algae from terrestrial wetland habitats of the coastal area of Hornsund bay, not farther than 3 km from the Werenskiöld

glacier, were collected. Composite sample was prepared by mixing all collected samples and filtering through a sieve (mesh 0,4 mm).

0,5 l of composite sample was directly spattered on the ice or snow at 7 localities, in a marked rectangle of a size ca 40 × 20 cm.

Localities were selected so as to represent different light and temperature conditions and ice substrata. Three localities (Upper snow-field, Lower snow-field and Werenskiöld) were situated on the snow or glacier surface, therefore the natural light was present there. The remaining four localities (Upper Kvisla-Locality 1, Upper Kvisla-Locality 2, Lower Kvisla and Tone cave) were situated under the glacier surface where no light could penetrate, at least no measurable calibre using standard Lux meter. The selected localities also differ in the type of the ice substratum they provided: firstly "Snow" substratum, secondly "Firn", a well-bonded, high-density snow older than one year; and thirdly the smooth "Ice"-cover. A map of all localities is in Fig 3 and their characteristics are described in Table 2.

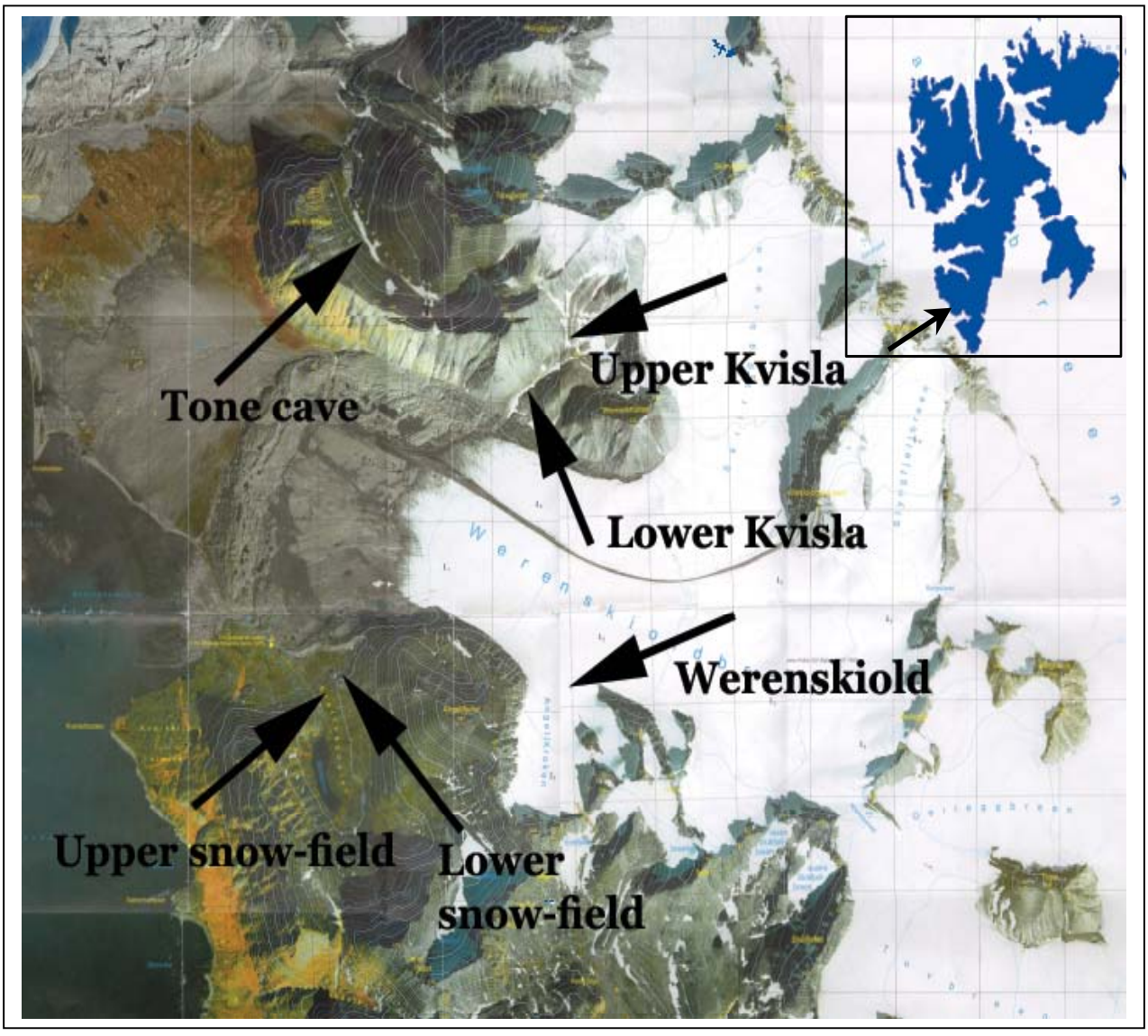


Fig 3 Map of Hornsund, Svalbard localities where the composite sample was spattered. The 1990-orthophotomap by Jania et al. (2002) - modified.

Table 2 Characterization of selected localities. The position was determined with an eTrex GPS navigator (Garmin, USA).

Locality	Geograph. position	Presence of light	Ice substratum	Characterization
Upper snow-field	N 77°03.941' E 15°09.756'	+	Snow	Snow-field on the slope of Mt. Gullilksenfjellet
Lower snow-field	N 77°03.971' E 15°09.779'	+	Snow	Snow-field on the slope of Mt. Gullilksenfjellet
Werenskiöld	N 77°04.087' E 15°19.331'	+	Ice	Ice area on the Werenskioldbreen glacier, in front of Eimfjelet
Upper Kvisla Locality 1	N 77°04'42" E 18°15'03"	-	Firn	Ice cap in the cave under Wernerbreen glacier
Upper Kvisla Locality 2	N 77°04'42" E 18°15'03"	-	Firn	Ice shelf in the cave under Wernerbreen glacier
Lower Kvisla	N 77°04'41" E 18°07'08"	-	Firn	High Ice shelf in the cave under Wernerbreen glacier
Tone cave	N 77°06.499' E 15°13.497'	-	Ice	Ice cap close to the exit from the cave under Tonefjellbreen

After approximately 1, 2 and 4 weeks, 20ml (2x) of the inoculated ice was picked up into sterile plastic tubes. Tubes were melted at room temperature and part of it was used for immediate microscopical examination with a field microscope (Leitz HM-Lux). The rest was fixed with 1,5% formaldehyde and later evaluated in laboratory: all viable cells were counted in 500 sight views and determined using an Olympus CX 40 light microscope (magnitude 400×). In filamentous algae and cyanobacteria cells within the trichome were counted, with an exception of cyanobacterium *Leptolyngbya*, where trichome census was made.

For statistical analysis numbers of viable cells were converted to the more suitable and comparable abundance-scale (scale from 1 to 7) describing the average number of cell per sight view (Table 3).

3.5. Statistical Analysis

Both data from freezing-desiccation experiment and "surviving of algae and cyanobacteria on ice substrata" experiment were tested using the multivariate direct ordination technique of canonical correspondence analysis (ter Braak and Šmilauer (2002). This method was chosen because its asymmetrical approach considers biological data as response variables, and relates them directly to the independent (environmental) variables (Legendre and Legendre 1998).

In the freezing-desiccation experiment both Principal Components Analysis (PCA) and Redundancy Analysis (RDA) were used to analyse the data. Whereas PCA is use-

ful for discerning patterns within the species viability data itself, RDA can be used to test hypotheses regarding the importance of external factors (locality, habitat and type of organism) in explaining variations in viability data (ter Braak 1987). The Monte Carlo permutation test – 499 permutations (ter Braak 1990) was used to test the statistical significance of the relationship between environmental variables and variations in species vitality.

In the experiment where survival of algae and cyanobacteria on various types of ice substrata were tested, Redundancy Analysis (RDA) with forward selection was used to create a model explaining variability in community structure and total abundance data. Monte-Carlo permutation test (499 permutations) was applied to compute significance of hypothetic relations. Abundance of each species was used as predicted values and locality, type of ice substratum and presence of light were used as predictors. Additionally, PCA was used for describing the axes of maximum variability in the multivariate data set.

All calculations and the ordination biplots were made using multivariate data analysis software CANOCO for Windows (ter Braak and Šmilauer, 2002) and CANODRAW 4.0.

4. Results

4.1. Isolation and Determination of Strains

Seventeen pure strains of cyanobacteria and eleven pure strains of green algae were isolated. Others, mainly green algal strains, were if possible determined into genera, but were not isolated into unialgal strains.

All isolated cyanobacterial strains were simple filamentous species from the order Oscillatoriales (Anagnostidis and Komárek 1988). Fourteen of these strains were identified as the genus *Phormidium* and therefore labelled as "Ph" and number of sample (Ph10, Ph12, Ph16, Ph21, Ph28, Ph31, Ph32, Ph38, Ph43, Ph50, Ph53, Ph54, Ph58, Ph68), two as the genus *Leptolyngbya* (LPP28 and LPP31) and one remained unidentified (Bl50). However, the determination of oscillatorian cyanobacteria is very difficult and demands a thorough morphological evaluation of quantitative and qualitative features.

Isolated strains of algae were mostly coccoid – *Chlorella* sp. (Chl16, Chl21, Chl57, Chl69), *Chlorella minutissima* (Chlmin48, Chlmin57, Chlmin59), cf. *Chlorosarcina* (Chlsar22, Chlsar24) and *Pseudococcomyxa simplex* (Pscoc 62). One strain was filamentous – *Klebsormidium* sp. (Kl16).

4.2. Morphology of Oscillatorian Cyanobacteria

Trichome width, cell length and cell width to length ratio were measured and statistically compared. In addition, strains colour, mat appearance, number of trichomes in sheath, trichome shape, presence of calyptra, terminal attenuation of trichome, shape of apical cell and constrictions at transverse walls were recorded. Summary of all observed features for all studied strains is described in Appendix 2 and their pictures in Appendix 3.

Measuring of trichome width (Fig 4), useful morphological feature when comparing into genera, and its statistical comparison distributed the strains into three groups. Same happened when compared the cell width to cell length (Fig 5), also very characteristic and practical feature for determining filamentous cyanobacteria. Cell length is a very changeable feature and therefore less suitable for determination (Volf 2002).

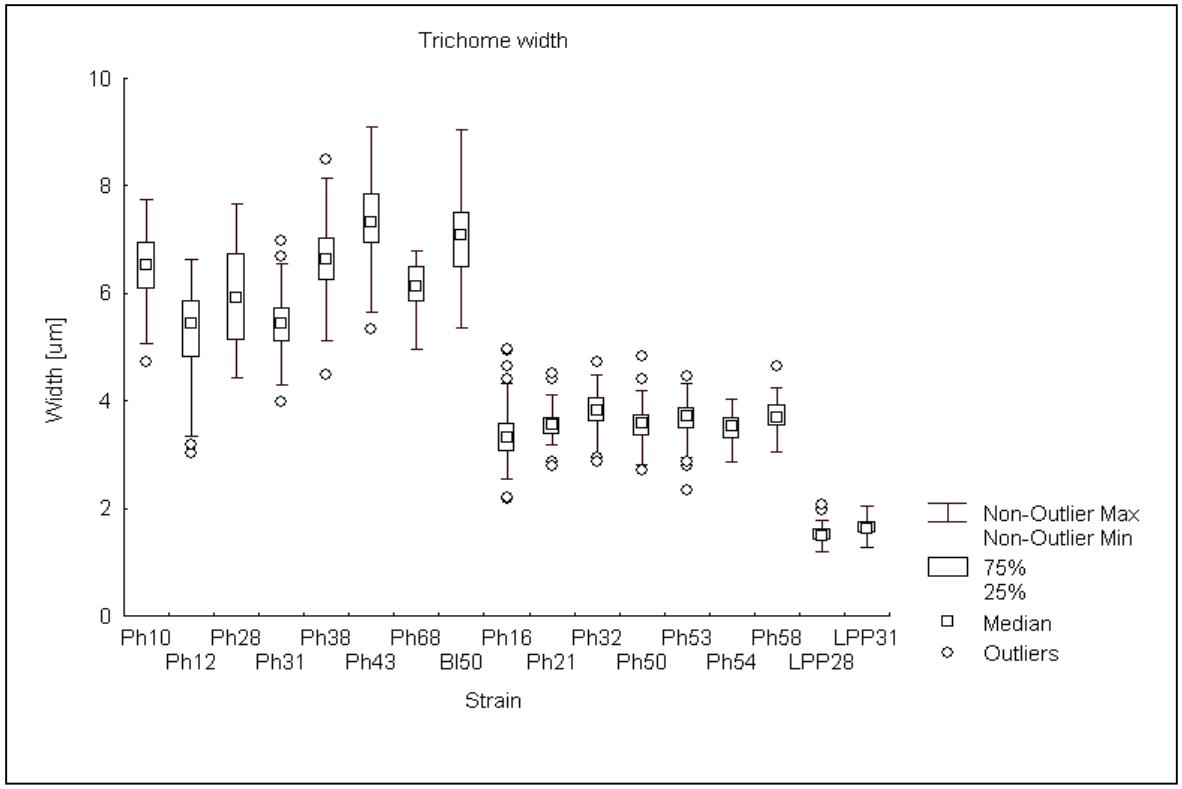


Fig 4 Comparison of trichome width of all studied oscillatorian strains using ANOVA/MANOVA ($p << 0,01$; $F = 886,29$)

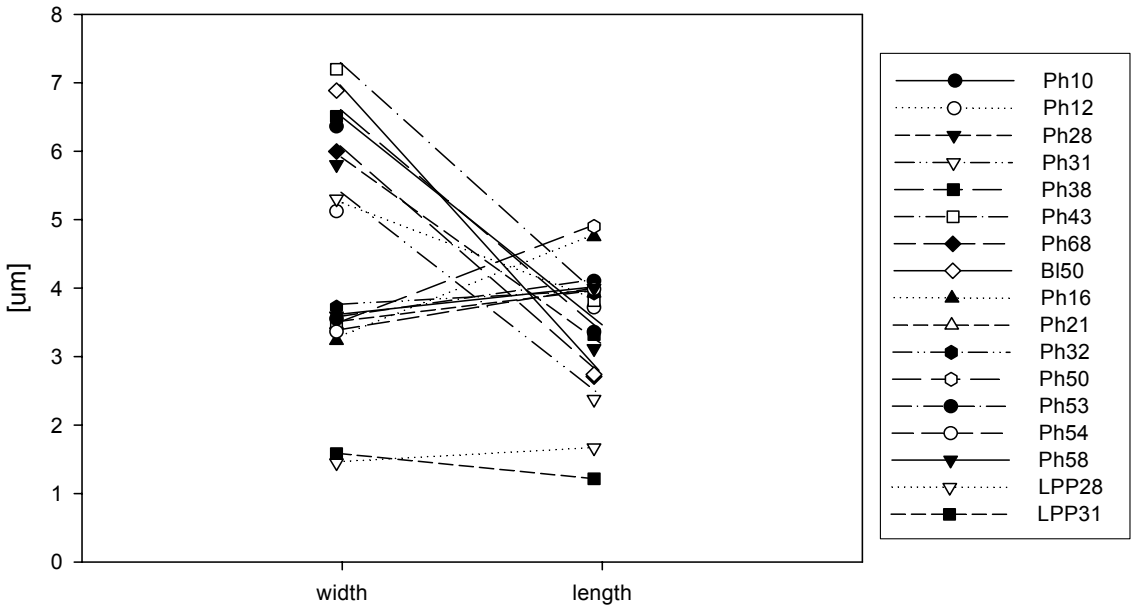


Fig 5 Mean cell width/ length ration of cells

The first cluster grouped together eight morphologically variable strains with thick trichomes. Seven of them (Ph10, Ph12, Ph28, Ph31, Ph38 and Ph43) were identified as species from the *Phormidium autumnale* sensu lato group. The last strain (Bl50), isolated from seepages, was completely different from others both in the type of macroscopical growth and in the microscopical trichome appearance. Therefore the Bl50 strain was separated from this group and described as morphotype A.

The strain Ph28, isolated from seepages, differed from other strains, mainly because of presence of distinct firm not adhering sheaths and gradually attenuated and curved apical parts. This type was described as morphotype B.

The rest six strains (Ph10, Ph12, Ph31, Ph38, Ph43 and Ph68) differed significantly in cell width ($p << 0,01$; $F = 97,11$), cell length ($p << 0,01$; $F = 41,90$) and cell width/length ratio ($p << 0,01$; $F = 40,42$). Nevertheless, they were morphologically similar with slight differences according to habitat origin. They were grouped together and described as morphotype C.

The second cluster grouped together seven strains: Ph16, Ph21, Ph32, Ph50, Ph53, Ph54 and Ph 58. All these strains were isolated from wetlands or seepages habitat with one exception in Ph16, which was isolated from ornithogenous soil. Even thought they significantly differed between each other in cell width ($p << 0,01$; $F = 38,84$), cell length ($p << 0,01$; $F = 24,31$) and cell width/length ratio ($p << 0,01$; $F = 26,61$), none of these strains were distinguishable from the others, when compared strain to strain with post hoc comparison Tukey honest significant difference test (HSD). This cluster grouped together morphologically very similar strains with characteristic macroscopical growth on plates. Therefore they were grouped together and described as morphotype D.

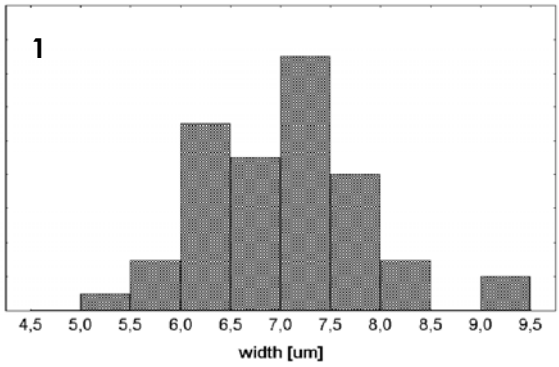
The third cluster grouped together two morphologically almost identical strains (LPP28 and LPP31) with thin trichomes. They were described as morphotype E.

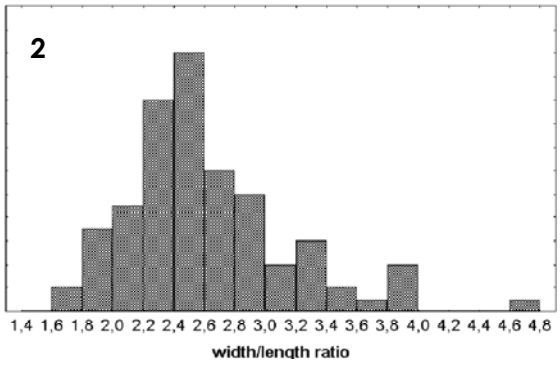
Morphotype A – Characteristics

strain Bl50, Fig 6

Brown trichomes were growing around the agar plate and formed circles. Mostly straight trichomes, 5,36-9,03 μm (median 7,08 μm) wide. Short cells, 1,64-4,71 μm (median 2,88 μm) length, always shorter than wide - 1,71-4,78 μm (median 2,51 μm) cell width/length ratio. Sheath present, distinct, sometimes not adhering. 1, rarely 2 or 3 trichomes in sheet were present. Calyptra absent, apical cell mostly attenuated and often curved. Gradual attenuation of apical parts often present.

MORPHOTYPE A





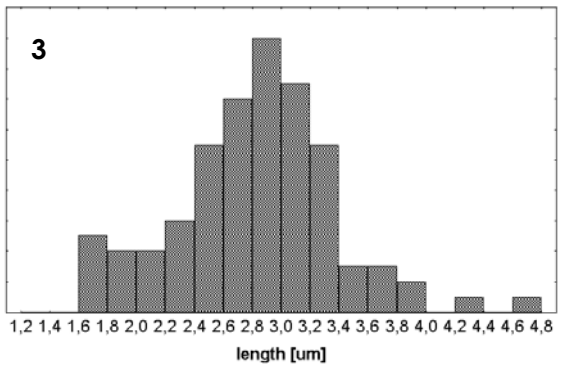
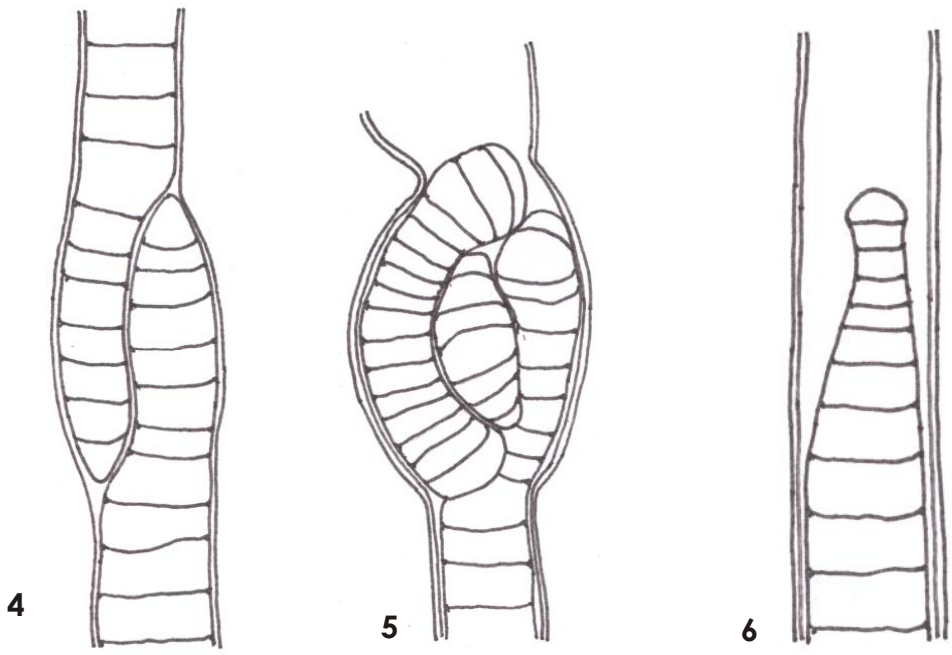


Fig 6 Morphotype A, 1 - cell width; 2 - cell width/length ratio; 3 - cell length; 4, 5 and 6 - drawings of typical trichomes



Morphotype B – Characteristics

strain Ph28, Fig 7

Ring macroscopical growth on the plates, typical for *P. autumnale* sensu lato. Mostly straight trichomes, 4,42-7,68 µm (median 5,92 µm) wide, with a slight terminal hook over 2-8 cells. Short cells, 1,82-5,74 µm (median 3,06 µm) length, mostly isodiametric - 0,95-3,83 µm (median 1,82 µm) cell width/length ratio. Sheath present, distinct, sometimes not adhering. Calyptra absent, apical cell gradually attenuated and curved.

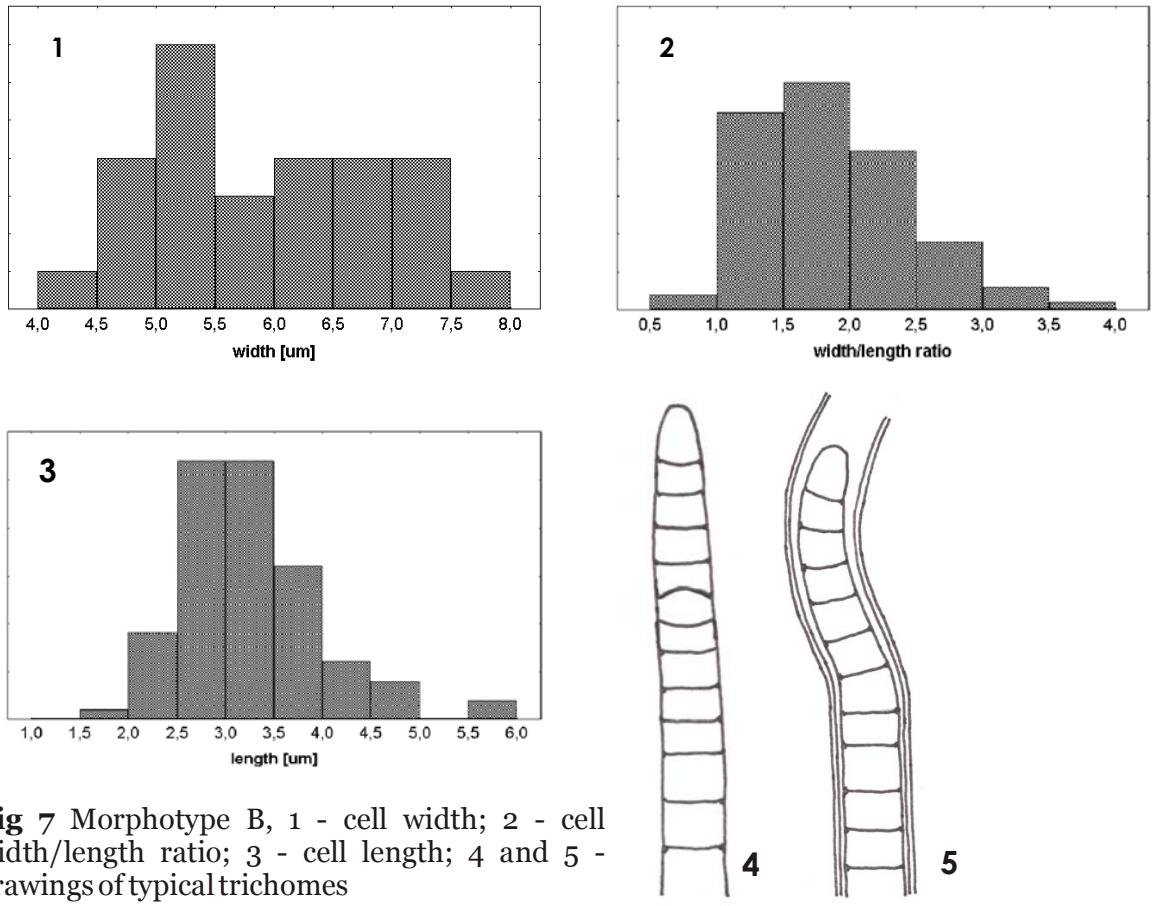


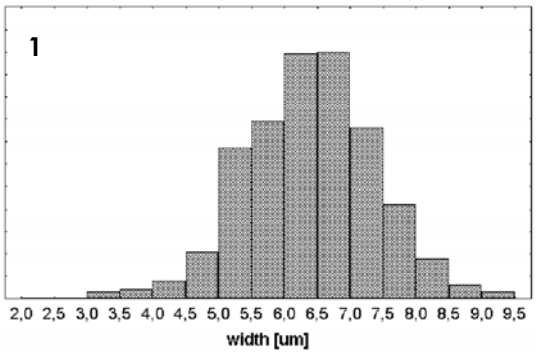
Fig 7 Morphotype B, 1 - cell width; 2 - cell width/length ratio; 3 - cell length; 4 and 5 - drawings of typical trichomes

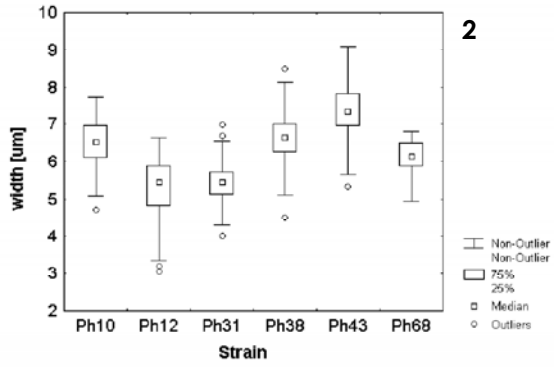
Morphotype C – Characteristics

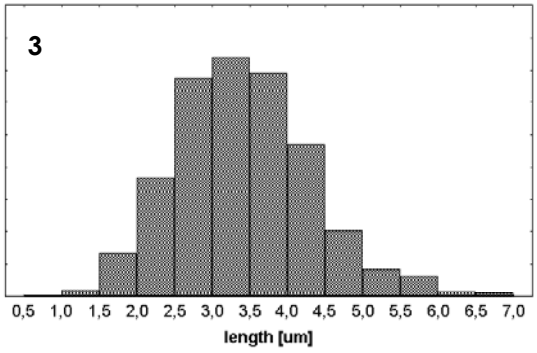
strains Ph10, Ph12, Ph31, Ph38, Ph43 and Ph68, Fig 8

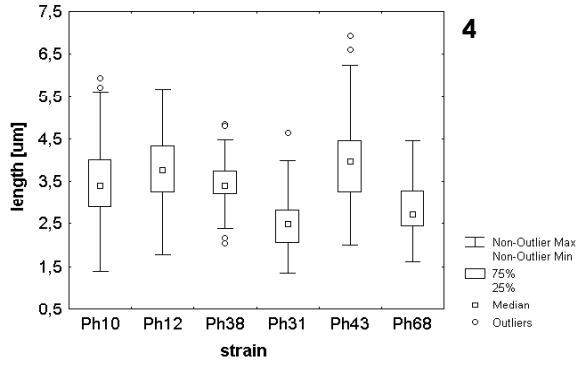
Ring macroscopical growth on the plates, characteristic for *P. autumnale* sensu lato. Mostly straight trichomes 3,03-9,09 µm (median 6,39 µm) wide with a slight terminal hook over 2-8 cells. Cells more or less isodiametric, 1,35-6,94 µm (median 3,23 µm) length, or mostly slightly shorter than wide - 0,53-4,82 µm (median 1,97 µm) cell width/length ratio. Thin sheath mostly present. Calyptra often present, apical cell slightly attenuated. Apical cell broadly rounded to slightly attenuated to slightly conical.

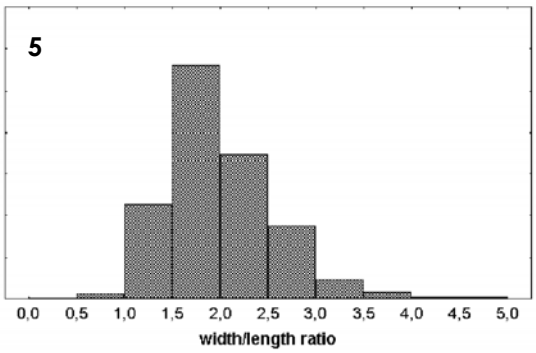
MORPHOTYPE C

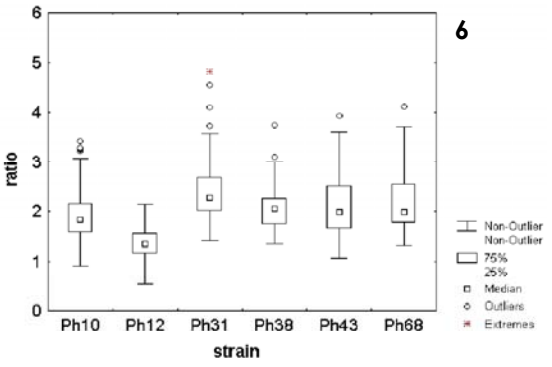












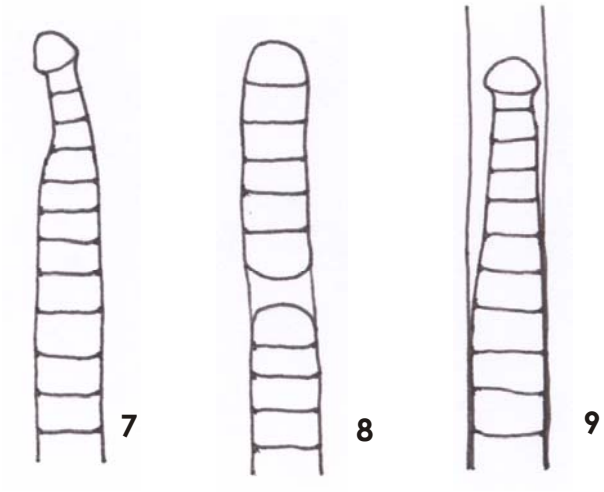


Fig 8 Morphotype C, 1 - cell width, 2 - box and whisker plots of cell width of each strain; 3 - cell length; 4 - box and whisker plots of cell length of each strain; 5 - cell width/length ratio; 6 - box and whisker plots of cell width/length ratio of each strain; 7, 8 and 9 - drawings of typical trichomes

Morphotype D - Characteristics

Strains Ph16, Ph21, Ph32, Ph50, Ph53, Ph54 and Ph58, Fig 9 and Fig 10

Light green mats growing in straight bands from one corner of the plate to the other. Trichomes usually vertically sticking out of the plate.

Light blue-green trichomes 2,18-4,96 μm (median 3,61 μm) wide, straight to flexuous, mostly isodiametric cells –1,99-8,12 μm (median 4,28 μm) length or cells little bit longer – 0,36-2,14 μm (median 0,84 μm) cell width/length ratio. Straight terminal region of trichome with no attenuation, sheath absent or thin, firm and distinct. Apical cell bluntly rounded to slightly conical, no distinct granulation of cells, calyptra absent.

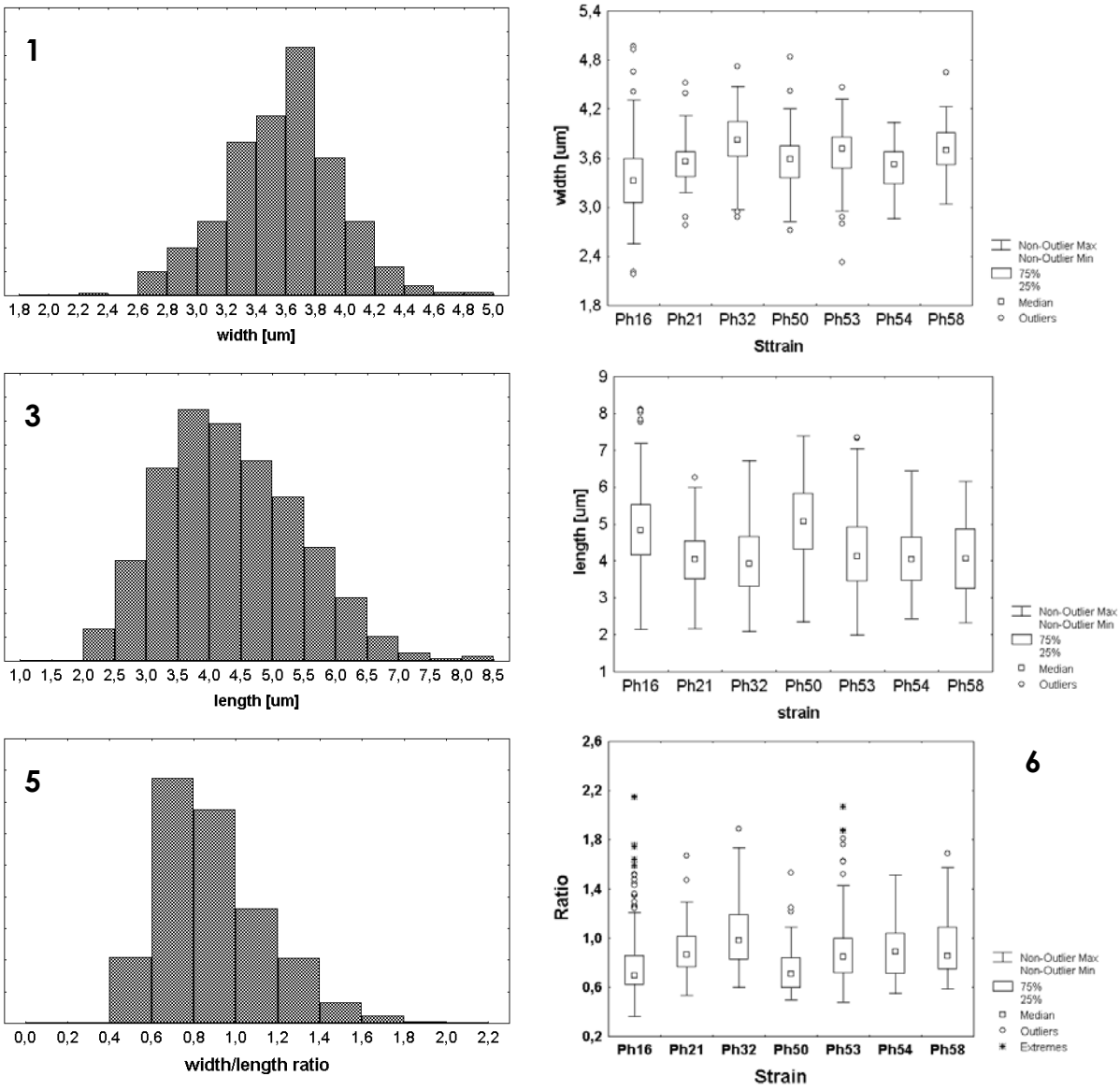


Fig 9 Morphotype D, 1 - cell width, 2 - box and whisker plots of cell width of each strain; 3 - cell length; 4 - box and whisker plots of cell length of each strain; 5 - cell width/length ratio; 6 - box and whisker plots of cell width/length ratio of each strain

MORPHOTYPE D

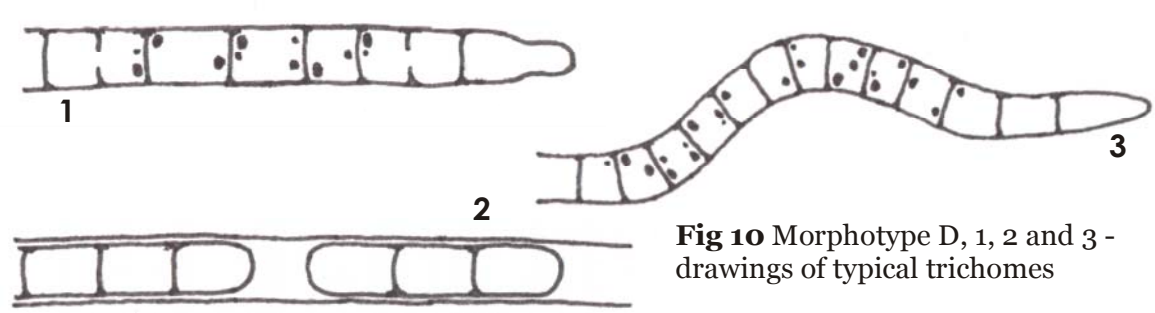
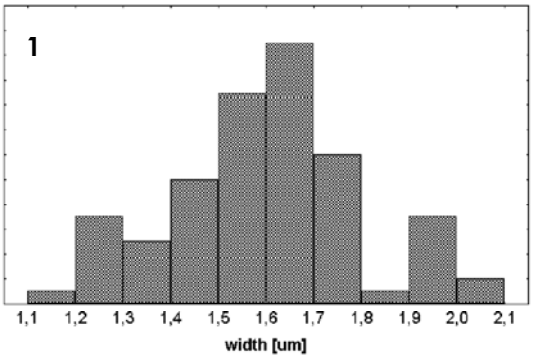


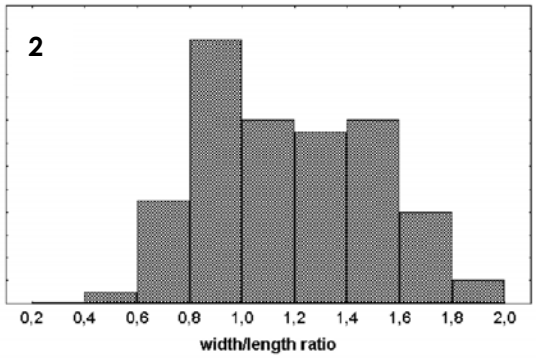
Fig 10 Morphotype D, 1, 2 and 3 - drawings of typical trichomes

Morphotype E – Characteristics

Strains LPP28 and LPP31, Fig 11

Minute twisted trichomes 1,20-2,08 μm (median 1,61 μm) width, cells approximately isodiametric, 0,89-2,51 μm (median 1,42 μm) length, cell width/length ratio: 0,50-1,96 μm (median 1,13 μm). Distinct constrictions at transverse wall, no attenuation, apical cell broadly rounded, sheath absent.





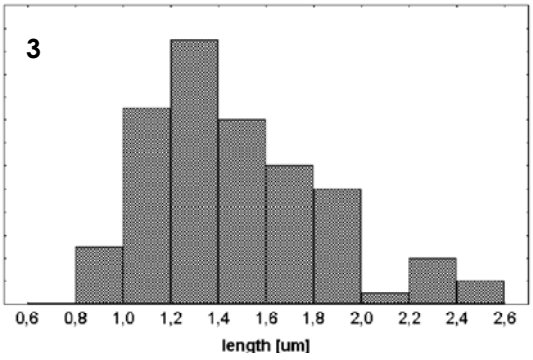


Fig 11 Morphotype E, 1 - cell width; 2 - cell width/length ratio; 3 - cell length; 4 - drawing of typical trichome

4.3. Freezing and Desiccation Tolerance of Strains

Fifteen strains of filamentous cyanobacteria and eleven strains of green algae were exposed to described freezing and desiccation regimes and their viability was tested. Summary graphs showing the viability of all cyanobacterial and algal strains are in Appendix 4, resp. Appendix 5.

It is evident that cyanobacterial strains were much more resistant to both freezing and desiccation than green algal strains. The viability of cyanobacterial strains was usually very high (on average around 90%), even when exposed to deep freezing. Sometimes the viability was higher than those of reference culture. When exposed to desiccation, the viability was usually lower and more diverse. Some strains seemed to be less tolerant to desiccation (viability around 20-40%) than others.

The viability of green algal strains after exposure to freezing was usually less than 50%, only when exposed to –4°C the viability was higher (on average 77%). When exposed to desiccation, the average viability was 14%. For both cyanobacteria and algae, the most injurious was the exposition to week desiccation at 20°C.

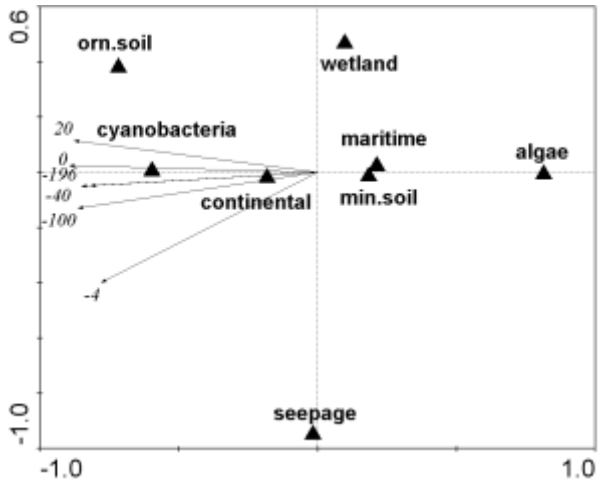


Fig 12 RDA diagram showing the response of cyanobacteria and algae to freezing (-4, -40, -100 and -196) and desiccation (0 and 20).

The response of strains to freezing and desiccation (Fig 12) was primarily determined by the type of organism (cyanobacterium or alga) exposed to the freezing or desiccation stress. In the RDA, this component captured 73,5 % of the total variance and was the only significant factor (p = 0,002; F = 66,726). The whole model was interpreting 77,1% of the total variance. The green algae were strongly negatively correlated with the deeper freezing.

However, whether the organism was a cyanobacterium or an alga was of such a high value in this analysis that the found trend could be negatively influenced by this fact and other significant factors may be hidden. Therefore a similar RDA analysis was made, where the environmental factors cyanobacteria and algae were set as co-variables, for both freezing and desiccation (Fig 13). The whole model was interpret-

ing 23,2%. Same was made for freezing tolerance (Fig 14), where the whole model interpreted 25,6%. Even though no environmental factor had significant influence in both models, there were clear tendencies apparent in Fig 12 and 13. It seemed that freezing favoured Antarctic microorganism, whereas those from maritime Antarctica seemed negatively correlated with deeper freezing. Additionally, algae and cyanobacteria from seepages seemed to be negatively correlated with deeper freezing and desiccation.

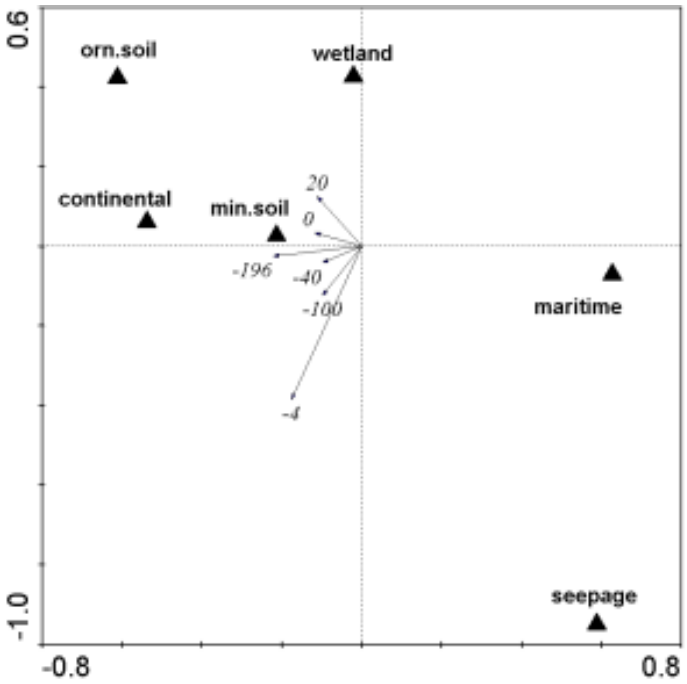


Fig 13 RDA diagram showing the response of cyanobacteria and algae to freezing (-4, -40, -100 and -196) and desiccation (0 and 20).

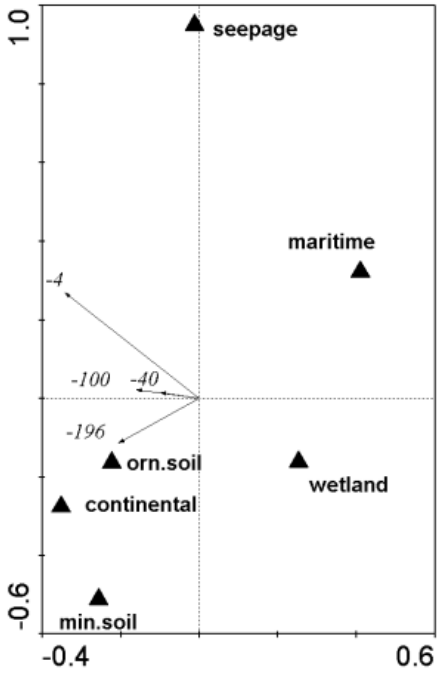


Fig 14 RDA diagram showing the response of cyanobacteria and algae to freezing.

Subsequently, PCA was used to compare either freezing or desiccation tolerance of both algae and cyanobacteria. The whole model interpreted 82,4%. As can be seen from the diagram on Fig 15, the tolerance to freezing was much more diverse among algae (marked by squares) than among cyanobacteria (marked by circles). Among cyanobacteria, tolerance to freezing was uniformly high.

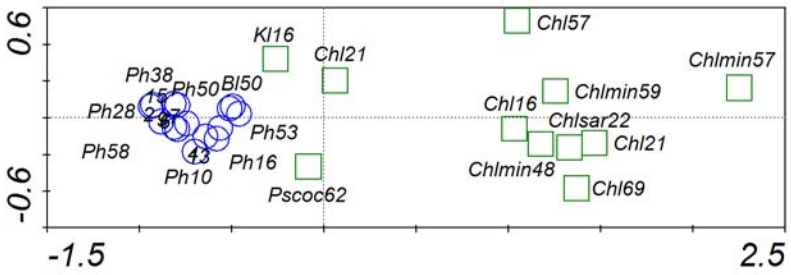


Fig 15 PCA diagram showing the response of cyanobacteria and algae to freezing.
Circles – cyanobacteria
Squares - algae

However, the tolerance to desiccation was more diverse among cyanobacteria than the freezing tolerance, as clear from PCA diagram on Fig 16. The whole model was interpreting 97,1%.

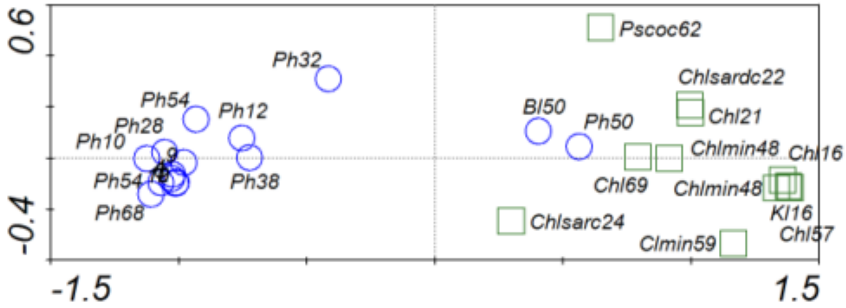


Fig 16 PCA diagram showing the response of cyanobacteria and algae to desiccation.
Circles – cyanobacteria
Squares - algae

Fig17 shows the RDA analysis of freezing and desiccation tolerance for cyanobacteria. The whole model was interpreting 33,5%. The habitat seepages captured 30,4% of the total variance and was the only significant factor (p = 0,002; F = 5,69). Cyanobacteria from this habitat seemed to be less tolerant to both freezing and desiccation than cyanobacteria from other habitats.

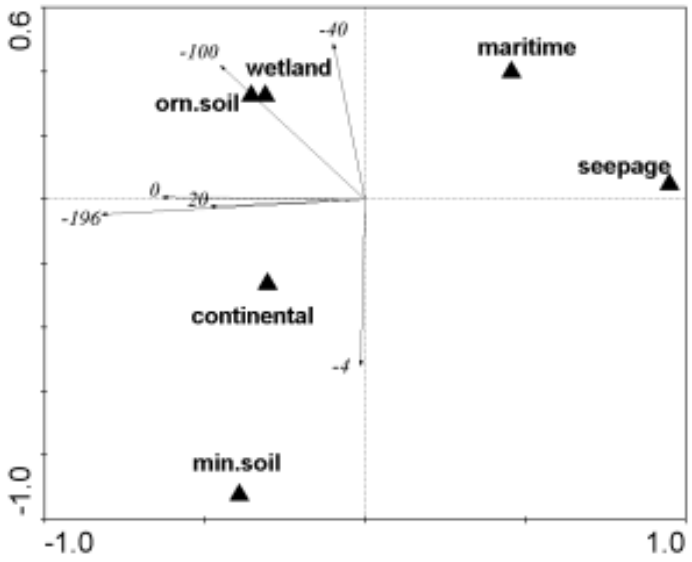


Fig 17 RDA diagram showing the response of cyanobacteria to freezing (-4, -40, -100 and -196) and desiccation (0 and 20).

When using the RDA to compare only the freezing tolerance of cyanobacteria among each other (Fig 18), it showed that again the habitat seepages was a significant factor (p = 0,002; F = 8,72), capturing 40,1% of total variance. The second significant factor was the geographical origin (continental and maritime Antarctica) of cyanobacteria (p = 0,026; F = 3,66), which captured 22% of total variance. Cyanobacteria isolated from continental Antarctica seemed to be more resistant to freezing than cyanobacteria isolate from maritime Antarctica. The whole model interpreted 53,3%.

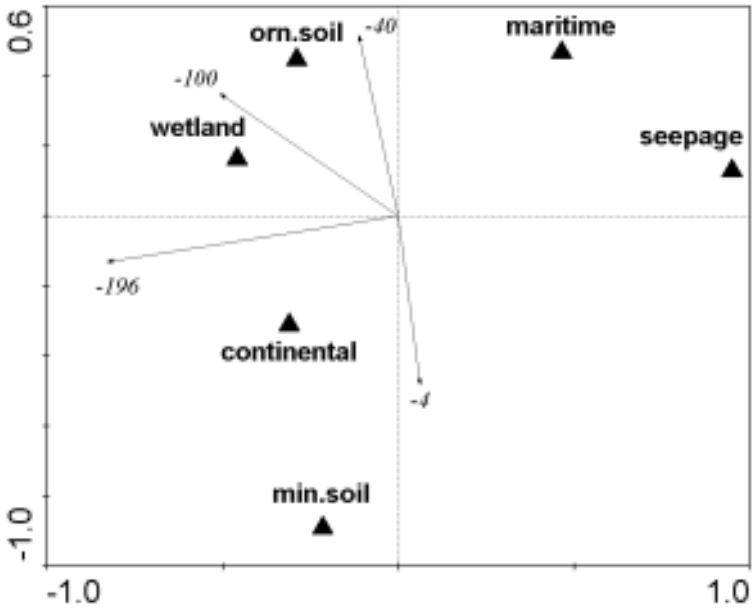


Fig 18 RDA diagram showing the response of cyanobacteria to freezing.

No significant components, however, significant trends, were found if the same analysis was applied to green algae.

4.4. Freezing and Desiccation Tolerance of Natural Samples

Observed changes in community structure were similar in all tested samples; therefore, in order to clarify the results, only three different samples are described in detail – two from wetlands (29 and 56) and one from mineral soil (33).

When exposed to freezing or desiccation, changes in community structure were observed after five days of cultivation at 6°C (described as "5 days" in graphs) and after another five days at 12°C (described as "10 days" in graphs).

Changes in communities of natural samples 29, 33 and 56 are shown in Fig 19, 20 and 21.

Natural sample 29 (Fig 19) was isolated from a wetland close to the Rothera station on Adelaide Island, continental Antarctica. Cyanobacteria captured more than 85% of total biomass in the control culture, whereof *Phormidum* sp. contained 75% and *Oscillatoria* sp. 10%. The rest (15%) was the biomass of green algae – *Chlorella* sp., *Prasiola crispa* and *Klebsormidium* sp. When frozen to –4°C, changes in community structure were very minor. Freezing to –4°C was the only regime that *Prasiola* was able to withstand. Deeper freezing (-40 and –100°C) reduced the biomass of the green alga *Chlorella*; the relative biomass of the rest was therefore higher. The relative biomass of green alga *Chlorella* was higher when cultivated longer in higher temperature (10 days). When directly immersed into liquid nitrogen (-196°C), only *Phormidium* was able to survive, or took the advantage of being the most abundant and resistant component of the sample and outcompeted the rest species. Desiccation was for green algae more injurious than freezing. Desiccation at 20°C was fatal for *Chlorella* and *Prasiola*.

Natural sample 33 (Fig 20) was isolated from a mineral soil on Trump Island, continental Antarctica. The control cultured contained of various green algae (*Chlorella* sp., *Klebsormidium* sp., cf. *Muriella*, *Prasiola crispa* and *Stichococcus* sp.), yellow-green alga *Xanthonem*a sp. and cyanobacterium *Leptolyngbya* sp., which contained less than 1% of the total biomass. Freezing to –4°C decrease the relative abundance of *Klebsormidium* and *Muriella* and increased the relative abundance of *Leptolyngbya* and *Chlorella*. As in the case on natural sample 29, *Prasiola* was able to restore its biomass only when exposed to –4°C. Deeper freezing markedly increased the relative biomass of *Leptolyngbya* and decreased the relative biomass of green algae. When subsequently cultivated at 12°C, the relative biomass of green algae begun to increase as they started to regenerate its damaged biomass. Desiccation was particularly injurious to green coccoid algae. However, the green filamentous alga *Klebsormidium* was very resistant to desiccation both at 0°C and at 20°C.

Natural sample 56 (Fig 21) was a mat isolated from the surface of mineral soil of King George Island, South Shetland Islands. The conditions of control culture favoured the growth of green algae cf. *Muriella*, *Prasiola crispa* and *Chlorella* sp. The latter was the most conspicuous component capturing ~ 85% of the total biomass.

Together with green algae, unidentified diatom, yellow-green alga *Xanthonema* and cyanobacterium *Leptolyngbya* were present. As in the case of natural sample 33, deeper freezing and desiccation markedly favoured the growth of *Leptolyngbya* compared to other components. After being cultivated at 12°C for another five days, the community began to restore its original structure.

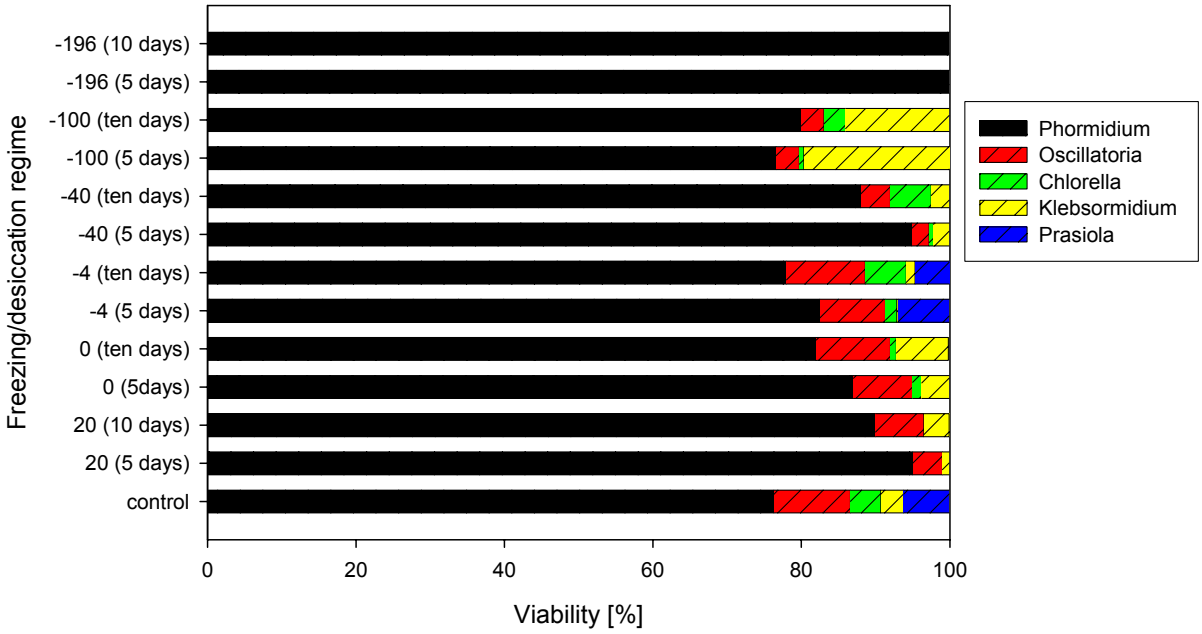


Fig 19 Changes in community structure in frozen or desiccated natural sample 29 from a wetland

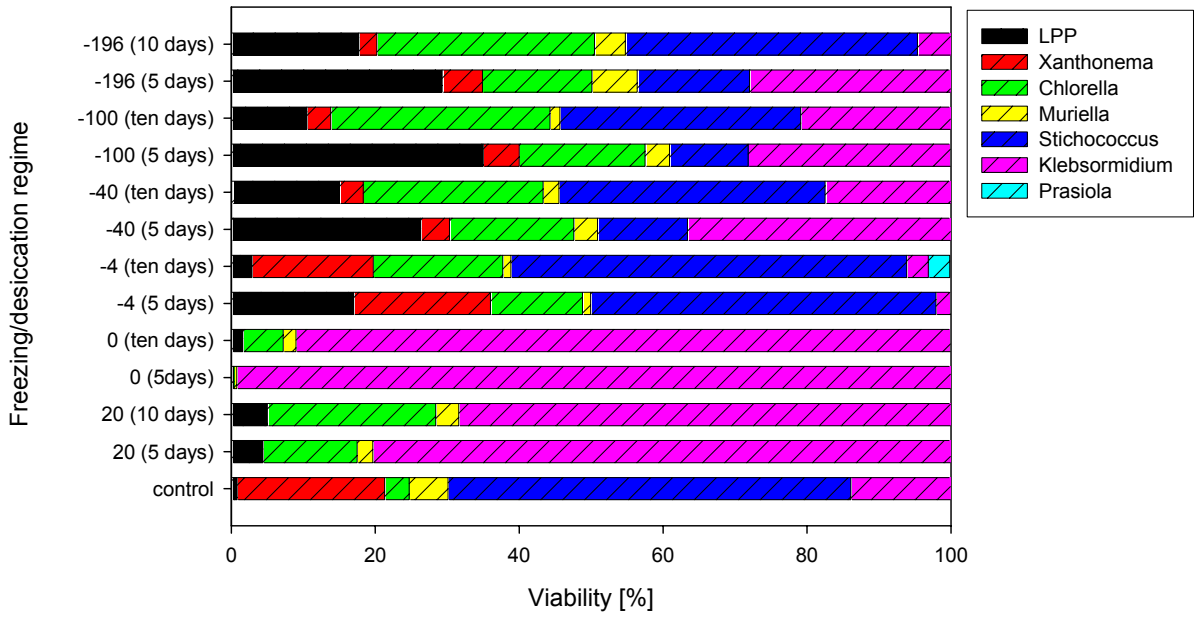


Fig 20 Changes in community structure in frozen or desiccated natural sample 33 from mineral soil

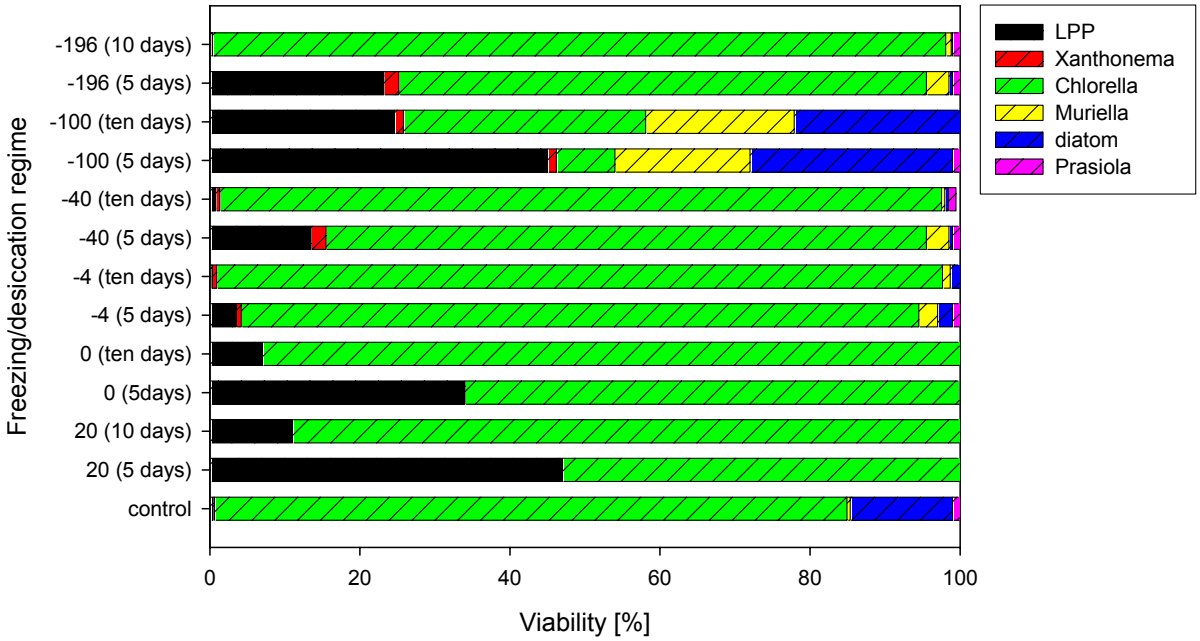


Fig 21 Changes in community structure in frozen or desiccated natural sample 56 from a wetland

4.5. Survival of Algal-Cyanobacterial Communities on Various Types of Ice Substrata

The composite sample of cyanobacteria and algae collected in Svalbard wetlands contained more than 40 genera of algae and cyanobacteria. The sample was spattered on the ice, firn and snow substrata in selected localities. Most of algal and cyanobacterial species were able to survive for a week on an ice substratum. After a month, most of the algae and cyanobacteria were dead. Nevertheless, some viable algae were found in all localities. Samples from Lower Kvisla cave could not be collected owing to weather conditions and will be collected next season. The most successful algae in surviving on some type of ice substratum were cyanobacteria *Phormidium* and *Leptolyngbya*, yellow-green alga *Tribonema* and some diatom species. While the viability of *Phormidium*, *Leptolyngbya* and *Tribonema* was uniformly high in all localities, diatoms were found only on some of them. From large amount of dead *Tribonema* trichomes and empty diatom frustules found, it was obvious, than less than 50% of cell survived the incubation.

Unsuccessful algae were mainly green filamentous algae, such as *Oedogonium*, *Zygnema*, *Mougeotia* and various green cococid algae.

RDA analysis (Fig 22) was used to analyse changes in community structure. The whole model was interpreting 53,5%. Three significant components were found. The first one – ice substratum (p = 0,004; F = 3,94) – captured 19,8% of total variance. The collection after month was the second significant factor (p = 0,016; F = 2,98) and captured 15,7% of total variance. The third significant factor was the firn substratum (p = 0,02; F = 2,86). It captured 5,2% of total variance.

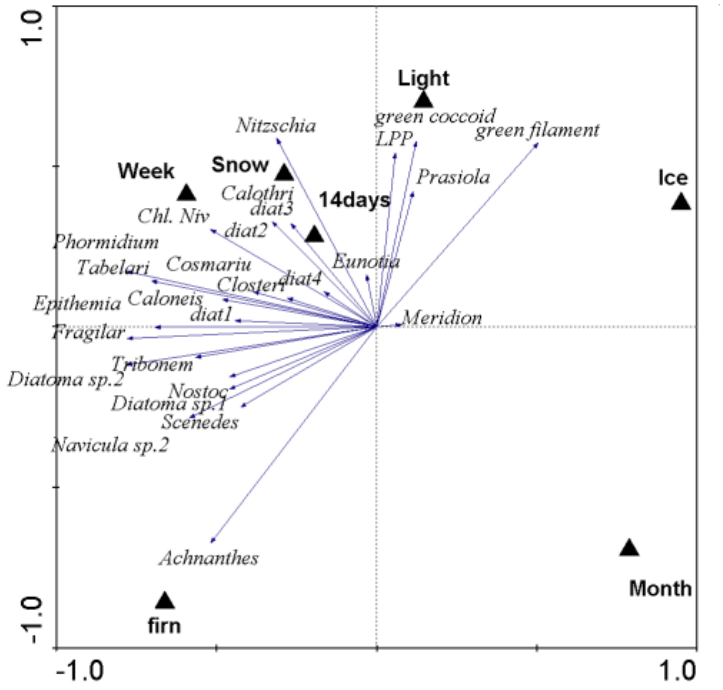


Fig 22 RDA diagram showing the changes in community structure after week, 14 days and month incubation on ice, firn and snow substrata in the presence or absence of light

The PCA (Fig 23) showed that the community structure in Tone cave locality and Werenskiöld locality, where ice was the substratum, very similar, thus the abundance was very low. The whole model was interpreting 54,9%.

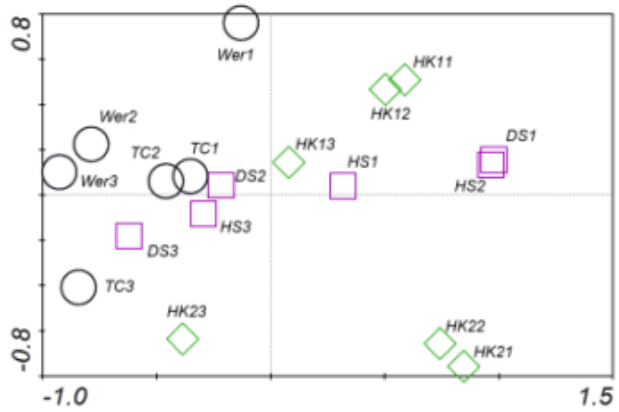


Fig 23 PCA diagram showing the similarities and differencies in community structure among localities and type of ice substrata. Circle – ice; square – snow; diamond - firn

The RDA analysis where changes in the community structure were compared according to the type of ice substratum is displayed in Fig 24. The whole model was interpreting 28,6% of the total variance. No significant components were found. Only the unidentified green coccoid alga and green filamentous alga showed a preference of ice substratum.

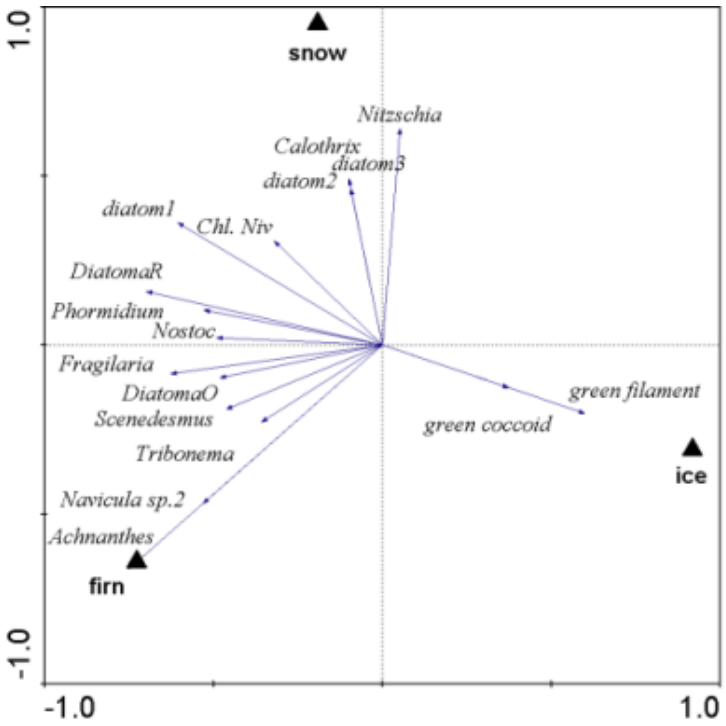


Fig 24 RDA diagram showing the changes in community structure after incubation on ice, firn and snow substrata

The RDA analysis, where changes in the community structure were compared according to the light conditions is in Fig 25. Presence or absence of light was not significant

and, as the only environmental factor, captured the total variance 8,2%. Only the unidentified green coccoid alga and green filamentous alga showed some preference of light.

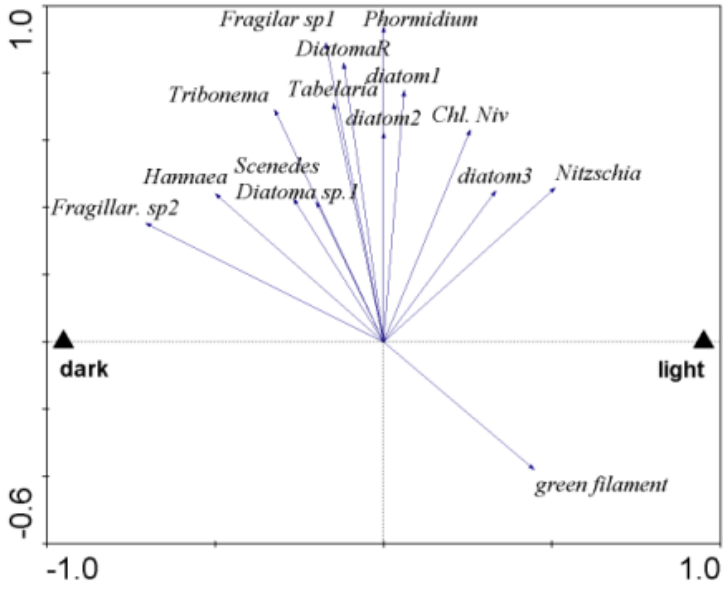


Fig 25 RDA diagram showing the changes in community structure after incubation in light and in darkness.

"Life forms" (cyanobacterium, diatom, yellow-green algae, green algae, flagellate, coccoid, filamentous, dormant stage, presence of sheet, rigid cell wall) were used instead of species in order to find out some correlations between "life forms" and environmental conditions. RDA analysis (Fig 26) showing changes in community structure was used. The whole model was interpreting 55,5%.

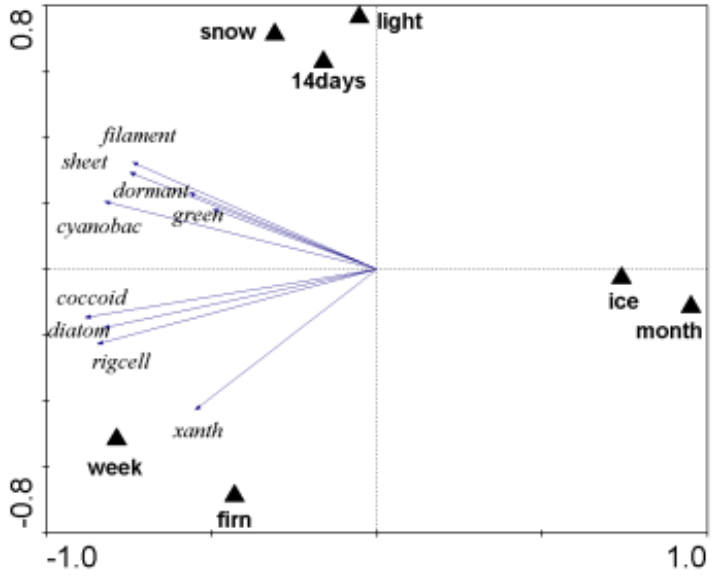


Fig 26 RDA diagram showing the changes in community structure in life forms after week, 14 days and month incubation on ice, firn and snow substrata in the presence or absence of light

5. Discussion

5.1. Isolation and Determination of Strains

Most of the isolated strains belonged to filamentous cyanobacteria from the order Oscillatoriales (Anagnostidis and Komárek 1988). This is in accordance with the general belief of the dominance of oscillatorian cyanobacteria in freshwater ecosystems in the Antarctic (e.g. Broady 1996) and the Arctic (Tang et al. 1997a; Vézina and Vincent 1997). Interestingly, these filamentous cyanobacteria also predominate in the most extreme polar environments (Friedmann 1993), including ice shelf communities (Priscu and Christner, in press). Oscillatoriales are also widely distributed in the Archean and Proterozoic fossil records (Schopf 1993).

Their dominance is mostly attributed to both a high tolerance for the extreme environment and a paucity of predators and competing species that are excluded by the inhibiting effects of low temperatures and potential freezing (Nadeau and Castenholz 2000). They also secrete mucilaginous organic compounds and bind together sediment particles, resulting in cohesive mats and films that would offer protected microhabitats for less tolerant biota during periods of extensive glaciations (Vincent et al. 2000a).

The dominant component was the genus *Phormidium*, with 14 isolated strains. *Phormidium* is the most successful cyanobacterium in immature fellfield soils. It plays an important role in soil-binding processes (Davey 1988) and is the most conspicuous component of perennial Antarctic lake ice (Priscu et al. 1998).

As for green algae, most of strains were isolated only if the natural samples were grown at higher temperature (12, 18°C). Generally, if microalgae are present in microbial mats, diatoms rather than green algae occur. The experimental conditions of cultures probably more favoured the growth of green algae (Buffan-Dubau et al 2001), which grow faster and have high nutrient uptake capabilities (Tang et al. 1997b).

All isolated strains of cyanobacteria and algae were able to grow both at 6 and 25°C; hence all of them can be considered psychrotolerant and not psychrophilic, which is in agreement with the fact that few true cyanobacterial psychrophiles associated with polar freshwater systems have been found so far (Tang et al. 1997a; Tang and Vincent 1999; Nadeau and Castenholz 2000; Nadeau and Castenholz 2001). The lack of cyanobacterial psychrophiles in freshwater and ice assemblages may be related to selection factors other than temperature (e.g. freeze-thaw tolerance, tolerances of high fluxes of solar radiation) that dictate which organisms survive and grow in these environments (Tang et al. 1997a; Vincent et al. 1997; Vincent 2000a).

Based on morphological features, most of microorganisms found in Antarctica have apparently cosmopolitan distribution. This hypothesis is supported by the fact

that these organisms have efficient dispersal abilities, and the relatively young age of the ice-free area (Vincent 2000a). However, Komárek (1999) attributed the assignment of Antarctic cyanobacterial taxa to cosmopolitan species to the use of taxonomic keys developed for temperate or tropical microflora. In support of this hypothesis that there are endemic cyanobacteria in polar environments (Vincent 2000b) are the fact that Antarctica has been more isolated than other parts of the world for several million years, the fact that dispersal processes which favour local species are more efficient than long-range dispersal processes, and that there has probably been environmental selection for adaptative strategies. The presence of endemic species in Antarctica has been recently confirmed by molecular data (Taton et al. 2003). Furthermore, Antarctic endemic species seem to be more abundant than estimated on the basis of morphological features (Taton et al. 2003).

Determination of Antarctic oscillatorian cyanobacteria into species is very troublesome as there is no monography or summarized work available.

Most of the scientists pay little attention to detailed determination of algae or cyanobacteria, particularly of such a complicated group as Oscillatoriales. Most of the *Phormidium* (or perhaps other filamentous cyanobacteria) species are determined as *Phormidium autumnale* or just *Phormidium* sp. This is not surprising regarding that there are few morphological features on the simple *Phormidium* trichome and that obtaining of unialgal strains is usually necessary for correct determination.

5.1.1. Morphotype A

Strains Bl50, the only representative of morphotype A, was isolated from a seepage on King George Island, South Shetland Islands. The only similar species characterized by presence of more than one trichome in sheath known from seepages is *Blennothrix lauterbachii* (Hieron and Schmidle) Anagn and Kom 1988 (*Hydrocoleum lauterbachii* Hieron and Schmidle 1901). Very short cells and gradual attenuation of apical part are typical features of the genus *Blennothrix*. However, according to Geitler (1932), the trichomes of *Hydrocoleum lauterbachii* should be much wider and shorter. Therefore it may be described as *Blennothrix* sp.

5.1.2. Morphotype B

Strain Ph28, the only representative of morphotype B, is similar to morphotype C, but had thick distinctive non-adhering sheaths. Additionally, calyptra was never present and trichomes were usually gradually attenuated and curved in the apical part. The latter is a typical feature of *Phormidium pseudopriestleyi* Anagn and Kom 1988 (*Oscillatoria priestleyi* West and West 1911) an endemic species from Antarctic seepages (Komárek 1999; Komárek and Komárek 2003). Thus, it might be described as *Phormidium pseudopriestleyi* but with distinct thick sheaths, which may be conse-

quence of culture conditions. However, this strain was difficult to grow and therefore difficult to determine properly.

5.1.3. Morphotype C

Five strains were isolated from soils (both mineral and ornithogenous) and one from a wetland. They can be identified as *Phormidium autumnale* sensu lato. Even though cosmopolitan *Phormidium autumnale* sensu stricto is the most recorded *Phormidium* species either from Antarctic soils (e.g. Davey 1988) or mats (e.g. de los Ríos et al. 2004), it should be only present in Antarctic streaming waters (Komárek 1999). Antarctic eutrophic soils, such as ornithogenous soils, are characterised by presence of black mats of *Phormidium attenuatum* (Fritsch) Anagn and Kom (*Lyngbya attenuata* Fritsch 1912) on the soil surface (Komárek 1999). Characteristics of all three strains isolated from ornithogenous soils (Ph 38, Ph43 and Ph68) agree with the original description of *Lyngbya attenuata* (Fritsch 1912). Two strains isolated from mineral soils (Ph10 and Ph12) and one from a wetland (Ph31) were very similar to those from ornithogenous soils, but little granulated, and therefore could be also identified as *Phormidium attenuatum*.

5.1.4. Morphotype D

This grouped together morphologically very similar strains of thin-trichomed *Phormidium* strains from wetlands and seepages, with one strain from ornithogenous soil. This morphotype fits best to the traditional species *Leptolyngbya scottii* (Fritsch) Anagn and Kom 1988 (*Lyngbya scottii* Fritsch 1912) or *Phormidium murrayi* (West and West) Anagn and Kom 1988 (*Lyngbya murrayi* West and West 1911).

Leptolyngbya scottii is a problematic taxon sporadically reported from Antarctic shallow meltponds (Broady et al. 1984; Mataloni and Pose 2001), seepages (Komárek and Komárek 2003) or soils (Komárek 1999). Its taxonomical position is also not very clear. Considering original description (West and West 1911), this species should be probably revised and renamed as *Phormidium scottii*. However, finding of thylakoid pattern is necessary for distinguishing of the genera *Phormidium* and *Leptolyngbya*. While some authors use the valid name *Leptolyngbya scottii* (Komárek 1999; Mataloni and Pose 2001), some authors use *Phormidium scottii* without further explanation (Worland and Lukešová 2000), and some use both names (Komárek and Komárek 2003). Thin non-adhering sheaths and wider trichomes are the main difference of morphotype D from description of *L. scottii*.

On the other hand, *Phormidium murrayi* is often reported from aquatic systems of continental Antarctica (Broady and Kibblewhite 1991; Quesada and Vincent 1997; Mataloni and Pose 2001; Mataloni and Tell 2002) but it has not been recorded at King George Island (Komárek 1999), from where four strains were isolated. Charac-

teristics of *P. murrayi* are very similar to description of this morphotype, mainly, cell width, length, characteristics of apical cells and sheaths and presence of granulation.

5.1.5. Morphotype E

Komárek and Komárek (2003) reported four *Leptolyngbya* species from King George Island seepages: *L. antarctica* (West and West) Anagn and Kom 1988 (*Phormidium antarcticum* West and West 1911), *L. glacialis* (West and West) Anagn and Kom 1988 (*Phormidium glaciale* West and West 1911), *L. borchgrevinkii* and *L. vincentii*. The last two *Leptolyngbya* are new species not yet described, hence impossible to determine (Komárek 1999). Characteristics of this morphotype, however, do not fit to description of both *L. antarcticum* and *L. glacialis*. Its characteristic fits more with description of cosmopolitan species *Leptolyngbya fragilis* (Gom) Anagn and Kom 1988 (*Phormidium fragile* Gom 1982) reported from Antarctic ponds (Mataloni and Pose 2001), lakes (Mataloni and Tell 2002), soils (Komárek 1999) and other various habitats throughout Antarctica.

Additionally, DNA from all oscillatorian strains was isolated and more detailed characterization bringing together molecular and morphological (both microscopical and ultrastructural) data is in preparation.

5.2. Freezing and Desiccation Tolerance of Antarctic Strains

In environments such as polar wetlands, physical stresses are likely to play an important role in defining the species composition and activity of biological communities. One of the most important stress factors affecting the composition of polar wetland communities is the freezing and desiccation period, connected with water availability (Davey 1989).

From the results obtained in this study, cyanobacteria from polar wetlands are much more resistant to both freezing and desiccation than green algae. This is in agreement with previous published papers (Hawes 1989; Hawes et al. 1992; Davey 1989), which showed that algae have higher rates of photosynthesis and lower resistance to freeze-thaw cycles, which predetermined algal mats to their annual character. The green algae were resistant only when exposed to mild freezing (-4°C), which simulated diurnal freezing during the autumn. These temperatures are supposed to cause no intensive damage to even the most susceptible Arctic or Antarctic organisms (Block 1990; Hawes 1990).

Based on field or experimental data it is known that cyanobacteria are able to withstand some intensity of freezing (Holm-Hansen 1963; Becker 1982; Davey 1988, 1989; Hawes 1990; Hawes et al. 1999) and desiccation (Jones 1977; Scherer et al.

1984, Potts and Morrison 1986; Hawes et al. 1992; Caiola et al. 1993; Caiola et al. 1996; Qiu et al. 2003). Recent studies showed that cyanobacteria from the entire world, including tropics, have the same remarkable ability to survive deep freezing (Lukešová et al., unpublished data). Therefore this is probably a typical feature of all cyanobacteria and not a special disposition of polar ones.

Cyanobacteria in this experiment were able to endure - without almost any harm - both immediate freezing to –196°C and slow cooling to –40°C and –100°C and subsequent rewetting, that can be more injurious than freezing (Biebl 1938).

At slow rates of cooling salt exclusion increases the osmotic potential of the remaining liquid medium (Morris and McGrath 1981; Hawes 1990). Intracellular ice nucleation cooling occur at high cooling rates, while its occurrence at slow rates of cooling depend on the temperature and duration of freezing exposure (Hawes 1990).

After exposure to freezing regimes, strains were kept in the dark to prevent light injury (Deming-Adams et al. 1990; Jacob et al. 1992). In the field, it is likely that most freezing events will occur at low light intensities, either at night in autumn during the diurnal freeze-thaw cycles or under snow during the long winter period of freezing (Davey 1989).

Desiccation was usually more injurious than freezing, especially for green algae that showed high mortality. More injurious was the desiccation at 20°C, fatal for some strains. Dry tolerance is determined mostly by the ability to restore photosynthesis and respiration after rehydration. Recovery from desiccation is rapid when conditions have favoured the accumulation of carbon reserves (Du Bois and Kapustka 1983, Coxson and Kershaw 1983).

Results showed that there are some differences between microorganisms isolated from continental and maritime Antarctica, and that the microorganisms isolated from seepages are less tolerant to freezing and desiccation than organisms from other habitats. To confirm this theory, the statistical analysis was performed for cyanobacteria and algae. While significant trends were found when compared cyanobacteria, no significances, but obvious trends were found when compared algae. This can be explained by the fact that all cyanobacterial strains used for this experiment were very similar, being filamentous oscillatorians, mostly of the genus *Phormidium*, while the composition of green algal strains were much more diverse, comprising different genera and different life forms (coccoid, filamentous). Thus, it is probable that differences among algal strains are primarily determined by the specific characteristics of each strain (species) rather than the habitat or locality origin. Hence, comparison of similar cyanobacterial strains seems to be more suitable for detection of differences between organism from different types of habitat and localities.

From the results obtained in this study, filamentous cyanobacteria isolated from the continental Antarctica are significantly more successful in surviving of deep-freezing than similar types of cyanobacteria isolated from maritime Antarctica. It seems that cyanobacteria in the Antarctic are selected to be more resistant in surviv-

ing prolonged freezing. However, if considering that average air temperatures (Fig 2, p.22) are lower on Signy Island (maritime Antarctica) than on Anvers Island (Palmer station) in continental Antarctica, it is evident that mere temperature is not the only determining factor. Moreover, meteorological air temperatures have little relation to the temperatures of the microhabitats (Chambers 1996, Longton and Holdgate 1967). More important is e.g. the large-scale topography of any site, and hence the potential for snow to settle and remain on the surface; snow also serves as a thermal blanket protecting the underlying habitats from the effects of declining air temperatures and in general, the deeper snow covering the site the less severe the winter temperatures experienced at, or near, the soil surface (Davey et al. 1992). The essential is that wintertime is longer in continental Antarctica than in maritime, and it has been shown that the time of freezing period is an important factor in survival of algae (Hawes 1990).

The results also showed that cyanobacteria isolated from seepages were significantly less tolerant to both freezing and desiccation. This may be explained by the fact that freshwater seepages communities are more structured and more stable in water level changes and therefore might offer better protection against freezing and desiccation. Hence, microorganisms inhabiting seepages may not have been such intensively selected on freezing and desiccation stress as in other habitats.

However, as these results are based on a limited number of strains, it is difficult to generalize, and more experiments with different strains and different organisms are necessary.

Different freezing and desiccation tolerance of various Antarctic algae have been already discussed by some authors according to the habitat origin. Davey (1989) suggested that higher rates of photosynthesis after freezing in the green alga *Prasiola crispa* related to the cyanobacterium *Phormidium autumnale* could be explained by different ecology of both microorganisms. While *Prasiola* is an opportunistic species occurring on ornithogenous soils, where regular re-growth is required, *Phormidium* was isolated from mineral fellfield soils, where it played an important role in soil binding processes. The difference in habitat was also demonstrated in their resistance to drying. *Phormidium*, occurring on habitat subjected to extensive periods of desiccation during summer, displayed greater rate of recovery from desiccation than *Prasiola*, preferring wet environment of ornithogenous soils (Davey 1989).

According to Becker (1982), Antarctic *Nostoc* from melt waters, where the temperature oscillates between a few degrees above and below zero, has photosynthetic limits at –5°C but is able to tolerate temperatures up to –70°C (Holm-Hansen 1963). On the other hand, aerophytic *Prasiola crispa* was able to perform photosynthesis at temperatures down to –15°C (Becker 1982).

Hawes et al. (1992) observed slower recovery of nitrogenase activity, photosynthesis and respiration after desiccation of *Phormidium*-dominated mats from Antarctic freshwater ponds than of *Nostoc*-dominated mats. They suggested that *Phormidium*

mats are distributed in pools and streams with reliable supply of water during summer, whereas growth of *Nostoc* mats is favoured in areas with lower water content (Hawes et al. 1992).

Testing the freezing and desiccation tolerance of cyanobacteria and algae has become important in terms of application in cryopreservation of culture collections (Taylor and Fletcher 1999). Freeze-drying is a practical and inexpensive means of preserving microorganisms which have been applied successfully in the areas of medical and industrial microbiology (McGrath et al. 1978).

5.2. Freezing and Desiccation Tolerance of Natural Samples

Mild freezing (-4°C) had usually little effect on community structure. However, the deeper freezing decreased the biomass of green algae and favoured the growth of cyanobacteria. Nevertheless, after subsequent cultivation in laboratory conditions (conditions favourable for growth) green algae very quickly regenerate their initial biomass.

It seems that cyanobacteria and algae have different life strategies how to avoid damage cause by winter freezing. While all cyanobacterial cells are very resistant, green algae rely on few resistant cells. When conditions change back to those favourable for growth, green algae took advantage from their higher rates o photosynthesis and quickly regenerate. These observations are in agreement with Hawes (1989, 1990).

However, the resistance to freezing or desiccation was not the only factor that predetermine changes in the communities structures. Growth rate, ability to grow in cultures conditions and compete to other species are also important factors that predetermine, which species will survive and which not. *Prasiola crispa* for example is known to be able to withstand intensive freezing (Davey 1989), however, in presented experiment, *Prasiola* was able to survive only mild freezing. This finding could be explained by the fact that it is very difficult to cultivate *Prasiola* species. It growths slowly in cultures and could be easily outcompeted by faster-growing species.

5.3. Survival of Algal-Cyanobacterial Communities on Various Types of Ice Substratum

Recent studies have brought strong evidence that bacteria and cyanobacteria from wetland communities are deposited via aeollian processes throughout Antarctica and serve as a source of organic material for icy ecosystems (Gordon et al. 1996; Gordon et al. 2000; Priscu et al. 1998). No similar research has been undertaken in the Arctic so far; nevertheless, it is probable that same could happen in the North where also strong katabatic winds are present. The species diversity is much lower in ice ecosys-

tem than those in wetland habitats. Hence, some organisms are not able to survive either in the aerosol or on the ice substratum. What predetermines some microorganisms to survive on the ice or snow is not very clear.

From the results obtained in this study, most of the algae and cyanobacteria from Svalbard wetlands were able to survive for a week or two on some ice substrata. The number of species that survived a monthly incubation was, however, limited. No algae or cyanobacteria, with an exception of an unidentified green coccoid alga and unidentified green filamentous alga, preferred distinct light conditions or substratum type. Unidentified green filamentous alga and unidentified coccoid alga were only found on Werenskiöld locality, which was irradiated and where ice was the type of substratum. Therefore, it would not be correct to deduce preferences of these algae from one observation. Nevertheless, some preferences of light of green algae compared to other algae were obvious.

The least suitable substratum for colonization by algae or cyanobacteria was the smooth ice of Werenskiöld and Tone cave localities. These two localities, completely different in other characteristics, had similar (and very low-abundance) community composition. Why the smooth ice was such inhospitable for microorganisms compared to firn and snow can be only hypothesized. The differences between snow and firn are minor and therefore differences between species that preferred snow or firn were very small. Actually, no species or life form was found to prefer either snow or firn. On one hand, the ice is very solid, probably does not enable movement of cells and entrap them on the ice surface, exposed to outside conditions (e.g. intensive light on the glacier surface) and they also might be more easily washed away. It did not happened on the studied localities, because dead remainders of communities were found. There also might be problem with liquid water availability on or in the ice. No one has observed living motile organism *in situ* in glacial freshwater ice yet (Price 2000). Firn and snow are, on the other hand, more structured, enable motility and might serve as a protection against destructive UV or intensive freezing. The availability of liquid water would be also less problematic in snow or firn than in or on the ice.

In this study, no common characteristics that enable cyanobacteria and algae to survive were found. Filamentous cyanobacteria (*Phormidium*, *Leptolyngbya*) were one of the most successful species in colonizing of the ice and snow. Green algae, on the other hand, were very unsuccessful. When compared with results of laboratory experiment of freezing and desiccation tolerance of similar Antarctic strains, species that were able to survive on the ice were those resistant to intensive freezing and desiccation. *Tribonema* was also quite successful alga in surviving on the ice even though its success might be affected by high amount of *Tribonema* trichomes in composite sample. Together with viable *Tribonema* trichomes, larger amount of dead trichomes were usually found, hence only small amount of *Tribonema* cells were able to survive the conditions on snow, firn or ice. Some *Tribonema* strains from temperate zone are

also able to withstand intensive freezing, particularly by formation of dormant stages (Machová 2004). Formation of these dormant akinetes was not observed in this study.

Species that were able to survive for month on ice, firn or snow are similar to those usually found in cryoconite holes on the glacier surface (e.g Säwström et al. 2002), permanently frozen lakes (Priscu et al. 1998) or ice shelves (Vincent et al. 2004). However, what predominates them to be such successful is not clear a needs further investigations.

Airborne dispersal of algae and cyanobacteria and their potential colonization of ice ecosystems is a very up-to-date problematic. Finding the mechanisms of successful survival of some organisms on or inside the ice could reveal more about the limits of life, life on prehistoric Earth and perhaps about possibilities of extraterrestrial life. More experiments focused on life processes of algae on ice are in progress.

6. Conclusions

Freezing and desiccation tolerance of cyanobacteria and algae isolated from various wetland habitats of continental and maritime Antarctica was assessed.

While filamentous cyanobacteria from the order Oscillatoriales were remarkably resistant to mild freezing, deeper freezing, supranatural freezing and desiccation, green algae showed high mortality after exposure to deeper and subnatural freezing and very high mortality when exposed to desiccation. Both cyanobacteria and algae were most damaged when desiccated at 20°C. However, green algae were capable of rapid regeneration of their damaged biomass.

It seems that cyanobacteria and algae have different life strategies how to avoid damage cause by winter freezing. While all cyanobacterial cells are very resistant, green algae rely on few resistant cells.

Subsequent using of multivariate statistical methods showed that cyanobacteria from maritime Antarctica are less resistant to deeper freezing than similar types from continental Antarctica. In addition, filamentous cyanobacteria from seepages habitat were significantly less tolerant to freezing and desiccation than cyanobacteria from true wetlands and ornithogenous and mineral soils.

In addition, *in situ* freezing of similar Arctic microbial communities from wetland habitats and their potential of colonization of various ice substrata was studied in late summer 2003 on Werenskiöld glacier in Wedel Jarlsberg Land, SW Spitsbergen.

While the conditions on snow or firn substratum enabled survival of some cyanobacteria and algae, the smooth ice surface was unsuitable for colonization by most of the species. The most successful species in surviving on ice substrata were again filamentous cyanobacteria from the order Oscillatoriales.

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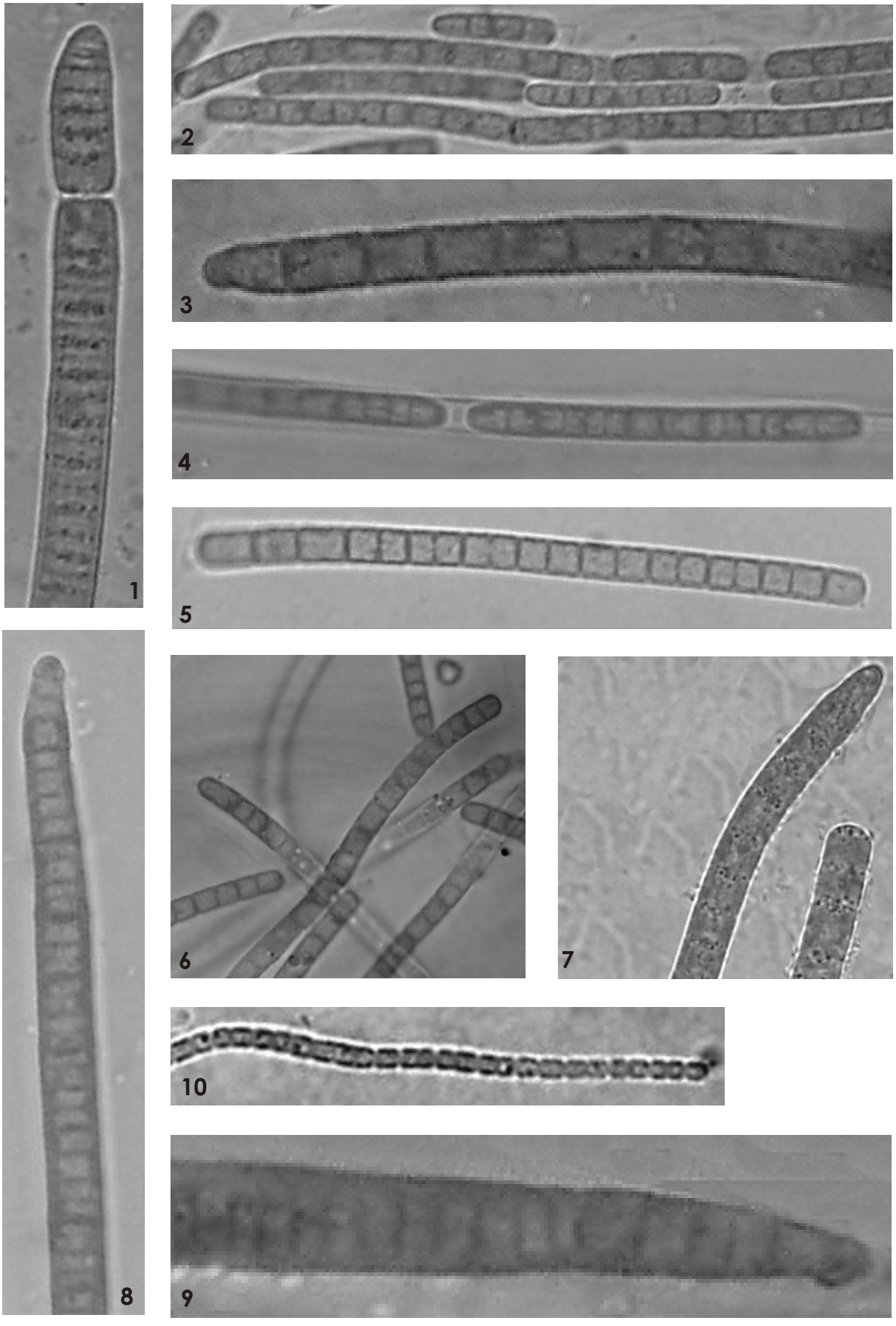
APPENDICES

	Locality	Location	Geographical position	Characterization	Habitat
10	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Black crust between stones	Mineral soil
12	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Soil crust	Mineral soil
16	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Mats from ornithogenic soil	Mineral soil
19	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Crust from frozen and desiccated seepage	Seepage
21	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Brown mats from shallow wetland	Wetland
22	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Black mats from shallow wetland	Wetland
24	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Black mats from shallow wetland	Wetland
28	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Black mats from seepage	Seepage
29	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Brown mat from shallow wetland	Wetland
31	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Black and brown mats from shallow wetland	Wetland
32	Rothera – Adelaide Island	Antarctic peninsula	67°34'S 68°08'W	Black mats from shallow wetland	Wetland
33	Trump Island	Antarctic peninsula	approx. 64°S 63°W	Soil crust between stones	Mineral soil
37	Palmer – Anvers Island	Antarctic peninsula	64°46'S 64°03'W	Soil crust between stones	Mineral soil
38	Palmer – Anvers Island	Antarctic peninsula	64°46'S 64°03'W	Quag from roockeries	Ornith. soil
43	Palmer – Anvers Island	Antarctic peninsula	64°46'S 64°03'W	Black mat on roockerie	Ornith. soil
48	Jubany – King George Island	South Shetland Islands	62°14'S 58°40'W	Soil crust	Mineral soil
49	Jubany – King George Island	South Shetland Islands	62°14'S 58°40'W	Brown mats from seepage	Seepage
50	Jubany – King George Island	South Shetland Islands	62°14'S 58°40'W	Brown mats from seepage	Seepage
53	Jubany – King George Island	South Shetland Islands	62°14'S 58°40'W	Mats from seepage	Seepage
54	Jubany – King George Island	South Shetland Islands	62°14'S 58°40'W	Mats from seepage	Seepage
55	Jubany – King George Island	South Shetland Islands	62°14'S 58°40'W	Red mats from seepage	Seepage
56	Jubany – King George Island	South Shetland Islands	62°14'S 58°40'W	Mats on soil	Wetlnad
57	Jubany – King George Island	South Shetland Islands	62°14'S 58°40'W	Grey mats	Wetland
58	Jubany – King George Island	South Shetland Islnads	62°14'S 58°40'W	Grey mats from dry pool	Wetland
59	Signy – Coronation Island	South Orkney Islands	60°43'S 45°36'W	Red ice with soil	Mineral soil
62	Signy – Coronation Island	South Orkney Islands	60°43'S 45°36'W	Brown mats from seepage	Seepage
69	Signy – Coronation Island	South Orkney Islands	60°43'S 45°36'W	Brown mats from seepage	Seepage

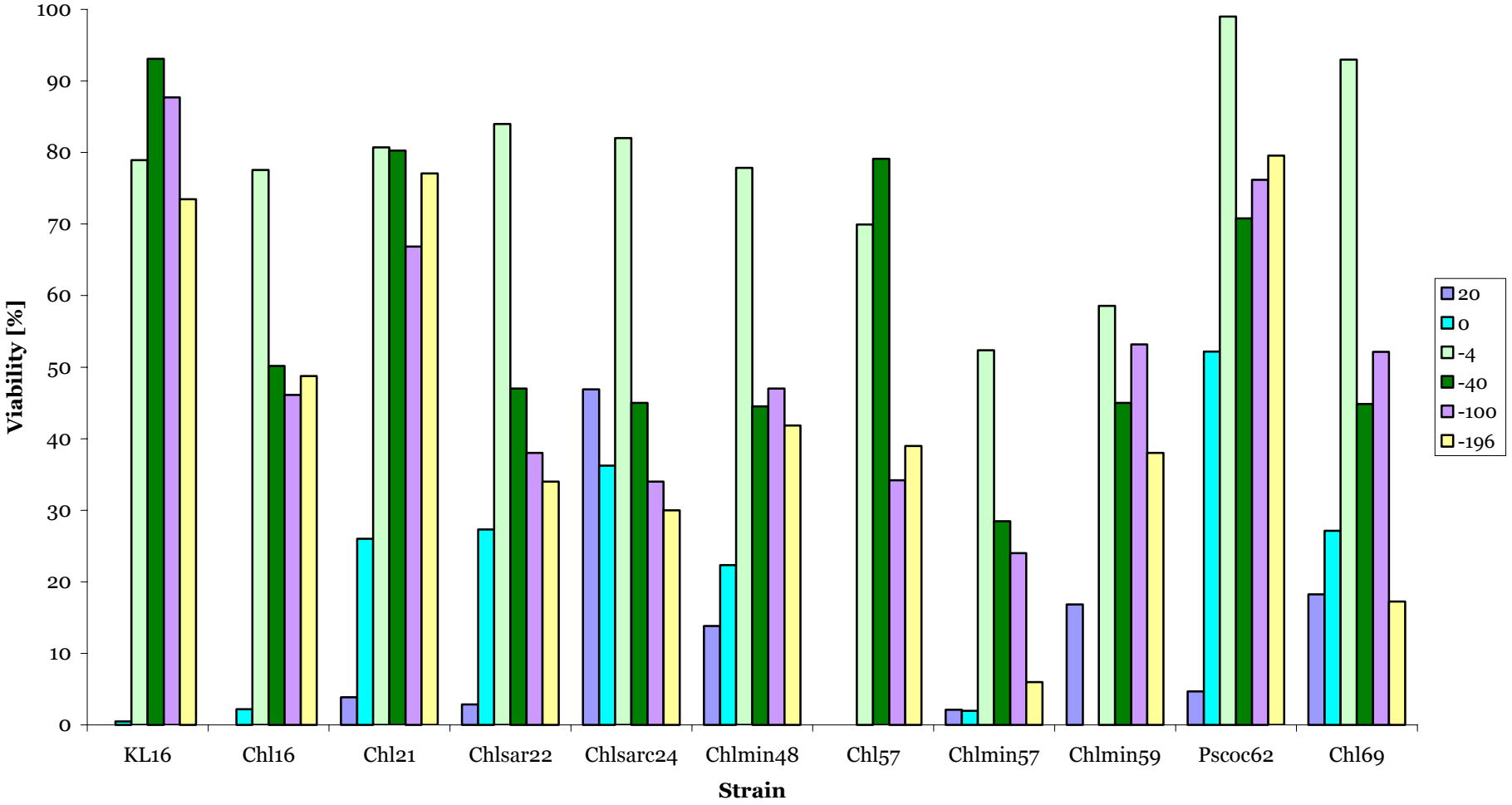
Appendix 1 List of all samples collected from Antarctic wetlands

Strain	Presence of calyptra	Number of trichomes in sheat	Trichome shape	Terminal attenuation of trichome	Shape of apical cell	Constrictions at transverse walls	Range in width of trichomes	Range of cell length	Ratio of cell width to cell length	Colour
Ph10	+	1	Mostly straight with a slight terminal hook	Slight over 2-8 cells	Broadly rounded to slightly attenuated to slightly conical	None	4,71 - 7,75	1,39 - 5,95	0,91 - 3,42	Greyish green
Ph 12	+	1	Mostly straight with a slight terminal hook	Slight over 2-8 cells	Broadly rounded to slightly attenuated to slightly conical	None	3,04 - 6,65	1,79 - 5,68	0,53 - 2,13	Greyish green
Ph 16	-	1	Slightly flexuous	None	Bluntly rounded to slightly conical	None	2,18 - 4,96	2,16 - 8,12	0,36 - 2,15	Light green
Ph 21	-	1	Mostly flexuous	None	Bluntly rounded to slightly conical	None	2,78 – 4,52	2,18 – 6,27	0,53 – 1,67	Light green
Ph 28	-	1	Mostly straight with a slight terminal hook	Slight over 2-8 cells, twice curved	Gradually attenuated	None	4,42 – 7,68	1,82 – 5,74	0,95 – 3,83	Brown-green
Ph 31	+	1	Mostly straight with a slight terminal hook	Slight over 2-8 cells	Broadly rounded to slightly attenuated to slightly conical	None	3,99 – 6,98	1,36 – 4,64	1,41 – 4,82	Blue-grey
Ph 32	-	1	Mostly straight with a slight terminal hook	None	Bluntly rounded to slightly conical	None	2,88 - 4,72	2,08 - 6,73	0,6 - 1,89	Light green
Ph 38	(+)	1	Mostly straight with a slight terminal hook	Slight over 2-8 cells	Broadly rounded to slightly attenuated to slightly conical	None	4,50 – 8,48	2,04 – 4,83	1,34 – 3,75	Brown-green
Ph 43	(+)	1	Mostly straight	Slight over 2-8 cells	Broadly rounded to slightly attenuated to slightly conical	None	5,33 – 9,09	2,00 – 6,93	1,07 – 3,93	Greyish green
Ph 50	-	1	Mostly straight, little flexuous	None	Flatly rounded to flatly conical	None	2,72 – 4,83	2,35 – 7,39	0,5 – 1,53	Light green
BI 50	-	1, rarely 2-3	Mostly straight	Slight over final 2-8 cells	Attenuated	None	5,36 – 9,04	1,65 – 4,71	1,71 – 4,78	Brown
Ph 53	-	1	Slightly flexuous	None	Bluntly rounded to slightly conical	Indistinct	2,33 – 4,46	1,99 – 7,34	0,48 – 2,07	Light green
Ph 54	-	1	Slightly flexuous	None	Bluntly rounded to slightly conical	Indistinct	2,86 – 4,04	2,43 – 6,44	0,55 – 1,52	Light green
Ph 58	-	1	Mostly straight	None	Bluntly rounded to slightly conical	None	3,04 – 4,65	2,32 – 6,17	0,59 – 1,69	Yellow green
Ph 68	(+)	1	Mostly straight with a slight terminal hook	Slight over 2-8 cells	Broadly rounded to slightly attenuated to slightly conical	None	4,95 – 6,8	1,61 – 4,46	1,3 – 4,12	Greyish green
LPP28	-	1	Mostly flexuous	None	Broadly rounded	Distinct	1,20 – 2,08	1,09 – 2,51	0,50 – 1,64	Light green
LPP31	-	1	Mostly flexuous	None	Broadly rounded	Distinct	1,26 – 2,04	0,89 – 1,93	0,80 – 1,96	Light green

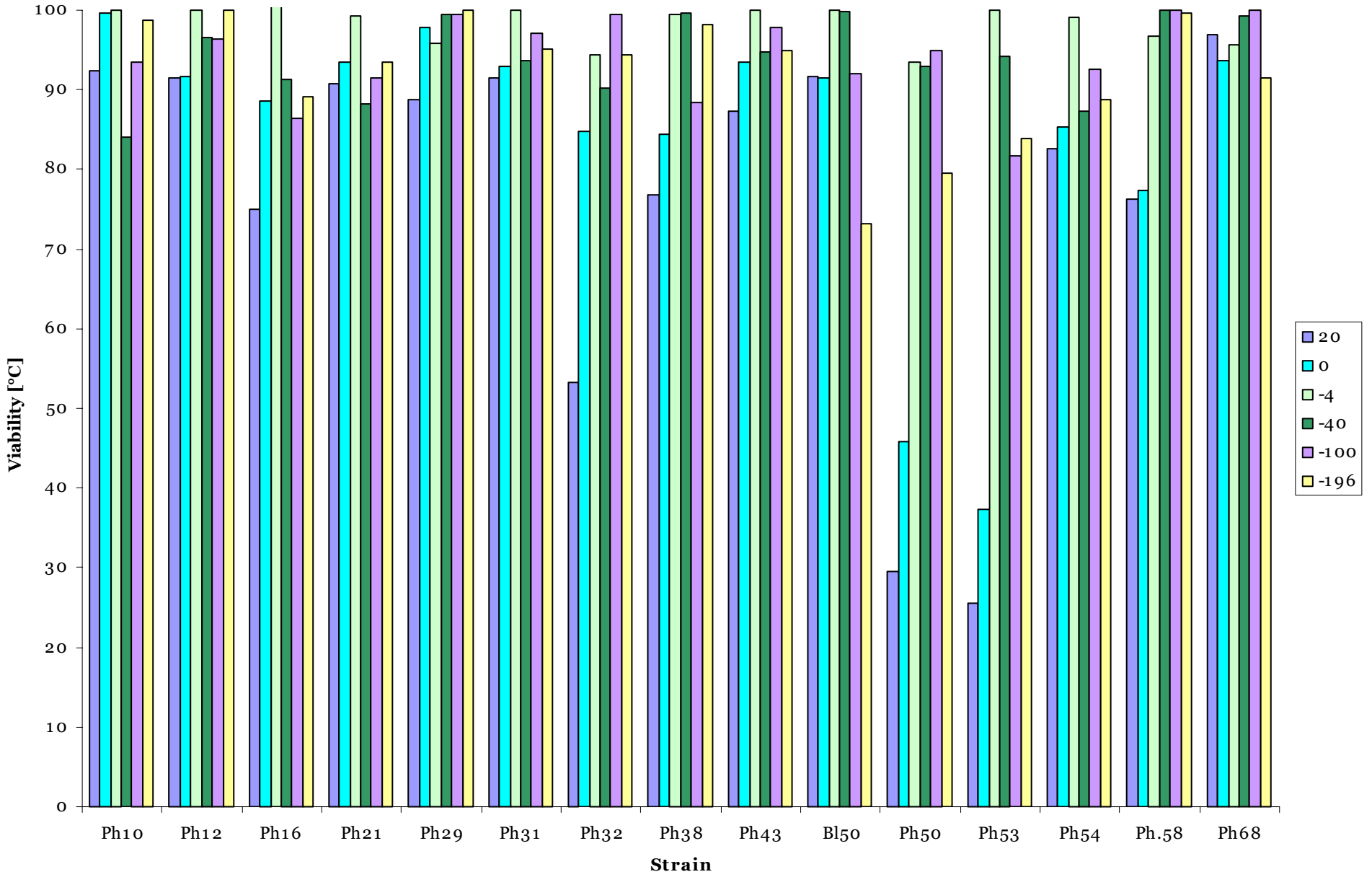
Appendix 2 Detailed morphology of each cyanobacterial strain. Compiled according to Broady et al. (1984) and Volf (2002) - modified. Brown – strains isolated from mineral soils; Green – strains isolated from ornithogenous soils; Blue – strains isolated from – true wetlands; Yellow – strains isolated from seepages



Appendix 3 Pictures of morphotypes: 1 - morphotype A; 2-6 - morphotype D; 7-9 - morphotype C; 10 - morphotype E



Appendix 5 Viability of algal strains after exposition to freezing (-4; -40; -100 and –196) and desiccation (0 and 20)



Appendix 4 Viability of cyanobacterial strains after exposition to freezing (-4; -40; -100 and –196) and desiccation (0 and 20)