CLAUDINEIA LIZIERI DOS SANTOS

ENVIRONMENTAL ATTRIBUTES IN ASSEMBLING CYANOBACTERIAL COMMUNITIES FROM THE McMURDO SOUND REGION, ANTARCTICA

Thesis presented to Botany Graduate Program of the Universidade Federal de Viçosa, in partial fulfillment of the requirements for the degree of *Doctor Scientiae*

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I dedicate this thesis...

…to the living memories of Rosane Euclydes Aguiar from whom this challenging and beautiful journey started…

AND

to Dr. Ian Hawes without which I would not have been able to get the end of this journey…

"In the long history of humankind (and animal kind, too) those who learned to collaborate and improvise most effectively have prevailed." (Charles Darwin)

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SANTOS, Claudineia Lizieri dos, D.Sc., Universidade Federal de Viçosa, março de 2014. **Atributos ambientais na montagem da comunidade de cianobactérias da região do McMurdo Sound, Antártica**. Orientador: Carlos Ernesto Gonçalves Reynaud Schaefer. Co-orientadores: Antônio Galvão do Nascimento e Adriano Nunes Nesi.

A variedade de ecossistemas microbianos que existe na Antártica representa uma oportunidade extraordinária para pesquisas sobre a ecologia, diversidade e evolução microbiana, principamente em termos de cianobactéria. Neste trabalho, foi avaliado o papel de atributos ambientais no controle da montagem de esteiras microbianas antárticas. Inicialmente foi caracterizado a diversidade cianobacteriana das esteiras ao longo de gradientes ambientais na região do McMurdo Sound, Antártica continental. Em seguida, foi avaliado o papel das variáveis ambientais na determinação da composição da assembléia de cianobactérias através da análise de amostras de água e esteiras de cianobactérias microbianas de 25 lagos distribuídos em quatro distintas áreas geográficas: McMurdo Ice Shelf, Ross Island e Upper e Lower Wright Valleys. Finalmente, foi realizado experimentos de laboratório para determinar a extensão com que a composição de espécies afeta a formação das esteiras microbianas. Vinte e nove morfoespécies foram identificadas e descritas no capítulo 1. Quatro foram designadas à ordem Chroococcales, três Nostocales e 22 Oscillatoriales. No segundo capítulo foram investigados os fatores que podem estar envolvidos na determinação da presença e/ou ausência de cianobactérias dentro da comunidade das esteiras microbianas em cada lago amostrado. Os lagos das regiões Ross Island, McMurdo Ice Shelf e Upper e Lower Wright Vallyes apresentaram algumas características específicas próprias de cada área em termos de fatores físico-químicos e diversidade de cianobactérias, embora em vários casos houve uma sobreposição considerável das características. A análise multivariada dos dados, com base em variáveis físico-químicas mostrou que os lagos de cada área amostrada tendeu a se agruparem por local, embora com considerável sobreposição, com os dois Wright Valleys e as duas zonas costeiras tendendo a ser mais semelhantes entre si. Este padrão tendeu a ser reproduzido em análises dos dados de biomassa e composição das espécies, onde foi possível indentificar táxons que foram amplamente espalhados por toda a região e outros que foram mais restritos por área. A importância da dispersão e condições de crescimento na condução desse padrão foi discutida. No terceiro capítulo foi desenvolvido um experimento para avaliar o papel de cepas de cianobactérias isoladas no processo de formação de esteiras microbianas. Seis cepas de cianobactérias foram utilizadas, em combinações variadas, para formar biofilmes em condições de laboratório: CYN-50 (Phormidium cf. autumnale); CYN-68 (Leptolyngbya A); CYN-65 (Leptolyngbya B); CYN-66 (Microcoleus sp.); CYN-67 (cf. Aphanocapsa) e CYN-72 (Nostoc sp.). O conteúdo de clorofila-a, exopolissacarídeo e matéria orgânica foi avaliado

e utilizado como medida de avaliação do desenvolvimento dos biofilmes. No final do experimento, observou-se que os biofilmes desenvolvidos mostraram-se diferentes em termos de sua morfologia e que as cianobactérias filamentosas “oscilatoriales” são requeridas para a formação de biofilme consistente, entretanto, todas as cepas morfologicamente diferentes produziram efeito nas matrizes dos biofilmes. Phormidium produziu o melhor biofilme desenvolvido. Em conclusão, este trabalho fornece um maior conhecimento sobre a taxonomia e ecologia de cianobactérias da Antártica na região do McMurdo Sound. Nós identificamos uma mistura de organismos amplamente distribuídos e tolerantes e outros com distribuições mais locais e requerimentos ambientais específicos. A significância destes em temos de gestão da biodiversidade antártica também foi discutida.

ABSTRACT

SANTOS, Claudineia Lizieri dos, D.Sc., Universidade Federal de Viçosa, March of 2014. **Environmental attributes in assembling cyanobacterial communities from the Mcmurdo Sound region, Antarctica**. Advisor: Carlos Ernesto Gonçalves Reynaud Schaefer. Co-Advisors: Antônio Galvão do Nascimento and Adriano Nunes Nesi.

The variety of microbial ecosystems that exist in Antarctica represents an extraordinary opportunity for research on microbial ecology, diversity and evolution, particularly in terms of cyanobacteria. In this work we focus on the role of environment in controlling cyanobacterial mat assembly, and begin by describing the cyanobacterial diversity of mats along environmental gradients in the McMurdo Sound region, continental Antarctica. We then evaluated the role of environmental variables in determining their composition by analysing water and microbial cyanobacterial mat samples from 25 ponds from four distinct geographic sites: McMurdo Ice Shelf, Ross Island and Upper and Lower Wright Valleys. Finally we undertook a series of laboratory experiments to determine the extent to which species composition affects mat formation. Twenty nine morphospecies are identified and described in chapter one. Four were assigned to the order Chroococcales, three to the Nostocales and 22 to the Oscillatoriales. In chapter two, we investigated the factors that appeared to determine the presence or absence of morphospecies within the cyanobacterial mat community at each sampled pond. Ross Island, McMurdo Ice Shelf and Upper and Lower Wright Valleys ponds each showed some specific features in terms of physical-chemical factors and cyanobacteria diversity, though in many cases there was considerable overlap. Multivariate analysis based on physic-chemical variables showed that the ponds from each site tended to cluster by site, though with considerable overlap, and with the two Wright Valley and two coastal sites tending to be more similar to each other. This pattern tended to be reproduced in analysis of biomass and species composition data, and we were able to identify taxa that were broadly spread across the region and others that were more restricted by area. The importance of dispersal and growth conditions in driving this pattern is discussed. In chapter 3 we developed an experiment to evaluate the role of isolated cyanobacteria strains on the mat-building process. Six cyanobacteria strains, in varying combinations, were used to grow mats under laboratory conditions: CYN-50 (Phormidium cf. autumnale); CYN-68 (Leptolyngbya A); CYN-65 (Leptolyngbya B); CYN-66 (Microcoleus sp.); CYN-67 (cf. Aphanocapsa) and CYN-72 (Nostoc sp.). The content of chlorophyll-a, exopolysaccharide and organic matter was evaluated to assess mat development. At the end of our experiment, we observed that the mats developments showed variety in terms of their

morphology, that oscillatorean cyanobacteria are required for coherent mat formation, but that different strains all produced effect mat matrices. P. autumnale produced the best developed mats. In conclusion, our findings provide an increased knowledge on the Antarctica cyanobacteria taxonomy and ecology in the McMurdo Sound region. We identified a mix of broadly tolerant, widely distributed organisms and other with more local distributions and specific environmental requirements. The significance of this in terms of management of Antarctic biodiversity is discussed.

1. GENERAL INTRODUCTION

Antarctica is a continent locked in ice, with almost 99.7% of current terrain covered by permanent ice and snow (Convey et al., 2008). The continent has been relatively isolated from the rest of the world since its separation from Gondwanaland about 85 Ma years ago, and final separation from South America about 35 Ma years ago (McLoughlin, 2001; Lewis et al., 2008.). As Antarctica became isolated from warmer waters and greenhouse gases declined in the atmosphere, the continent cooled down and glaciers began to form (Tripati et al., 2005; Fernández-Carazo et al., 2012). As climate cooled, the continent lost much of its temperate biota and, for the last 10 million years, has largely been a cold desert (Lewis et al., 2008). Since the ice sheet formation, Antarctica has remained isolated from other continents, but has continued to support a range of habitats, which are home for a variety of species, most of which are microorganisms (Convey et al., 2008; Fernández-Carazo et al., 2012).

Today ice-free areas of Antarctica cover a tiny proportion (0.32%) of the continent (British Antarctic Survey, 2004; Convey et al., 2008). All these habitats, including terrestrial and aquatic, face extended seasonal snow and/or ice cover mainly during winter, which restricts periods of biological activity, but also can protect the biota from extremes of temperature and wind abrasion (Convey et al., 2008).

Antarctic biota encounters a range of environmental stresses which has been described as one of the most remote, harsh and challenging for terrestrial life (Convey, 2006). Besides the extreme temperature and wind abrasion, these include lack of liquid water leading to desiccation, nutrient limitation due to poorly developed soils and catchments, repeated freeze thaw cycles, bright sunlight exposure during summer and prolonged darkness during winter (Moorhead et al., 2005; Mueller, 2005; Vincent, 2007; Convey et al., 2008). Antarctic contains the coldest and driest deserts on earth (for example the McMurdo Dry Valleys) (Vincent, 2004).

In the polar regions, extreme cold is an overarching stress because it drastically modifies the physical-chemical environment of living cells, with effects on biochemical reaction rates, substrate transport, membrane fluidity, and conformation of macromolecules, such as DNA and proteins (Zhao et al., 2007, Rodrigues & Tiedje, 2008). Moreover, additional physical and chemical stresses are imposed by ice crystal formation, water loss and increasing solute concentrations once the freezing point is crossed (Varin et al., 2012).

Although harsh climate conditions predominate on the Antarctic continent, there is still a great diversity of aquatic habitats in Antarctica, ranging from the benthos, water-column and fluctuating sea-ice cover of the circumpolar Southern Ocean to diverse lakes and ponds, and intermittently wet soils which sustain Antarctic biodiversity (Wynn-Williams, 1996). Even the most arid regions, such as the McMrudo Dry Valleys, are home to perennially ice-covered, endorheic, meromictic lakes and ponds, which are home to microbially-dominated communities (Taton et al., 2003; Sutherland & Hawes, 2008). Antarctica is the only continent that is dominated by microbial communities, mostly based on cyanobacteria, algae, mosses and lichens). Only two vascular plants are known from the Antarctic, both restricted to coastal regions of the Antarctic Peninsula (Convey, 2006). Due cyanobacteria being highly tolerant of extreme conditions and quickly responsive to sudden moisture availability after desiccation and/ or freezing in cold deserts, they often dominate these Antarctic habitats (Hawes et al., 1992).

Cyanobacteria are photosynthetic bacteria that require light, liquid water, air and some mineral nutrients for growth. They serve as primary colonizers of soils newly exposed by glacial retreat. Some taxa (e.g. Nostoc sp.) can fix atmospheric nitrogen that can locally enrich the predominantly oligotrophic biotopes (Vincent, 2000). The occurrence of cyanobacteria in Antarctica has been recorded since one of the first research expeditions conducted on the continent in the early twentieth century (as reported in Broady & Kibblewhite, 1991). Cyanobacteria are uniquely acclimated to the extreme conditions that Antarctic places offer (Taton et al., 2003; Sutherland & Hawes, 2008) with many ecophysiological and strategic adaptations that enable them to survive, colonize and even flourish in what is often considered to be a biologically hostile environment (Wynn-Williams, 1996). The abundance of cyanobacteria in Antarctic ecosystems has been related to both adaptive capacity, with high tolerance to extreme environment, as well as, the lack of predators and competing species which are eliminated by the effect of low temperature and freezing environment (Nadeau & Castenholz, 2000).

Among the strategies launched by cyanobacteria for their survival within hostile conditions, the production and excretion of extracellular exopolysaccharides (EPS) has been proposed as a protective response (Tamaru et al., 2005). EPS comprise a variety of organic macromolecules of high molecular weight such as polysaccharide, proteins, nucleic acids, phospholipids, uronic acids, jointly with other non-polymeric constituents of low molecular weight (Zhang & Fang, 2001). Several strains of cyanobacteria have been reported to be

capable of synthesizing exocellular polysaccharides as an additional structure of surface that differs in thickness, consistency and appearance (Phillipis & Vincenzini, 1998).

The production of EPS is generally considered to be directly related to environmental restraints on the microorganism and it is involved in the protection of a number of factors such as desiccation, freezing and ultraviolet irradiation (Phillipis & Vincenzini, 1998; Tamaru et al., 2005). The main function assigned to capsules or other investments of polysaccharide origin is to serve as a barrier between the organism and its immediate contact with the environment (Phillipis & Vincenzini, 1998). EPS production is also involved in the organization of cyanobacteria into mats (also termed biofilm), which might also provide a strategy that favors survival under adverse conditions (Rickard et al., 2003; Garcia-Meza et al., 2005). Mats are typically found attached to a solid substratum in a moist or liquid environment from which those nutrients are obtained (Rickard et al., 2003).Mats are functional consortia between cells (aggregate) of different microbial species, which present greater metabolic activity than the isolated species, and togerthe modify the light, nutrient and moisture regime within the mat in ways that can benefit individual growth and survival. The coaggregation of species and the specific interactions in the biofilm environment are an important mechanism to the formation of communities that optimizes cellular interactions, colonization of organisms and allows the set of strains to survive and proliferate under conditions where cells (species) isolated would show reduced growth (Rickard et al., 2003).

Several studies have recorded that the maritime and coastal microbiota of Antarctica has relatively high species diversity, but this becomes depauperate further inland and south where life exists near its limits (Wynn-Williams, 1996). The extreme stresses imposed by winter freezing on further inland must play a role in structuring biological communities (Hawes et al., 1999).

Owing to its limiting climate conditions and its habitats that have remained isolated for hundreds of thousands of years or longer, Antarctica offers an immense field laboratory for fundamental process research with global implications (Wynn-Williams, 1996; Vincent, 2000). The variety of Antarctic microbial ecosystems is of singular importance to research in evolutionary ecology and, is some unique opportunities for research on microbial evolution (Vicent, 2000). Although the studies on Antarctic cyanobacteria have increased on the last decade, many Antarctic locations have not been recorded yet. This present work was divided in 3 chapters and, we initially explored the variety of cyanobacteria strains from different Antarctic areas and on this topic, a morphological description of each morphotype found was

made. We also investigated the process (e.g. random e/or deterministic) that can be involved in determining the presence and/or absence of cyanobacteria communities at any one site. A quick method of assessing mat formation to demonstrate the effect of certain cyanobacterial strains on the mat-building also was developed through this research.

1.2. General description of sampling sites

The sampled ponds for this work are spread in the McMurdo Sound region of the Antarctic continent, including ponds on Ross Island, the McMurdo Ice Shelf and McMurdo Dry Valleys (Lower and Upper Wright Valley) (Figure 1 and 2).

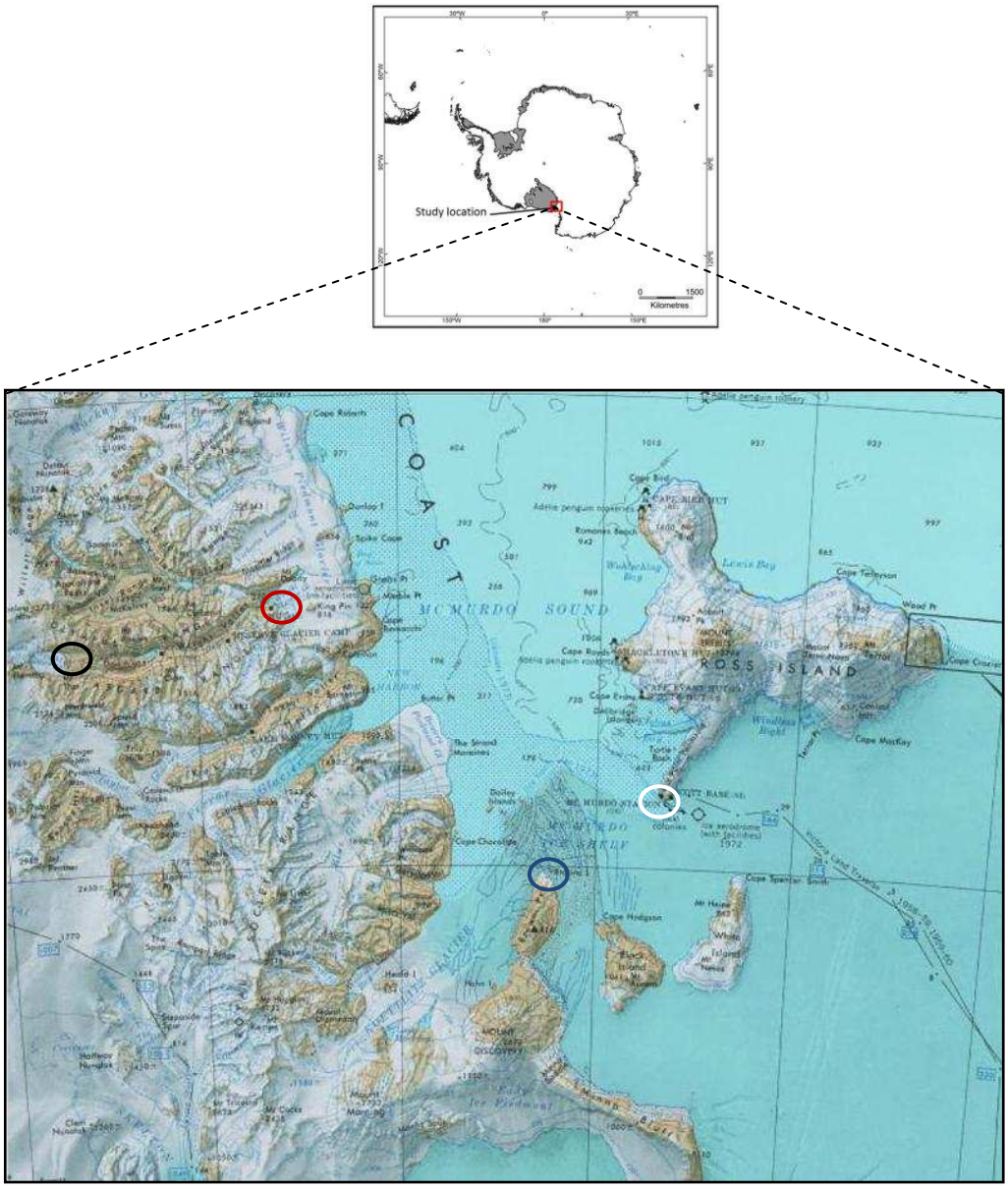

Figure 1. Map of the McMurdo Sound region showing the location of the four field areas: Upper Wright Valley (black ellipse); Lower Wright Valley (red ellipse); Ross Island (white ellipse); McMurdo Ice Self (blue ellipse). Inset, map of Antarctic continent modified from Simões et al., 2004.

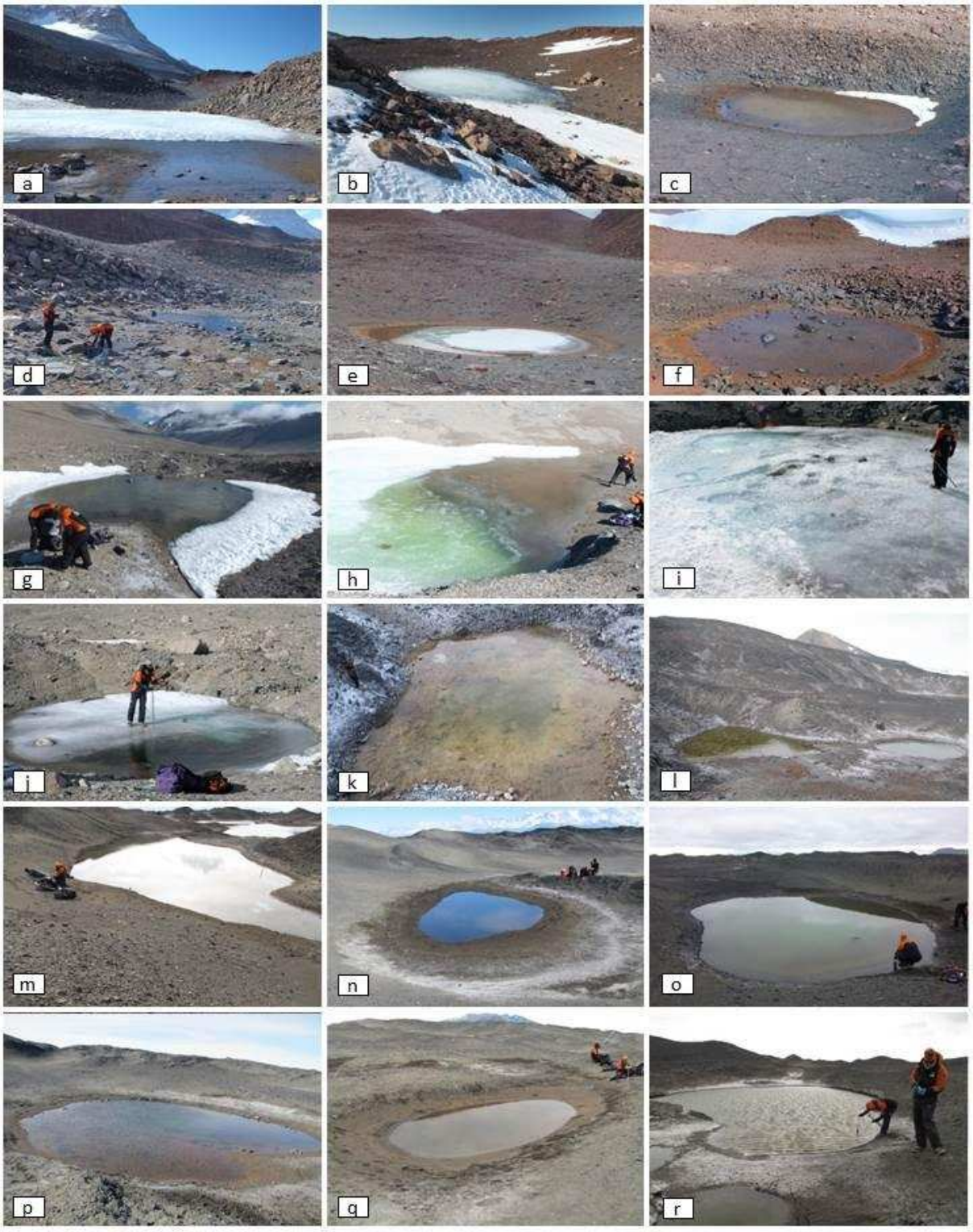

Figure 2. a-r: Photographs of the sampled ponds. a-f: Upper Wright Valley ponds. g-j: Lower Wright Valley ponds. k-l: Ross Island ponds; m-r: McMurdo Ice Shelf ponds.

McMurdo Dry Valleys

McMurdo Dry Valleys which experiences some of the most adverse environmental conditions in Antarctica is located in Southern Victoria Land, on the Wesern coast of the Ross Sea. The McMurdo Dry Valleys became a hyper-arid desert about 14 million years ago, and has remained dry ever since, despite the coming and going of the Ross Ice Shelf (Lewis et al., 2008). This makes the McMurdo Dry Valley region a very old habitat, with plenty of time to accumulate and adapt species.

Wright Valley

The Wright Valley is one of the conspicuous ice-free areas and it is located in the McMurdo Dry Valleys. The Wright Valley is 45 km long and 7 km wide and is an enclosed basin. The Olympus and Asgard mountain ranges lie to the north and south respectively, and stand up to 2000 m above the valley floor. The Upper Wright and Lower Wright glaciers terminate at either end of the valley. The valley lies west to east, and perpendicular to the sea. Much of the valley floor is covered in glacial moraine and till, and soils are derived from this and bedrock. Major materials in these are sandstone, granite, diorite, dolerite and basalt. The dry valleys are too far inland to see marine animals; however mummified seals do occur (Campbell & Claridge, 1987).

Upper Wright Valley

Also known as The Labyrinth is an area at the west end of Wright Valley, furthest from the sea, and is so named because large troughs have been carved through the valley by sub-glacial water flow (Healy 2005). The bed rock is mostly composed of dolerite, and little detritus or sediment is present (Torii et al., 1989). The area is at 800 - 1000 m elevation, and lies at the base of the Upper Wright Glacier (Healy, 2005). More than 60 ponds exist in depressions of the troughs, and these are fed by melt from snow and the glacier. The geochemistry of the ponds has been documented, and ponds vary from fresh to saline, with salts accumulated by atmospheric deposition, and concentrated by evaporation.

Lower Wright Valley

The Lower Wright Valley lies at the eastern, seaward end. The Lower Wright Glacier terminates at this end, into Lake Brownworth at its base. The Onyx River runs inland from Lake Brownworth to Lake Vanda in the central valley. A number of ponds exist which are fed

by glacial melt and the limited precipitation that reaches the valley, including those around Bull Pass (Webster et al., 1994).

Ross Island (Hut Point),

Ross Island is an island formed by four volcanoes in the Ross Sea near the continent of Antarctica, off the coast of Victoria Land in McMurdo Sound. Hut Point Peninsula is about 20 kilometers long and 2 to 4 kilometres wide. It consists of a series of en echelon lines of volcanic cones that extend in a south-southwest direction from Mount Erebus, Ross Island. The cones are composed of basanite and basanitoid lavas with lesser amounts of hawaiite and phonolite (Kyle & Treves, 1974). The Hut Point site is one of the principal sites of early human activity in Antarctica. It is an important symbol of the Heroic Age of Antarctic exploration and, as such, has considerable historical significance. Some of the earliest advances in the study of earth sciences, meteorology, flora and fauna in Antarctica are associated with the Discovery Expedition based at this site (Management Plan for Antarctic Specially Protected Area 2010).

McMurdo Ice Shelf, Bratina Island

The McMurdo Ice Shelf (MIS) is a small 1500 km^2 ice shelf located in the southwest corner of the Ross Sea, near to Bratina Island. The MIS is one of the most extensive surface ablation areas in Antarctica (Swithinbank, 1970), yet it does not ablate entirely due to a unique characteristic of the area – nourishment of the floating ice shelf by the freezing of seawater beneath (Debenham, 1920). MIS is almost completely covered by rock debris and this debris is derived from an unusual source – the seafloor below the ice shelf. Debris are incorporated into the ice during basal freezing, which are slowly moved to the ice surface as the surface ice ablates. The topography is undulating with hundreds of ponds existing where meltwater has collected between the troughs. The ponds come in all sizes, from small lakes 100's of meters long, to small pools 2-5 m in diameter. Some lose their ice cover in summer, others retain it, and some may lose it in some years and keep it in others. The physical and chemical properties are greatly varied, with some hyper saline ponds existing within meters of freshwater ponds (Hawes & Howard-Williams, 2003). The location is inaccessible to penguins, however at least one breeding pair of skua nests around the ponds that were sampled.

2. References

British Antarctic Survey. 2004. Antarctica, 1:10 000 000 scale map. BAS (Misc) 11. (http://www.antarctica.ac.uk/Resources/schoolzone/resources/Factsheets/index.html). British Antarctic Survey, Cambridge, UK.

Broady, P. A. & Kibblewhite, A. L. 1991. Morphological characterization of Oscillatoriales (Cyanobacteria) from Ross Island and southern Victoria Land, Antarctica. Antarctic Science 3: 35-45.

Campbell, I. B.; Claridge, G. G. C. 1987. Antarctica: Soils, Weathering Processes and Environment. Elsevier: Amsterdam, 368p.

Convey, P. 2006. Antarctic terrestrial ecosystems: responses to environmental change. Polarforschung 75: 101-111.

Convey, P.; Gibson, J. A. E.; Hillenbrand, Claus-Dieter; Hodgson, D. A.; Pugh, P. J. A.; Smellie, J. L. & Stevens, M. I. 2008. Antarctic terrestrial life - challenging the history of the frozen continent? Biology Reviews 83:103-117.

Debenham, F. 1920. A new mode of transportation by ice: The raised marine muds of South Victoria Land (Antarctica). Quarterly Journal of the Geological Society of London 75: 51-76.

Fernández-Carazo, R.; Namsaraev, Z.; Mano, Marie-Jose; Ertz, D. & Wilmotte, A. 2012. Cyanobacterial diversity for an anthropogenic impact assessment in the Sør Rondane Mountains area, Antarctic. Antarctic Science 24: 229-242.

Friedmann, E.I. 1982. Endolithic microorganisms in the Antarctic cold desert. Science 215: 1045-1053.

García-Meza, J.V.; Barrangue, C. & Admiraal, W. 2005. Biofilm formation by algae as a mechanism for surviving on mine tailings. Environmental Toxicology and Chemistry 24:573-581.

Hawes, I.; Howard-Williams, C. & Vincent, W.F. 1992. Desiccation and recovery of cyanobacterial mats. Polar Biology 12: 587-94.

Hawes I. & Schwarz A.-M. 1999. Photosynthesis in an extreme shade environment: Benthic microbial mats from Lake Hoare, a permanently ice-covered Antarctic lake. Journal of Phycology 35: 448-459.

Hawes, I. & Howard-Williams, C. 2003. Pond life on the McMurdo Ice Shelf, one of the world's strangest ecosystems. Water & Atmosphere 11: 18-19.

Healy, M. 2005. The Physical and Geochemical Properties of Glacial Meltwater Ponds in the Dry Valleys, Antarctica. University of Auckland, Auckland.

Kyle, P. R. and Treves, S.B. 1974. Geology of Hut Point Penisula, Ross Island. Antarctica Jornal 9: 232-234.

Lewis, A.R.; Marchant, D.R.; Ashworth, A.C.; Hedenäs, L.; Hemming, S.R.; Johnson, J.V.; Leng, M. J.; Machlus, M. L.; Newton, A. E.; Raine, J. I.; Willenbring, L. K.; Williams, M. & Wolfe, A. P. 2008. Mid-Miocene cooling and the extinction of tundra in continental Antarctica. PNAS 105: 10676 - 10680.

Management Plan for Antarctic Specially Protected Area. 2010. Hut Point, Ross Island, Measure 10, Annex. No. 158.

McLoughlin, S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. Australian Journal of Botany 49:271-300.

Moorhead, D.; Schmeling, J. & Hawes, I. 2005. Modelling the contribution of benthic microbial mats to net primary production in Lake Hoare McMurdo Dry Valleys. Antartic Science 17: 33-45.

Mueller, D. R.; Vincent, W. F.; Bonilla, S. & Laurion, I. 2005. Extremotrophs, extremophiles and broadband pigmentation strategies in a High Arctic ice shelf ecosystem. FEMS Microbiology, Ecology 53: 73-87.

Nadeau, T. L. & Castenholz, W. F. 2000. Characterization of psychrophillic oscillatorians (Cyanobacteria) from antarctic meltwater ponds. Journal of Phycology 36: 914-923.

Phillipis, R. & Vincenzini, M. 1998. Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiology Reviews 22: 151-175.

Rickard, A. H.; Gilbert, P.; High, N. J.; Kolenbrander, P. E. & Handley, P. S. 2003. Bacterial coaggregation: an integral process in the development of multi-species biofilms. TRENDS in Microbiology 11: 94-100.

Rodrigues, D. F. & Tiedje, J. M. 2008. Coping with our cold planet. Applied and Environmental Microbiology 74: 1677-1686.

Simões, J. C.; Arigony-Neto, J. & Bremer, U. F. 2004. O uso de mapas antárticos em publicações. Pesquisa Antártica Brasileira 4: 191-197.

Sutherland, D. L. & Hawes, I. 2008. Annual growth layers as proxies of past growth conditions for benthic microbial mats in a perennially ice-covered Antarctic lake. FEMS Microbiology, Ecology 67: 279-292.

Swithinbank, C. W. M. 1970. Ice movement in the McMurdo Sound area of Antarctica. IAHS SCAR Publication 86: 472-487.

Tamaru, Y.; Takani, Y.; Yoshida, T. & Sakamoto, T. 2005. Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium Nostoc commune. Applied and Environmental Microbiology 71: 7327-7333.

Taton, A.; Grubisic, S.; Brambilla, E.; De Wit, R. & Wilmotte, A. 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Applied and Environmental Microbiology 69: 5157-5169.

Torii, T.; Nakaya, S.; Matsubaya, O.; Matsumoto, G. I.; Masuda, N.; Kawano, T. & Murayama, H. 1989. Chemical characteristics of pond waters in the Labyrinth of Southern Victoria Land, Antarctica. Hydrobiologia 172: 255-264.

Tripati, A.; Backman, J.; Elderfield, H. & Ferretii, P. 2005. Eocene bipolar glaciation associated with global carbon cycle changes. Nature 436: 341-346.

Varin, T.; Lovejoy, C.; Jungblut, A. D.; Vincent, W. F. & Corbeil, J. 2012. Metagenomic Analysis of Stress Genes in Microbial Mat Communities from Antarctica and the High Arctic. Applied and Environmental Microbiology 78: 549-559.

Vincent, W. F. 2000. Cyanobacterial dominance in the polar regions, p. 321-340. In: Whitton, B. & Potts, M. (eds.). Ecology of the Cyanobacteria: their diversity in space and time. Kluwer Academic Press, Dordrecht, The Netherlands.

Vincent, W. F.; Mueller, D. R. & Bonilla, S. 2004. Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the high Artic. Crybiology 48: 103-112.

Vincent W.F. 2007. Cold tolerance in cyanobacteria and life in the cryosphere, p 287-301. In: Seckbach J (ed), Algae and cyanobacteria in extreme environments. Springer, Heidelberg, Germany.

Zhang, T. & Fang, H. H. P. 2001. Quantification of extracellular polymeric substances in biofilms by confocal laser scanning microscopy. Biotechnology Letters 23: 405-409.

Zhao, D.; Chen, X.; He, H.; Shi, M. & Zhang, Y. 2007. Gene cloning and sequence analysis of the cold-adapted chaperones DnaK and DnaJ from deep-sea psychrotrophic bacterium Pseudoalteromonas sp. SM9913. Acta Oceanologica Sinica 26:91-100.

Webster, J. G.; Brown, K. L. & Vincent, W. F. 1994**.** Geochemical processes affecting meltwater chemistry and the formation of saline ponds in the Victoria Valley and Bull Pass region, Antarctica. Hydrobiologia 281: 171-186.

Wynn-Williams, D. D. 1996. Antarctic microbial diversity: the basis of polar ecosystem process. Biodiversity and Conservation 5: 1271-1293.

CHAPTER 1

Descriptions of morphospecies of cyanobacteria from benthic microbial mats of ponds in the McMurdo Sound region

1. Introduction

The Cyanobacteria (Cyanophyceae, blue-green algae) are an ancient group of micro-organisms that comprises unicellular to multicellular species which possess chlorophyll a and perform oxygenic photosynthesis (Schopf, 2000; Frey, 2012). They are found in almost all terrestrial and aquatic biotopes (Whitton & Potts, 2000) and they are well known for their ability to dominate habitats in temperate and extreme environments (Pearl & Huisman, 2008; Wood et al., 2010).

In Antarctica, cyanobacteria are widespread in lakes, ponds, streams, soils and on the surfaces and within rocks, where they can form macroscopically visible crusts or thin biofilms (Friedmann, 1982; Vincent, 2000; Andersen et al., 2011; Hawes et al., 2011). They often dominate the biomass and biological productivity of Antarctic regions due to their adaptation to the polar environment (Vincent, 2000; Jungblut et al., 2009).

Antarctic cyanobacteria tolerate a wide range of climatic conditions and harsh physicochemical parameters (e.g. high salinities, UV radiation, extended periods of darkness, freezing and desiccation) (Zakhia et al., 2009). They have developed several stress response mechanisms and they are highly responsive to sudden moisture availability after desiccation and freezing (Hawes et al., 1992; Vincent, 2007) which gives them the impressive ability to colonise and dominate these regions.

Many authors have reported on the diversity of Antarctic cyanobacteria using morphological and molecular methods. Both cosmopolitan and endemic taxa have been reported (Broady & Kibblewhite, 1991; Komárek, 1999; Taton et al., 2003, 2006a,b; Jungblut et al., 2005; Comte et al., 2007). Taxonomy of cyanobacteria has changed substantially in the last two decades with the introduction of molecular phylogenetics, but the true identities of many cyanobacteria remains an enigma and the number of taxa recorded in Antarctica is unclear (Garcia-Pichel, 2009; Frey, 2012). Also, many Antarctic locations have not yet been investigated. Unlike molecular methods, classifications based entirely on morphological features such whether a cyanobacterium is filamentous, colonial or unicellular, and whether or not it possess heterocytes do not reflect evolutionary relationships (Nadeau et al., 2001) but the possession of consistent features and common characters can be used to identify and sort them into recognisably distinct groups.

The present research presents descriptions of the morphology of cyanobacteria from pond mat communities sampled from ponds along inland to coastal gradients in the McMurdo Sound region. Twenty five ponds were sampled, including many never previously sampled.

This work will help extend knowledge of the distribution of Antarctic cyanobacteria morphospecies and these data can be compared with future molecular analyses to increase the understanding of cyanobacteria identities.

2. Materials and Methods

Sampling sites

Samples of benthic microbial mats from 25 Antarctic ponds distributed along inland-coastal gradients in the McMurdo Sound region (Ross Island, McMurdo Ice Shelf, Lower Wright Valley and Upper Wright Valley) were collected, in summer season 2012. The area location, nominal longitudes and sampling description for each pond were described in the Table 1. Samples were collected from the pond margins using a cut-off 20 mL syringe. Cores were taken with care to collect the mat only and no underlying sediment. The resulting mat core was carefully lifted from the sediment and transferred to a plastic container and kept chilled while in the field and subsequently frozen (-20°C) for return to New Zealand.

Sample analysis

For morphological identification, benthic mat samples were thawed and a subsample was directly observed by light microscopy. Remaining sample material was placed in 50 ml sterile polycarbonate bottles (Biolab, NIWA) containing the mineral nutrient medium MLA (Bolch & Blackburn, 1996) for future observation and maintained at laboratory temperatures of about 21°C. No attempt was made to isolate cyanobacterial morphospecies into unialgal cultures.

Cyanobacterial morphospecies were identified using an Olympus light microscope (BX51, Olympus) at up to 1000x. Ten morphological criteria were used to describe the morphotypes (trichome shape, number of trichomes in sheath, presence or absence of terminal attenuation of trichome, calyptra on mature apical cell, shape of apical cell, presence or absence of constrictions at transverse walls, granules, branching, range in width of trichomes and range of cell length) with reference to Komárek & Anagnostidis (1989); Komárek & Anagnostidis (2000); Komárek & Anagnostidis (2005) and other sources from literature (Broady & Kibblewhite, 1991; Taton et al., 2011; Strunecký et al., 2011). The cyanobacterial morphospecies were documented by photomicrography.

The percentage of frequency of occurrence of the morphospecies for each studied area was calculated by: FO = (Npi x 100)/Ntp; where FO= Frequency of occurrence, Npi= number of pond that the morphospecie was found and, Ntp= total number of studied pond.

Table 1. Locations of study sites and mat description

Area	Location			Mat description			
	Pond	Lat.	Long.	Colour	bilayer	cohesive	thickness
Upper Wright Valley	L26	77 33 03.2	160 43 24.0	1	1	2	1
	L09	77 33 02.4	160 44 26.2	2	2	3	2
	L15	77 33 13.1	160 43 01.2	3	1	3	2
	L16	77 32 29.0	160 45 20.0	1	1	1	2
	E9	77 31 35.1	160 46 16.7	2	1	1	1
	E4	77 31 21.3	160 44 17.9	2	1	1	1
Lower Wright Valley	LW2	77 26 43.9	162 41 30.3	1	1	3	2
	LW12	77 27 00.1	162 39 00.5	1	1	3	2
	LW13	77 27 02.0	162 38 52.5	1	2	3	2
	LW14	77 27 02.8	162 38 44.7	1	2	3	2
	LW15	77 27 03.1	162 38 35.3	1	2	3	2
	LW16	77 27 03.0	162 38 28.6	1	2	3	2
	LW18	77 27 02.7	162 38 19.3	1	1	2	2
Ross Island	OHP	77 51 20.9	166 41 25.7	1	1	2	2
	HP1	77 50 36.6	166 38 41.6	1	1	2	1
	HP2	77 50 41.8	166 38 36.8	1	2	3	2
	HP3	77 50 41.4	166 38 35.5	1	2	3	2
McMurdo IceShelf	FH	78 00.962	165 33.070	2	2	2	1
	Nostoc	78 00.832	165 33.288	2	1	2	1
	P70	78 00.892	165 33.136	1	2	3	2
	Skua	78 00.798	165 33.113	2	2	3	2
	Orange	78 00.838	165 33.339	1	2	3	2
	P70E	78 00.949	165 33.082	2	1	2	1
	Brack	78 00.947	165 32.723	1	2	3	2
	Salt	78 00.963	165 32.734	4	1	2	2

Colour: 1 = orange; 2=brown; 3=black;4=green
Bilayer: 1= no; 2= yes
Degree of cohesion: 1=flake; 2=sheet; 3= mat
Thickness: 1= thin; 2=thick

3. Results and Discussion

On the basis of microscope observations, twenty nine cyanobacterial morphospecies were distinguished in the samples. Four were assigned to the order Chroococcales, 22 to Oscillatoriales and three to Nostocales. No members of the Stigonematales were encountered. The morphotypes are designated 1-29 and a summarized morphological and morphometric description with data on site of occurrence of each morphotype are shown in Table 2. Photomicrographs are shown in Figures 1, 2 and 3.

OSCILLATORIALES

Morphotype 1 (Fig.1 A1-A2)

Description: Trichome which can form short filament (4 to 16 celled) or rarely trichome solitary. Cells are cylindrical or up to barrel-shaped, ± isodiametric by fragmentation of trichome, sheaths lacking, immotile, and constricted at the cross-walls.

Remarks: This morphotype was assigned to genus Borzia and appeared in 11 of the studied samples. Borzia sp. was previous documented in continental Antarctic systems by Hodgson et al. (2001) and Broady (2005). Despite of the increasing availability of information about Antarctic cyanobacteria diversity, it was not found literature that has reported this morphospecie on the Antarctica Peninsula habitats, suggesting that this morphotype has restricted distribution.

Morphotye 2 (Fig.1 B)

Description: Trichome forming fine filament, a little flexuous, elongated, broadly constricted at the cross-walls, immotile, without firm sheaths, simple. Necridic cells, akinetes, aerotopes and heterocytes were not observed. Cells barred-shaped or sometimes almost isodiametric.

Remarks: The morphotype 2 was found at only one sample and based on the classical literature we suggest that this strain contains resemblance with genus Komphovorum. Although the width description to this genus made by Komárek & Anagnostidis (2005) is a little wider than the width observed to the morphotype recorded in the present work.

Morphotype 3 (Fig.1C)

Description: Filamentous trichome, thin, cylindrical, straight or slightly flexuous, without sheaths, not constricted at the cross-walls, ± gradually attenuated and bent or coiled at

the ends. Trichome deeply motile, with intense gliding in the direction of the longitudinal axis forwards and backwards or waving (oscillation). Cells longer than wide, sometimes with large prominent granules. Apical cells usually conical, hooked or bent.

Remarks: The possession of consistent characters observed in this strain allowed the assignment to Geitlerinema cf. ionicum and the morphotype was observed at nine samples. Studies from Antarctic Peninsula (Komárk, 1999) and Antarctic continental (Taton et al., 2006a) have documented the presence of Geitlerinema sp., which show wide distribution of this morphospecie across Antarctica.

Morphotype 4 (Fig.1 D1-D2)

Description: Trichomes usually straight or a little waved, consisting of few to several cells, with broadly constrictions at cross-walls. Trichomes without sheaths. Motility lacking. Cells usually cylindrical with rounded ends, sometimes almost barred-shaped, apical cells not differentiated.

Remarks: This morphotype would separate in two morphospecies by using the length of the trichome, which sometimes possessed long filament (Fig.1 D1) and occasionally short trichome (Fig.1 D2). However, we have not found consistent character to separate these morphospecies. Morphotype 4 was recorded at 14 samples and it was assigned to genus Pseudanabaena sp.

Morphotype 5 (Fig.1 E1-E2)

Description: Filament very short, few-celled (3 – 6 celled), thin, straight or slightly curved. Cell cylindrical, constantly longer than wide, sometimes granules at the ends were present. Trichome constricted, not attenuated, motility, trembling-like.

Remarks: This strain also contains similarity to genus Pseudanabaena and appeared at eight of the studied samples.

Morphotype 6 (Fig.1 F1-F3)

Description: Trichome very thin, not attenuated at the ends, with firm, thin sheaths (Fig.1 F2) or not (Fig1. F1), presence constantly of prominent granules at the ends (Fig. 1 F3), with indistinct trembling.

Remarks: Filaments allocated to morphotype 6 were thinner than morphotype 4 but similar to width of filaments belonging to morphotype 5. However, morphotype 6 forms long filament while the possession of morphotype 5 is characterized by constantly short filament. The features found in morphotype 6 are also acceptable to genus Pseudanabaena and was recorded at 14 samples.

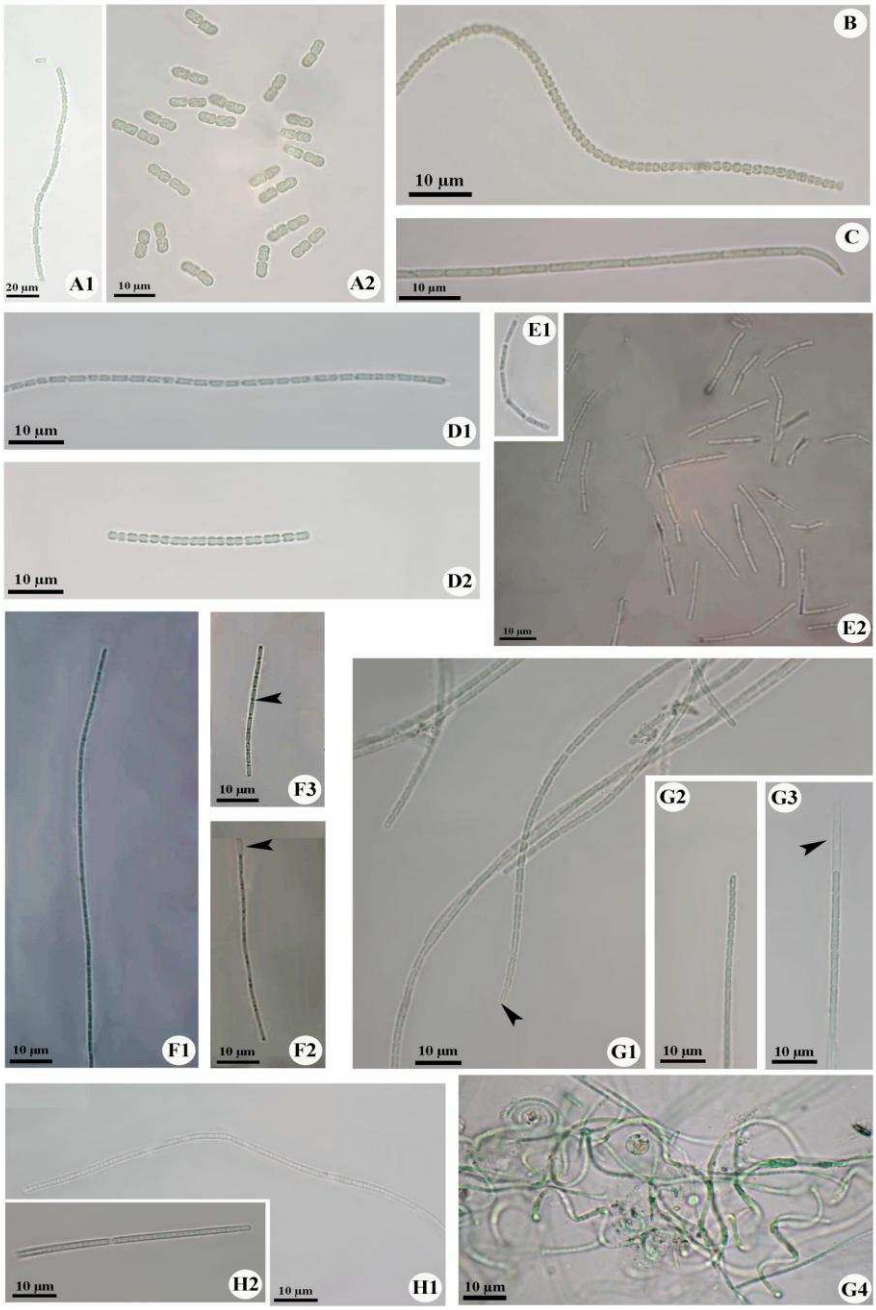

Figure1. Morphospecies. (**A-G**) morphospecies belonging to the Oscillatoriales order: (**A1-A2**) Borzia sp. (mph.1); (**A1**) formation of filament; (**A2**) short trichome; (**B**) cf. Kamvophorum sp. (mph. 2); (**C**) Geitlerinema sp. (mph. 3); (**D1-D2**) Pseudanabaena sp. (mph. 4); (**D1**) long filament; (**D2**) short filament; (**E1-E2**) cf. Pseudanabaena (mph 5); (**E1**) detail of trichome; (**F1-F3**) cf. Pseudanabaena (mph 6); (**F2**) ends of trichome with sheath (arrow); (**F3**) ends of cell with granule (arrow); (**G1-G4**) Leptolyngbya sp. (mph 7); (**G1**) ends of trichome with slightly attenuation (arrow); (**G2**) filament without sheath; (G3) end of trichome with sheath (arrow); (G4) coiled filaments; (**H1-H2**) Leptolyngbya sp. (mph 8). Mph= morphotype.

Morphotype 7 (Fig.1 G1-G4)

Description: Filamentous trichome, straight or flexuous, finely waved, long, with sheath (Fig.1 G3) or not (Fig.1 G2), the most not attenuated at the ends but attenuation present in some filaments (Fig.1 G1). Trichomes immotile or with indistinct trembling. Cells the most cylindrical, however, also isodiametric or a little longer than wide.

Remarks: Morphotype 7 was assigned to genus Leptolyngbya sp. and this morphotype also could include more than one morphospecies by the possession of trichomes very densely spirally coiled, which was occasionally observed in this work (Fig. G4). Komárek (2007) has separated and classified the spirally coiled trichome belonging to genus Leptolynbya as Leptolyngbya borchgrevinkii. Morphotypes 7 were found at all samples studied.

Morphotype 8 (Fig. H1-H2)

Description: Filaments nearly straight, sometimes slightly curved, pale blue-green, not constricted at cross-walls. Cells of various lengths, sometimes longer than wide but also wider than long. Apical cell somewhat elongated, sometimes rounded.

Remarks: This strain was assigned to genus Leptolynbya sp. as well. The major characteristic separating the morphotype 7 from 8 was the cells shape, which is nearly square at the morphotype 8 and slightly cylindrical or isodiametric at the morphotype 7. Morphotype 8 was observed in 22 samples.

Morphotype 9 (Fig.2 A)

Description: Trichome long, curved, with sheath, thin, straight at the ends, not attenuated, and not constricted.

Remarks: This morphotype was distinguished from 6, 7 and 8 morphotypes by cell length. Morphotype 9 contains cells constantly longer than wide (approximately 5x longer than wide). This strain might belong to genus Leptolynbya and we recorded this morphotype at only one of the studied samples.

Morphotype 10 (Fig.2 B1-B3)

Description: comprised filaments solitary or in small free clusters, thin, slightly curved, long with false branching with very thin sheath. Trichomes were cylindrical, slightly

constricted at cross-walls, not attenuated towards the ends. Cells cylindrical, ± isodiametric or slightly longer or shorter than wide, end cells rounded.

Remarks: This morphotype was characterized by the presence of false branching and the characters observed at this strain allowed to assignment to Plectolyngbya cf. hodgsonii, which has been recorded by Taton et al. (2011) in Antarctic ecosystems as well. We have recorded morphotype 10 at five samples.

Morphotype 11 (Fig.2 C1-C4)

Description: Filaments long, flexuous or slightly curved, sometimes irregularly coiled, richly and repeatedly branched, somewhat constricted at the cross walls. Sheath thick, slightly widened from trichomes. Cells always shorter than wide.

Remarks: This strain also could belong to genus Plectolyngbya and the major characteristic to distinguish morphotype 11 from morphotype 10 was the cell width, which is wider at morphotype 11. We recorded this strain at only two samples.

Morphotype 12 (Fig.2 D)

Description: Trichome straight, distinctly constricted at the cross wall, cell rounded up to barrel-shaped, shorter than wide to isodiametric, with prominent granules, apical cells widely rounded.

Remarks: This morphospecie might be assigned to genus Leptolyngbya with resemblance to Phormidum priestley as described by Komárek & Anagnostidis (2005) and it was observed at 17 of the studied samples.

Morphotype 13 (Fig.2 E)

Description: Filaments densely joined and entangled with one another with irregularly wavy forming tallus. Trichome not attenuated to the ends, slightly constricted at the cross-walls. Cells cylindrical, mostly longer than wide, apical cells mainly conical.

Remarks: We have suggested that this strain contains characteristic which could be assigned to genus Schizothrix sp. and it was found at three samples.

Morphotype 14 (Fig.2 F)

Description: Trichome cylindrical, straight or slightly waved, sometimes a little screw-like coiled at the ends, not constricted, motile, with gliding, oscillation. Filament cell very short, wider than long, without sheaths.

Remarks: The observed characteristics allowed the assignment of this morphotype to genus Ocillatoria sp. and appeared at three of the studied samples.

Morphotype 15 (Fig.2 G)

Description: Trichome straight, cylindrical, not constricted, slightly attenuated at the end (outer cell). Filaments cells were always shorter than wide, somewhat thickened outer cell wall.

Remarks: The possession of consistent characters in this morphotype also allowed the assignment to genus Ocillatoria. Although the width of morphotype 15 being only a little broader than morphotype 14, the cell length of morphotype 15 is constantly longer than morphotype 14. Moreover, the shape of apical cell also differs comparing the two morphotypes. Apical cell of morphotype 15, range to round to slightly elongated while in morphotype 14 is broadly rounded. We have recorded morphotype 15 at only one sample.

Morphotype 16 (Fig.2 H)

Description: Tallus olive green, thick, weakly curved, not constricted at the cross-walls, not attenuated at the ends. Filament cell always wider than long. Apical cell rounded with slightly thickened outer cell wall.

Remarks: This morphotype contains characteristics which could be assigned to genus Oscillatoria as well. The main feature that separate morphotype 16 from the other morphotypes designated to this genus in this study was the cell width. Morphotype 16 possessed cells wider than morphotypes 14, 15 and 17. This morphospecie was recorded at only two samples.

Morphotype 17 (Fig.2 I)

Description: Filament straight, not constricted, slightly attenuated and somewhat hooked at the ends. Cell were always shorter than wide.

Remarks: This morphotype also was assigned to Oscillatoria genus and was distinguished from Morphotype 14, 15 and 16 by the apical cell and trichome width. Morphotype 17 was thinner compared to 14, 15 and 16 morphotypes and additionally, the apical cell contains greatly thickened outer cell wall. Morphotype 17 was recorded at two of the investigated samples.

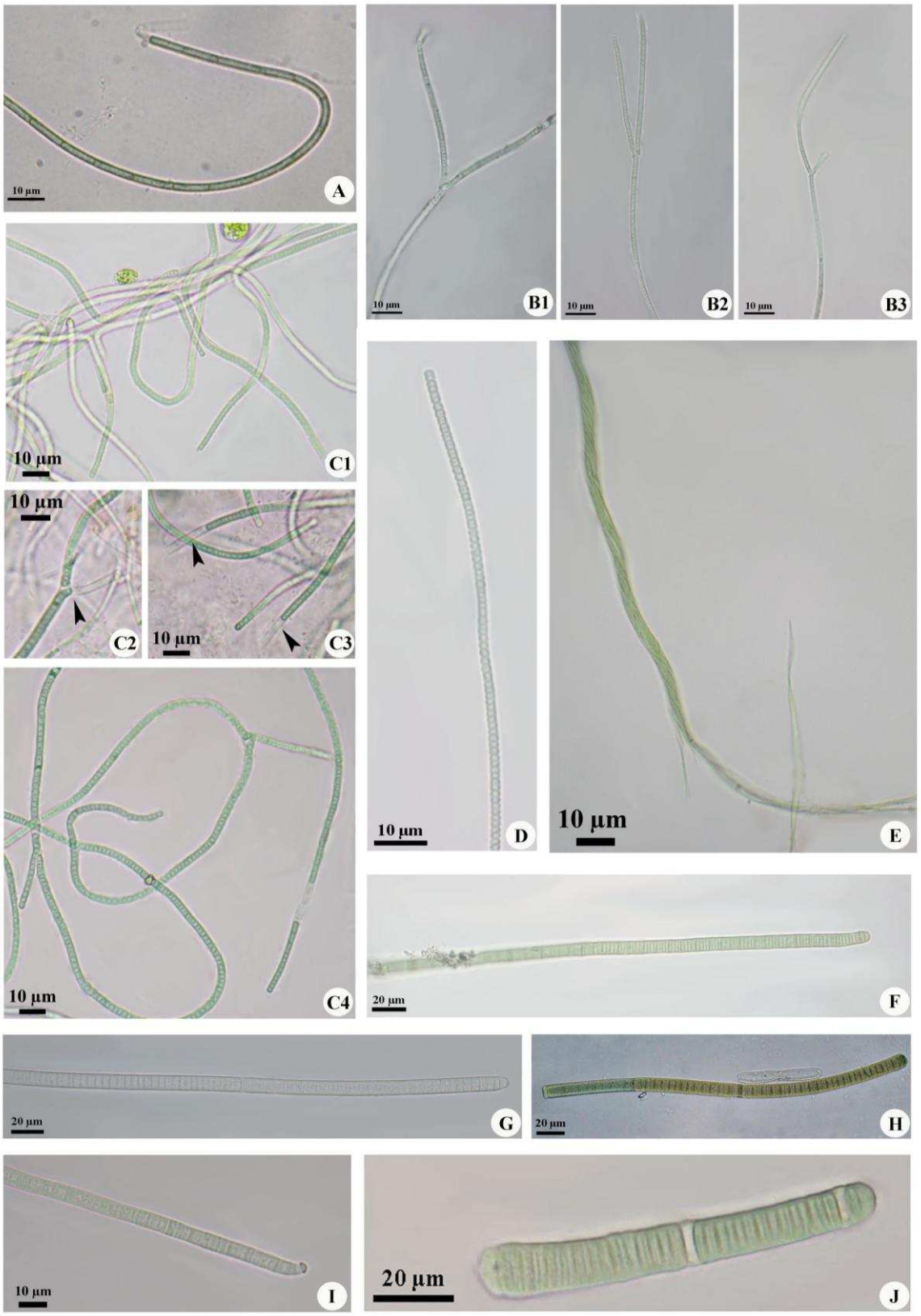

Figure 2. Morphospecies. (**A-J**) morphospecies belonging to the Oscillatoriales order: (**A**) cf. Leptolyngbya (mph 9); (**B1-B3**) Plectolynbya cf. hodgsonii (mph. 10); (**C1-C4**) cf. Plectolynbya (mph 11); (**C2**) detail of false branching; (**C3**) ends of filament with sheath (arrow); (**D**) Leptolyngbya with resemblance to P. prestleyei (mph12); (**E**) cf. Schizothrix (mph 13); (**F**) Oscillatoria sp. (mph. 14); (**G**) Oscillatoria sp. (mph. 15); (**H**) cf. Oscillatoria (mph. 16); (**I**) Oscillatoria sp. (mph 17); (**J**) Crinalium sp. (mph. 18). Mph = morphotype.

Morphotype 18 (Fig.2 J)

Description: Filament single, trichome straight or slightly wavy, cylindrical, not attenuated towards ends, slightly constricted at cross-walls, cells very shorter, always shorter than wide, terminal cells widely-rounded.

Remarks: The strain was assigned to Crinalium cf. glaciale and we have observed morphospecie 18 at only one sample. Crinalium sp. was earlier described by Broady & Kibblewhite (1991) dwelling Antarctica glacier.

Morphotype 19 (Fig.3 A)

Description: Filament long, variously curved but sometimes straight, not constricted, sheath present. Cell of filament were short, constantly wider than long with rounded apical cell.

Remarks: The presence of hyaline and thick sheath at the morphotype 19 suggests that this strain could belong to Lyngbya genus. The strain was recorded at 3 samples.

Morphotype 20 (Fig.3 B1-B3)

Description: Filamentous trichomes, straight or slightly curved, not or only slightly constricted at cross walls, presence of sheath (Fig.3 B3) or absence (Fig.3 B1-B2). Cells quadratic or longer than wide, occasionally with large granules. Terminal cells rounded at the ends (Fig.3 B2) or conical-shaped (Fig.3 B1).

Remarks: The regular occurrence of characters observed in this morphotype allowed the assignment to Phormidium cf. murrayi. This morphospecie was previous reported by Mataloni et al. (2005) and Komárek & Elster (2008). However, recently research has separated Antarctic strains which have been previously identified as Phormidium cf. murrayi to a new genus and classified as Wilmottia cf. murrayi (Strunecký et al., 2011). Additionally, by the position in phylogenetic trees the new genus is separated genetically from all related oscillatorialean genera and comprising only one species up to now (Strunecký et al., 2011). The presence of the morphotype 20 was detected at 14 samples.

Morphotype 21 (Fig.3 C)

Description: Thick filament, mostly straight but sometimes slightly curved (especially at the ends) with somewhat hooked end, not constricted. Filaments cells were the most wider than long, rarely longer than wide, with dispersed granules.

Remarks: This strain was assigned to Phormidium cf. autumnale and it is very often documented from many Antarctic systems (Broady & Kibblewhite, 1991; Mataloni et al., 2005; Komárek & Elster, 2008; Corrêa, 2012). In this study we have identified Phormidium cf autmnale at 17 of studied sites.

Morphotype 22 (Fig.3 D)

Description: Filament straight or slightly curved, not constricted, slightly attenuated at the ends and also was assigned to genus Phormidium.

Remarks: The distinction of the morphotypes 21 and 22 were made by using cells shape and size. Filaments designated to morphotype 22 were mostly thinner than filaments assigned to morphotype 21. Additionally, morphotype 22 contains cells nearly quadratic or sometimes longer than wide while morphotype 21 possessed cells mostly wider than long. The characters observed in the morphotype 22 suggest the assignment of this strain to Phormidium cf. setchellianaum. The presence of the morphospecie was detected at six samples.

NOSTOCALES

Morphotype 23 (Fig.3 E)

Description: Filamentous, isopolar, more or less straight or curved. Trichomes uniserial, cylindrical, constricted at cross walls, presence of heterocytes in more or less regular distances from one another. Cells slightly barrel-shaped which the length never exceeds the width. Heterocytes somewhat differ in their size from vegetative cells (slightly larger than vegetative cells).

Remarks: This strain was assigned to genus Nodularia sp. Different morphospecies belonging to Nodularia have been describe from different regions of the Antarctica by Broad (2005); Taton et al. (2006a), Komárek & Elster (2008). In the present work, morphotypes allocated to genus Nodularia were recorded at six of the studied samples.

Morphotype 24 (Fig.3 F1-F4)

Description: Filamentous tallus, widely constricted with intercalary heterocytes, slightly coiled, long, in cluster forming dense mat/colony. Colony morphology changes

during development. The mucilage of the colony is firm, wide and sometimes yellowish green to brownish. Cells are barrel shaped with a uniform shape and size along trichome bright blue-green (after growing in culture medium, Fig. 3 F1- F4) or olive green (field sample Fig.3 F2-F3).

Remarks: By the trichome characterization the morphotype was assigned to genus Nostoc and appeared at nine sites. Despite we have identified morphotypes belonging to genus Nostoc at only nine of the 25 studied ponds, this morphospecie has been documented in a wide range of Antarctic habitats (Broad, 2005; Taton et al., 2006a, Komárek & Elster, 2008, Fernández-Carazo et al., 2012; Corrêa, 2012).

Morphotype 25 (Fig.3 G1-G2)

Description: Filaments heteropolar, differentiated into basal and apical parts, simple, solitary or in small groups but not in common mucilage. Trichome unbranched, constricted at the cross wall with terminal heterocytes and widened basal part. Apical part composed from narrow, long, hyaline cells. Hair formation at the apical ends of filaments was observed. Sheaths always present. Cells slightly barrel-shaped.

Remarks: The characters observed in this strain suggest that the morphotype belong to genus Calothrix and we recorded this morphospecie at only one site. This morphospecie also has been previous identified from different Antarctic regions (Broad, 2005; Komárek & Elster, 2008; Martineau et al., 2013), however, with distinct designation of morphospecies.

CHROOCOCCALES

Morphotype 26 (Fig. 3 H)

Description: Groups of cells (only few-celled) surrounded by mucilagionous envelopes; colonial slime fine, diffluent, homogeneous and colourless. Cell widely oval, bright blue-green with homogeneous or granular content.

Remarks: The persistent characteristics observed in the strain have led us to assign the morphotype 26 to genus Chroococcus and this morphospecie was observed at two of the studied samples.

Morphotype 27 (Fig. 3 I)

Description: Group of cells forming colonies with numerous, sparsely arranged cells; colonial mucilage colourless, indistinct margin, formless, cells with individual gelatinous envelopes. Cells widely rounded.

Remarks: By the features observed, this morphotype could be assigned to genus Aphanocapsa and was identified at seven samples.

Morphotype 28 (Fig. 3 J)

Description: Group of cell forming colonies, mucilage colourless, indistinct margin, formless, cells with individual gelatinous envelopes. Cells rounded.

Remarks: The morphotype 28 was distinguished from morphotype 27 mainly by colony shape. Morphotype 28 possessed colony with densely arranged cells while morphotype 27 comprising sparse colony. We have suggested that this strain also belongs to genus Aphanocapsa and was recorded at five of the studied samples.

Morphotype 29 (Fig. 3 L)

Description: Group of agglomerated cells forming colonies, colonial mucilage colourless, indistinct margin, formless. Cells range to round to slightly elongated.

Remarks: The morphotype 29 also might belong to genus Aphanocapsa, and the major characteristic to distinguished morphotypes 29 from 27 and 28 was the cell size. Morphotype 29 was approximately 1.7 µm wide, morphotype 27 about 2.26 µm and morphotype 28 approximately 3µm (see Table 2). The morphotype 29 was found at two sites.

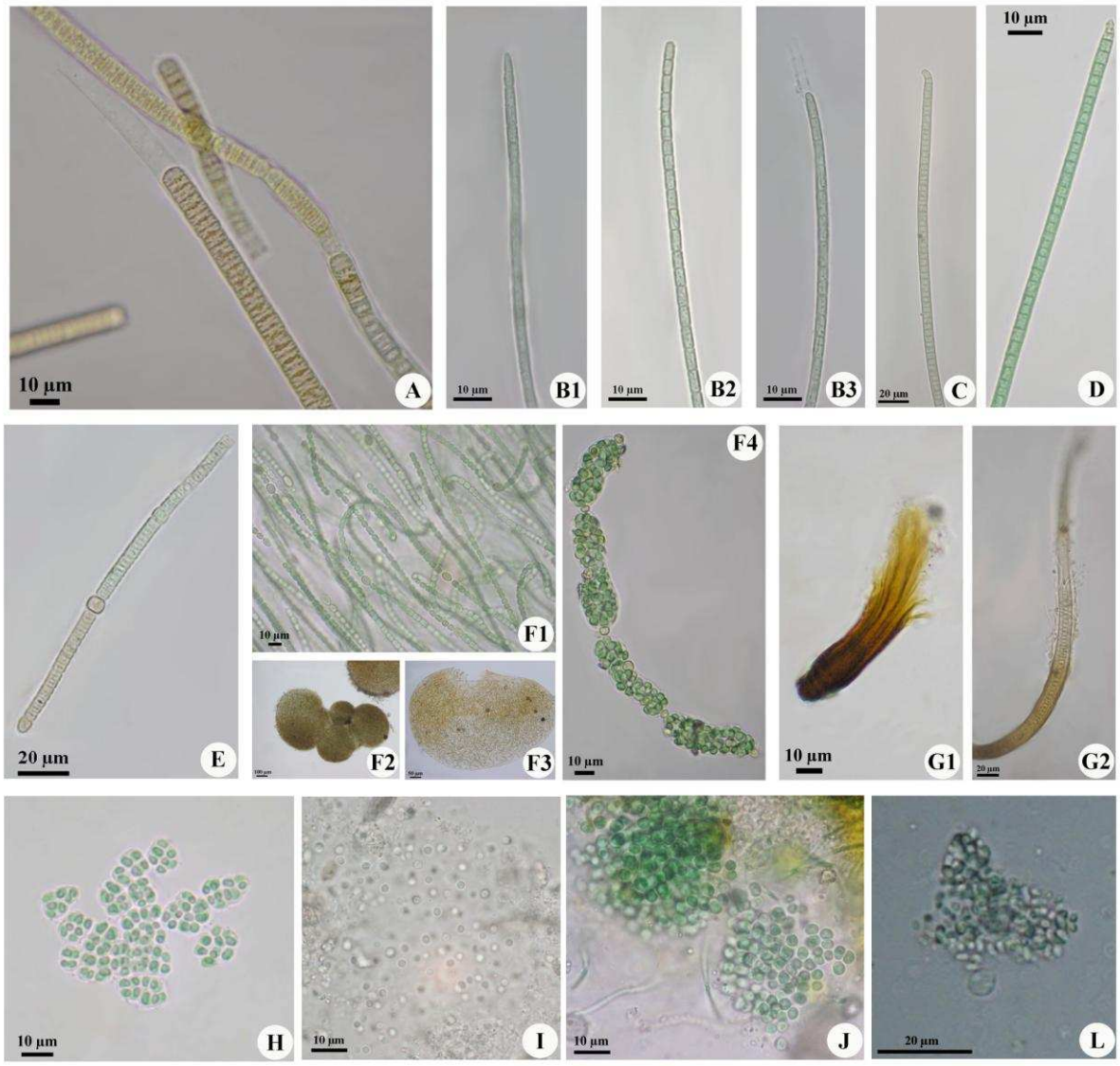

Figure 3. Morphospecies. **(A-D)** morphospecies belonging to the Oscillatoriales order: (**A**) cf. Lyngybya (mph.19); (**B1-B3**) Phormidium cf. murrayi (mph. 20); (**C**) Phormidium cf. autumnale (mph 21); (**D**) Phormidium cf. setchellianum (mph. 22). (**E-G**) morphospecies belonging to the Nostocales order: (**E**) Nodularia p. (mph. 23); (**F1-F4**) Nostoc sp. (mph. 24). (**G1-G2**) Calothrix sp. (mph. 25). (**H-L**) morphospecies belonging to the Chroococcales order: (**H**) Chroococus sp. (mph. 26); (**I**) cf. Aphanocapsa sp. (mph. 27); (**J**) cf. Aphanocapsa (mph. 28); (**L**) cf. Aphanocapsa (mph. 29). Mph=morphotype.

Table 2. Characteristic of the morphotypes from benthic microbial, along inland-coastal gradients in the McMurdo Sound region

Mph.	Trichome shape	Number of trichomes in sheath	Terminal attenuation of trichome	* Presence of calyptra	Shape of apical cell	Constrictions at transverse walls	Granules	Branching	Range in width of trichomes (µm)	Range of cell length (µm)	Sites of occurrence	Designation	Figure
1	Straight or slightly curved	Sheathless	-	-	Rounded up to barrel-shaped	+	+	-	2.23 – 3.33	2.33 – 4.0	L9; L15; L16; L26; LW2; LW12; LW13; LW14; LW15;LW16; LW18	Borzia sp.	1 A1-2
2	Slightly waved		-	-	Broadly rounded	+	+	-	1.50-1.85	1.13-1.85	OGE	cf. Konvophorum	1 B
3	Straight or slightly screw-like coiled		Slightly attenuated or bent	-	Conical up to hooked	-	+	-	1.04-1.53	3.47-5.23	HP3;BRK;NSTC; OGE; FH; LW12; LW14;LW16;LW18	Geitlerinema cf. ionicum	1 C
4	Straight or slightly curved		-	-	Slightly barrel-shaped	+	+	-	1.08-1.61	1.50-4.72	HP2;HP3;OGE;NSTC; FH; E4; E9; LW2; LW12;LW13;LW14; LW15;LW16;LW18	Pseudanabaena	1 D1-2
5			-	-	Cylindrical	Slightly constricted	+ (cell ends)	-	0.81-1.08	4.08-5.94	OHP; SALT; P70-E; L9;L26;LW2; LW13;LW16	cf.Pseudanabaena	1 E1-2
6		1	-	-	Cylindrical		+ (cell ends)	-	0.93-1.07	2.14-5.18	OHP;OGE;P70-E;E4; L9; L15; L16; L26; LW12; LW13; LW14; LW15; LW16; LW18	cf. Pseudanabaena	1 F1-3
7			Facultative	-	Cylindrical or conical-rounded		+	-	1.19-1.89 (0.76 apical cell)	1.42-5.04	HP1;HP2;HP3; OHP; BRK; NSTC; OGE; SALT; P70; P70-E; FH; SKUA; E4;E9;L9; L15;L16;L26;LW2;LW12;LW13;LW14; LW15; LW16; LW18	Leptolyngbya sp.	1 G1-4
8			-	-	Rounded or Hemispherical	-	-	-	1.22-1.53	0.76-2.40	HP1;HP2;HP3; OHP; BRK; NSTC; OGE; P70; P70-E; FH; SKUA;E4;E9;L9;L15;L16;L26;LW12;LW13;LW14; LW15; LW16	Leptolyngbya sp.	1 H1-2
9	Curved		-	-	Cylindrical	-	-	-	2.5	11 – 12.8	LW16	cf. Leptolgynbya	2 A
10	Straight or slightly curved		-	-	Rounded	Slightly constricted	-	False branching	1.34-.1.62	1.15-1.89	BRK; SALT; LW13; LW14; LW18	Plectolyngbya cf. hodgsonii	2 B1-3
11	Slightly curved		Not or slightly attenuation	-			-	False branching	1.89-2.66	1.11-1.70	L15, L16,	cf. Plectolyngbya	2 C1-4
12	Straight		-	-			+	-	1.50-1.85	1.50-2.0	HP2;HP3; BRK; NSTC; OGE; SALT; P70;P70-E; FH; E9;L15;LW2;LW12; LW14; LW15; LW16; LW18	cf. Leptolyngbya with resemblance to Phormidum prestleyei	2 D

13	Slightly irregularly wavy	More than one	-	-	Cylindrical or conical-rounded	Slightly constricted	+	-	1.51-1.60	4-5.3	E4; E9; LW12	cf. Schizothrix	2 E
14	Straight	Sheathless	Slightly attenuation slightly	+	Broadly rounded	-	Finely granulated	-	8.23-10.5	1.76-3.52	NSTC;FH;OGE	Oscillatoria sp.	2 F
15	Straight			+	rounded to slightly elongated	-	-	-	9.41	3.52-4.70	HP3	Oscillatoria sp.	2 G
16	Slightly curved			+	rounded	-	Finely granulated	-	10.6-12.0	3.0 -4.23	HP2;HP3	cf Oscillatoria	2 H
17	Straight			+		-		-	5.5-6.42	1.42-2.2	OGE;FH	Oscillatoria sp.	2 I
18	Slightly curved		-	-	Widely rounded	Slightly constricted	-	-	11-15	1.87-2.85	E9	Crinalium sp.	2 J
19	Straight or slightly curved	1	-	-	Rounded	-	+	-	7.8	3.2	HP2; P70; NSTC	cf. Lyngbya	3 A
20		1 (facultative sheath)	+ (slightly attenuation at the apical cell)	-	Cylindrical up to tapering	Slightly constricted	+	-	2.22-3.33	4.81-7.40	HP2; OHP;BRK; NSTC; OGE; FH; SKUA;P70-E; P70; E4; LW12; LW14; LW15; LW16	Phormidum murrayi also termed as Wilmottia murrayi)	3 B1-3
21	Straight			+ (Thickened outer cell wall)	Elongated, rounded or hooked	-	+	-	5.0-7.0	2.5-4.73	HP1;HP2; HP3; OHP;BRK; NSTC; OGE; FH; SKUA; SALT;P70-E; P70; L16; LW12;LW13; LW14; LW16	Phormidum cf. autumanale	3 C
22				+ (thickened outer cell wall)	Elongated to conical-rounded	-	+	-	4.54-5.0	3.8-6.66	HP1; OHP;FH;SKUA; NSTC;P70;	Phormidium cf. setchellianaum	3 D
23	Straight or slightly curved	1	-	-	Barrel (shorter than wide)	+	Finely granulated	-	4.94-5.88 (7.0 heterocysts)	2.7	HP2;FH;OGE;BRK; NSTC;SKUA	Nodualaria sp.	3 E
24	Irregularly coiled	Densely agglomerate forming colony	-	-	Broadly rounded	+	-	-	4.34	3.26-4.3	HP2;HP3; OHP;FH; OGE;NSTC; P70-E;P70;L15	Nostoc sp.	3 F1-4
25	Straight or slightly curved	1	+	-	Narrow and elongated	+	-	-	4.70 -5.88	4.11	L15	Calotrix sp.	3 G1-2
26	-	Colony (few cells)	-	-	all cells are broadly rounded	-	-	-	2.42-2.85 (diameter)		L15; LW16	Chroococcus sp.	3 H
27		Colony (densely packed cell)	-	-		-	-	-	2.0 – 2.5 (diameter)		L9;L26;LW12; LW13;LW14;LW15; LW16	cf. Aphanocapsa	3 I
28			-	-		-	-	-	2.4 -3.8 (diameter)		HP2; SALT; L9;L15;LW18	cf. Aphanocapsa	3 J
29			-	-	Elongated	-	-	-	1.6-1.8 (diameter)		HP3; E4	cf. Aphanocapsa	3 L

*Presence of calyptra = thickened membrane on mature apical cell

Distribution of morphospecies

In general, Oscillatoriales cyanobacteria, mainly morphotypes assigned to genus Leptolynbya (morphotypes 7 and 8) were widely spread in Upper and Lower Wright Valleys, Ross Island and MIS and showed the highest frequency of occurrence in the four studied areas (Fig. 4). Conversely, morphospecies assigned to order Nostocales were mostly recorded on Ross Island and MIS while only one of the studied sites from Upper Wright Valley and none from the Lower Wright Valley contained a strain belonging to this order (Fig. 4). Conversely, representatives of the order Chroococcales were most frequent in Upper and Lower Wright Valleys and were recorded at only two ponds from Ross Island and one site of the MIS area (Fig. 4).

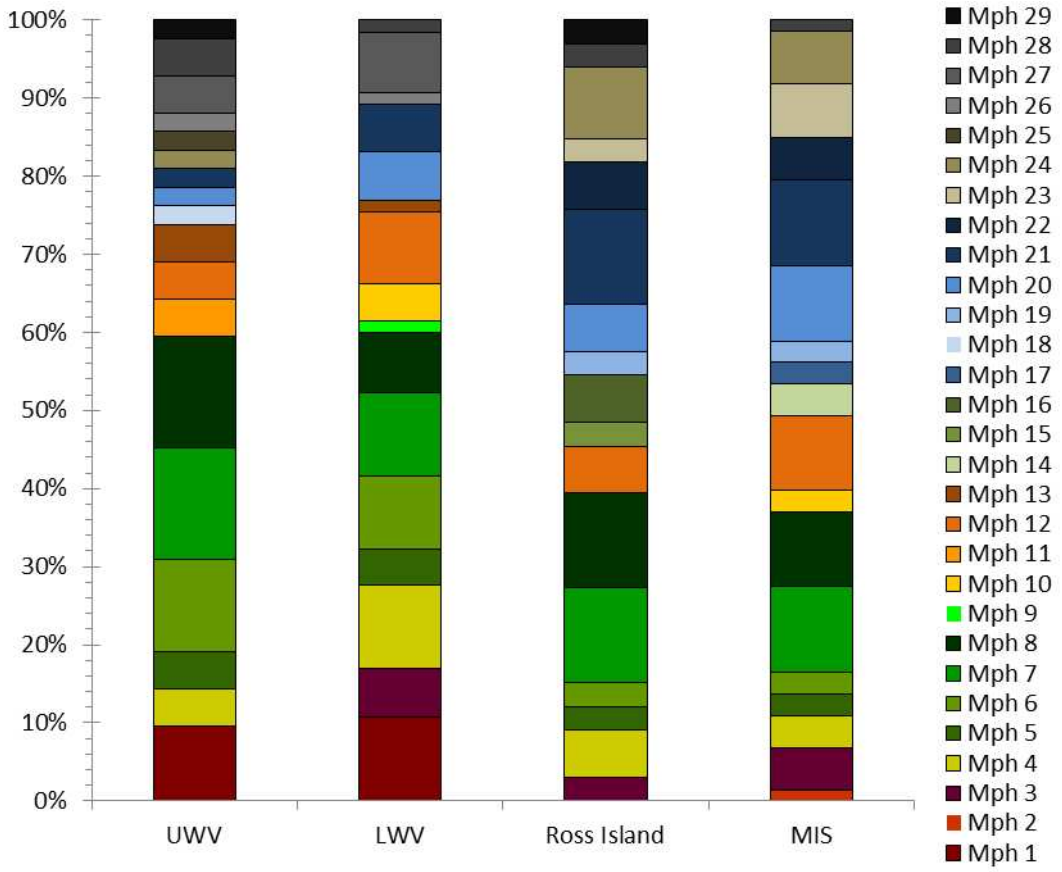

Figure 4. Community structure of the studied areas showing the frequency of occurrence (%) of each morphospecie UWV = Upper Wright Valley; LWV= Low Wright Valley; Ross Island and MIS= McMurdo Ice Self. Mph = morphotype.

Molecular researches have recognised that the genotype analyses from different biotopes yield wider diversity than is recognisable from phenotype identification (Taton et al., 2003; 2006a). However, according to Komárek (2007) the traditional definition and naming of species in agreement to the botanical nomenclatoric rules is still necessary and the only acceptable method for characterisation of cyanobacterial taxonomic units (generic and subgeneric; ecologically as well as morphologically).

We considerate that for the future work a complementary molecular research on these studied areas is important for connect the genotype with phenotypes characters and give a reliable designation of names (genus and species) for the morphotypes recorded through this work. Moreover, we do not have isolated the strains and all the observation and description were made from the field samples which sometimes contained numerous sediments and strong aggregation of cyanobacteria filament. These facts may have contributed to an overlook of the sample omitting the presence of the others morphospecies.

On the basis of the ease with which cyanobacteria tolerate desiccation, and can be expected to be well dispersed within the area by strong winds, there is an expectation that there is an equal opportunity for all taxa to colonize all water bodies and that the sites can be considered to form a metacommunity (Wilson 1992). This relates to the "everything is everywhere" hypothesis, which is challenged when connectivity between habitats within a metacommunity is low (Verleyen et al., 2009). The finding that the four sites contain different taxa rasies a number of questions. The strong similarities found in the cyanobacteria distribution amongst the four studied areas suggest that they are part of a metacommunity, but are the differences due to species sorting along the variations of the environmental, physical-chemical properties of the water body, of the climatic characteristics of each site, or is the dispersal of propagules less effective within the McMurdo Dry Valleys than expected? Is everything equally everywhere? It has been argued that the forces that define the cyanobacterial communites include both deterministic and stochastic processes (Sloan et al., 2006). The factors that are involved in determining the cyanobacterial community has been a concern for microbial ecology and it is a challenge for future work. The next chapter of this thesis will explore this question.

4. Concluding remarks

Our results showed, using our morphological approach, that the four studied areas have similar taxomonmic richness (15-17 morphospecies) share many morphospecies, but

that each site contains distinct floristic elements that are rare or absent from others. The expected high degree of airborne connectivity between the sites, the tolerance of cyanobacteria to desiccation, and the similarities of the community suggests that the four sites may approach a metacommunity. Distinct site-specific differences between sites, which are consistent across ponds within each site, suggest that either growth conditions within sites are selecting different taxa or dispersal of some taxa is constrained by dispersal.

5. References

Andersen, D. T.; Sumner, D. Y., Hawes, I.; Webster-Brown, J. & Mckay, C. P. 2011. Discovery of large conical stromatolites in Lake Untersee, Antarctica. Geobiology 9: 280-293.

Bolch, C. J. S. & Blackburn, S. I. 1996. Isolation and purification of Australian isolates of the toxic cyanobacterium Microcystis aeruginosa. Journal of Applied Phycology 8: 5-13.

Broady, P. A. & Kibblewhite, A. L. 1991. Morphological characterization of Oscillatoriales (Cyanobacteria) from Ross Island and southern Victoria Land, Antarctica. Antarctic Science 3: 35-45.

Broady, P. A. 2005. The distribution of terrestrial and hydro-terrestrial algal associations at three contrasting locations in southern Victoria Land, Antarctica. Algological Studies 11: 95-112.

Comte. K., Sabacka, M., Carré-Mlouka A, Elster J, Komárek J. 2007. Relationships between the Arctic and the Antarctic cyanobacteria; three Phormidium-like strains evaluated by a polyphasic approach. FEMS Microbiology, Ecology 59: 366-376.

Fernández-Carazo, R.; Namsaraev, Z.; Mano, Marie-Jose; Ertz, D. & Wilmotte, A. 2012. Cyanobacterial diversity for an anthropogenic impact assessment in the Sør Rondane Mountains area, Antarctic. Antarctic Science 24: 229-242.

Frey, W. (ed). 2012. Syllabus of Plant Families - A. Engler's Syllabus der Pflanzenfamilien. 13th ed. Part 1/1: Blue-green Algae, Myxomycetes and Myxomycete-like organisms, Phytoparasictic protists, Heterotrophic Heterokontobionta and Fungi. 178p.

Friedmann, E. 1. 1982. Endolithic microorganisms in the Antarctic cold desert. Science 215: 1045-1053.

Garcia-Pichel, F. 2009. Cyanobacteria. – In: Schaechter, M. (ed.): Encyclopedia of Microbiology. Pp. 107-124. – Academic Press, Oxford.

Hawes, I.; Howard-Williams, C. & Vincent, W.F. 1992. Desiccation and recovery of cyanobacterial mats. Polar Biology 12: 587-94.

Hawes, I.; Sumner, D. Y.; Andersen, D. T. & Mackey, T. J. 2011. Legacies of recent environmental change in the benthic communities of Lake Joyce, a perennially ice-covered Antarctic lake. Geobiology 9: 394-410.

Hodgson D.A.; Vyverman W. & Sabbe K. 2001. Limnology and biology of saline lakes in the Rauer Islands, Eastern Antarctica. Antarctic Science 13: 255-270.

Jungblut A.-D.; Hawes I.; Mountfort, D.; Hitzfeld, B.; Dietrich, D.R.; Burns, B.P. & Neilan, B.A. 2005. Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environmental Microbiology 7: 519-529.

Jungblut, A.-D.; Lovejoy, C. & Vincent, W. F. 2009. Global distribution of cyanobacterial ecotypes in the cold biosphere. The International Society for Microbial Ecology Journal 4: 191-202. Friedmann, E.I. 1982. Endolithic microorganisms in the Antarctic cold desert. Science 215: 1045-1053.

Komárek, J. & Anagnostidis, K. 1989. Modern approach to the classification system of cyanophytes. 4 - Nostocales. Archiv für Hydrobiologie Supplement 56: 247-345.

Komárek J. 1999. Diversity of cyanoprokaryotes (cyanobacteria) of King George Island, maritime Antarctica-a survey. Archiv fuer Hydrobiologie 94: 181-193.

Komárek, J. & Anagnostidis, K. 2000. Cyanoprokaryota. 1. Teil, Chroococcales. In Ettl, H., Gartner, G., Heynig, H. & Molllenhauer, D. (eds), Süsswasserflora von Mitteleuropa vol 19. Gustav Fisher, Jena: 548.

Komárek, J. & Anagnostidis, K. 2005. Cyanoprokaryota. 2. Teil, Oscillatoriales. In Budel, B., Krienitz, L., Gartner, G. & Schagerl, M. (eds), Süßwasserflora von mitteleuropa, Band 19/2 Spektrum Akademischer Verlag, Elsevier GmbH. München, German.

Komárek, J. 2007. Phenotype diversity of the cyanobacterial genus Leptolyngbya in the maritime Antarctic. Polish Polar Research 28: 211-231.

Komárek, J. & Elster, J. 2008. Ecological background of cyanobacterial assemblages of the northern part of James Ross Island, Antarctica. Polish Polar Research 29: 17-32.

Martineau, E.; Wood, S.A.; Miller, M.R.; Jungblut, A. D.; Hawes, I.; Webster-Brown, J. & Packer, M.A. 2013. Characterisation of Antartic cyanobacteria and comparison with New Zealand strains. Hydrobiologia 711: 139-154.

Mataloni, G.; Vinocur, A. & Pinto, P. D. T. 2005. Abiotic characterization and epilithic communities of a naturally enriched stream at Cierva Point, Antarctic Peninsula. Antarctic Science 17: 163-170.

Nadeau, T. L., Milbrandt, E. C. & Castenholz, R. W. 2001. Evolutionary relationships of cultivated Antarctic Oscillatoriaceans (cyanobacteria). Journal of Phycology 37:650-654.

Pearl, H. W. & J. Huisma. 2008. Blooms like it hot. Science 320: 57-58.

Schopf, J.W. 2000. The fossil record: tracing the roots of the cyanobacterial lineage. – In: Whitton, B.A. & Potts, M. (eds.): The Ecology of Cyanobacteria. Their Diversity in Time and Space. Pp. 13-35. - Kluwer Acad. Publ., Dordrecht.

Sloan, W. T.; Lun, M.; Wooddcock, S.; Head, I.M.; Nee, S. & Curtis, T. P. 2006. Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environmental Microbiology 8: 732-740.

Strunecký, O.; Elster, J. & Komárek, J. 2011. Taxonomic revision of the freshwater cyanobacterium „Phormidium“ murrayi = Wilmottia murrayi. Fottea 11: 57-71.

Taton, A.; Grubisic, S.; Brambilla, E.; De Wit, R. & Wilmotte, A. 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys,

Antarctica): a morphological and molecular approach. Applied and Environmental Microbiology 69: 5157-5169.

Taton, A.; Grubisic, S.; Balthazart, P.; Hodgson, D.A.; Laybourn- Parry, J. & Wilmotte, A. 2006a. Biogeographical distribution and ecological range of benthic cyanobacteria in East Antarctic lakes. FEMS Microbiology, Ecology 57: 272-289.

Taton, A.; Grubisic, S.; Ertz, D.; Hodgson, D. A.; Piccardi, R.; Biondi, N.; Tredici, M.R.; Mainini, M.; Losi, D.; Marinelli, F. & Wilmotte, A.. 2006b. Polyphasic study of Antarctic cyanobacterial strains. Journal of Phycology 42: 1257-1270.

Taton, A.; Wilmotte, A.; Šmarda, J.; Elster, J.; Komárek, J. 2011. Plectolyngbya hodgsonii: a novel filamentous cyanobacterium from Antarctic lakes. Polar Biology 34: 181-191.

Vincent, W. F. 2000. Cyanobacterial dominance in the polar regions, p. 321-340. In: Whitton, B. & Potts, M. (eds.). Ecology of the Cyanobacteria: their diversity in space and time. Kluwer Academic Press, Dordrecht, The Netherlands.

Vincent W.F. 2007. Cold tolerance in cyanobacteria and life in the cryosphere, p 287-301. In: Seckbach J (ed), Algae and cyanobacteria in extreme environments. Springer, Heidelberg, Germany.

Zakhia, F.; Jungblut, A.-D.; Taton, A.; Vincent, W. F. & Wilmotte, A. 2009. Cyanobacteria in cold environments. In: Margesin, R., F. Schinner, J. C. Marx & C. Gerday (eds), Psychrophiles: from biodiversity to biotechnology. Springer, New York: 121-135.

Whitton, B. A. & Potts, M. (eds). 2000. The ecology of cyanobacteria [electronic resource]: their diversity in time and space. Kluwer Academic, Boston.

Wilson, D. S. 1992. Complex interactions in metacommunities, with implications for biodiversity and higher levels of selection. Ecology 73: 1984-2000.

Wood, S. A.; Heath, M.; Kuhajek J. & Ryan, K. 2010. Fine scale spatial variability of anatoxin-a and homoanatoxin-a production in benthic cyanobacteria; implication for monitoring and management. Journal of Applied Microbial Ecology 109: 2011-2018.

CHAPTER 2

The role of physical-chemical properties of Antarctic ponds in determining the composition of cyanobacterial assemblages

1. Introduction

A great diversity of meltwater ponds are widespread throughout Antarctica and in many areas, like the McMurdo Dry Valleys, they are important components of the aquatic environment (Howard-Williams & Hawes, 2007; Quesada et al., 2008). The Antarctic climate has a fundamental control on the dynamic of these ponds. Many are hydrologically isolated, and influx of water and nutrients from outside is dependent on local melting of snow and ice (Hawes et al., 1993). In addition, climate determines whether ponds are fully or partially covered by ice year-round or have complete open water in summer, with seasonal freeze and thaw cycles (Vincent, 1987; Hawes et al., 1993; Howard-Williams & Hawes, 2007). This single feature has a wide range of implications for the physical, chemical and biological properties of ponds, and determines that for the most part dispersal of organisms to and from is dependent on Aeolian processes (Vincent, 1987).

When melted, these ponds provide a habitat for microbial growth (Hawes et al., 1999a). Developing microbial communities are mostly dominated by cyanobacteria but also include several microscopic species, as phytoflagellates, chlorophytes and diatoms (Vincent, 2000). The dominant cyanobacterial communities in these ponds typically takes the form of a variety of microbial mats covering the substrate (Vincent, 2000). Cyanobacterial mat communities dominate the total biomass and biological productivity (Vincent, 2000; Jungblut et al., 2005) and have a fundamental role in the functioning of these systems (Mountfort et al., 2003, Howard-Williams & Hawes, 2007). They have been assumed to control most of the biological flux of carbon, nutrients cycling and energy in dynamic aquatic systems (Wynn-Williams, 1996; Vincent, 2000).

Microbial mats in Antarctic ponds must cope with a variety of stresses. Desiccation provides a range of drying times for the pond mats from weeks, to years (Hawes et al., 1993). Also, these microorganisms must withstand frequent freeze-thaw cycles, and tolerate high variability in a wide range of chemical conditions (Howard-Williams & Hawes, 2007; Hawes et al., 2013). Such stressors impose an overarching need for tolerance of extreme conditions. However, within this extremophilic frame, the question of what determines mat composition within any given pond is as yet uncertain. Wynn-Williams (1996) argues that composition generally reflects the environmental chemical and physical conditions of the habitat, and that microbial communities can be used as key organisms to monitor ecosystems of Antarctica and to assess the climate change effects or other perturbations on Antarctic continent. Other studies argue that the impact of environmental conditions on mat composition is less direct

(Sutherland, 2009; Hawes et al., 2011). The relative importance of small differences in performance under ambient conditions in determining the composition of microbial communities is a contentious issue (Sloan et al., 2006), and it may be questioned whether conditions in Antarctic ponds are ever stable enough for long enough for competitive interactions to influence species compositions at low ambient growth rates.

The composition of cyanobacteria community has, however, been studied in several Antarctic localities, from the continental areas (Broady & Kibblewhite, 1991; Taton et al., 2003; Jungblut et al., 2005; Taton et al., 2006; Fernández-Carazo et al., 2012) to the Antarctic Peninsula and/or Maritime (Izaguirre et al., 1993; Komárek, 1999; Mataloni et al., 2000, Llames &Vinocur, 2007; Komáreck et al., 2008) including a large diversity of habitats. The majority of these studies focused on the diversity and endemism of the Antarctic microflora, either by morphological and/or molecular tools, but some of them reported the mat composition related to the environmental properties. Komárek (1999) documented that the composition of cyanobacteria species is related to its specific microbiotopes (e.g. creeks, seepages, coastal swamps, wet rocks, etc.) and is dependent on different ecological properties of individual species and their life strategies. In addition, Taton et al. (2006) reported, on the basis of morphological approach, a confined occurrence of cyanobacteria into specific liminological proprieties in lakes from different regions of Eastern Antarctica. Also, 47% of the morphospecies found in that study were restricted to only one sample, suggesting that the cyanobacterial communities of these lakes are not equally distributed, yet, the ubiquity of several cyanobacteria morphospecies has been also suggested from these studies to imply a well distributed flora.

The concept of metapopluation (Wilson 1992) may be appropriate to Antarctic aquatic communities, particularly as they occur as isolated, often ephemeral units within landscapes with little hydrological connectivity. Within metapopulations, it is argued that dispersal efficiency across distance may vary, and thus local community characteristics may develop over time through stochastic rather than deterministic processes (Verlmulen et al., 2009). Which factors actually at drive the cyanobacteria distribution in Antarctica – is it dispersal, niche separation, or a mixture of the two. These two main mechanisms have been advanced to explain the variation of the community structure and composition; differentiation of niches along ecological gradients that select for specific species and differentiation amongst species with broadly overlapping niches depending on limiting dispersal over geographical distance (Leibold et al., 2004). At the heart of this debate is the extent to which communities are

structured by the immediate environment, or whether they are determined by the probability of colonization – or a combination thereof.

Studies which have adopted the neutral theory to explain the community composition highlight the importance of the distance between the sites for community structure, emphasizing the role of dispersal and the colonization chance of the taxa (Hubbell, 2001; Sloan, 2006; Allison and Martiny, 2008). On the other hand, the deterministic line based on the niche theory considers the environmental heterogeneity the principal cause of the gradual replacement of species on a regional scale, and that communities would be altered by environmental characteristics and biotic interactions (Kraft et al., 2007; Jones & Hallin, 2010). However, the relative importance of each one of these process for the structuring of communities, without excluding the importance of the other has been examined as well (Leibold et al., 2004).

Understanding the process and characteristics that favor or limit cyanobacterial taxa to succeed in Antarctica environments is crucial for predicting future changes, as well as, understanding how certain areas are important to the Antarctica ecosystem. The conservation management planning of Antarctic systems requires information that can be used to reduce likely threats to biodiversity (Terauds et al., 2012). In this context, research on the factors controlling species and diversity distribution are important to identify areas that are representative of the Antarctic biodiversity and predict futures threats (Margules & Pressey, 2000)

Thus, to better manage Antarctic biodiversity conservation and design positive measures for preserving local and/or regional biodiversity, it is important to understand the whole environmental complexity and how it acts in structuring and maintenance of biodiversity (Leibold et al., 2004). To enhance our understanding of cyanobacteria community composition and consequently, optimize the values of these Antarctic ecosystems, this work aimed to:

(i) Describe the composition and distribution of cyanobacteria morphotypes from sub-regions of the McMurdo Sound and their ecological preferences;

(ii) Determine whether cyanobacteria composition is related to environmental conditions (physical and chemical variability of ponds), geographic position or a mixture of the both.

2. Materials and Methods

2.1 Sample sites

Twenty five ponds were sampled in locations along inland-coastal gradients in the McMurdo Sound region during the 2012-2013 summer season. The working area comprised ponds from Ross Island, McMurdo Ice Shelf (MIS), Lower Wright Valley (LWV) and Upper Wright Valley (UWV). The location and descriptions of each pond are described in Table 1 and the estimating distances between the four study areas are shown in Table 2.

2.2 Pond sampling

GPS location, water temperature, conductivity, dissolved oxygen (DO), depth, length and breadth of pond were determined in the field. Water and benthic mat samples were taken from each pond for physical, chemical and biological analyses. Water samples were taken to determine the content of dissolved inorganic carbon (DIC); dissolved reactive phosphorus (DRP); dissolved organic phosphorus (DOP); content of nitrogen (NO_3-N and NH_4^+) and planktonic chlorophyll (Chl a). Benthic mat samples were collected to characterize the content of Chl-a, phycoerythrin (Pe) and phycocyanin (Pc) pigments; extracellular polymeric substances (EPS), organic matter and for description of the composition of cyanobacteria community.

2.2.1 Measurements of temperature, pH, electrical conductivity, DO and depth

The determination of water temperature, pH, electrical conductivity and dissolved oxygen were read in field using a calibrated portable meter (Hach HQ 40d). The pond depth was determined by using a meter stick where possible, or estimated from pond edge slope and size where the centre could not be reached.

2.2.2 Determination of dissolved inorganic carbon

Sub-samples (1 ml) of undisturbed water were taken for DIC analysis using a surgical syringe. The sample was dispensed into gas tight tubes containing pre-dispensed aliquots of phosphoric acid (0.2 ml) and returned to New Zealand for later determination by infra-red gas analysis (Hawes et al., 2011).

2.2.3 Determination of phosphorus and nitrogen content

One liter of water was collected into an acid-washed polyethylene bottle and returned to the laboratory for processing. Water was vacuum filtered through a GF/F filter and the

filtrate frozen and returned to New Zealand for later analysis. The, NO_3-N, NH_4^+, DON and DRP and DOP were determined using an Astoria autoanalyser, as described in Hawes et al. (2011).

2.2.4 Determination of planktonic chlorophyll-a

For determination of planktonic Chl a samples (250 ml) were collected onto Whatman GF/F filters and stored frozen and returned to New Zealand for later analysis. Filters were ground in cold 95% acetone, extracted at 4°C for four hours then the supernatant separated by centrifugation. Chlorophyll a was estimated using a Perkin Elmer spectrofluorometer, using excitation at 430 nm and emission at 675 nm, and calibrated with standard solutions of chlorophyll a (Hawes et al., 2011).

2.2.5 Determination of benthic pigments, EPS and organic matter

Quintuplicate mat cores (area 1.54 cm^2) were taken using a cut-off 10 ml syringe, from each pond studied. Each replicate was equally spaced around the pond margin, and care was taken to collect the mat only and no underlying sediment. The resulting mat core was carefully lifted from the sediment and transferred to a plastic container, and frozen for return to New Zealand. On return to New Zealand, these samples were freeze-dried and ground to a fine powder and weighed. Weighed subsamples were taken for analysis of chlorophyll-a, phycobilins, EPS and organic matter characterization.

Chlorophyll-a pigment

For the chlorophyll a determination, weighed subsamples were transfered in eppendorf to which was added 1 ml of 90% acetone. Samples were left overnight in in the fridge, and then centrifuged at 1250 g for 15 min. 0.5 ml of supernatant was diluted x5 with fresh 90% acetone, and the fluorescence of chlorophyll-a in the supernatant was read at 663 nm in a Turner Desings Aquafluor fluorometer, without acidification. Chlorophyll-a concentration was calculated based on calibrations using standard solutions of Chl a and the results were converted to µg cm^{-2} using the total sample and aliquot weights.

Phycobilin pigments

For the Pc and Pe determination, subsamples stored in erpendoff were added of 1.5ml of Tris buffer (0.1M and pH 8.0) and cleared by centrifugation. The fluorescence of the supernatant was measured on a Perkin-Elmer fluorometer using excitation/emission wavelength combinations of 557/643 nm for Pe and 621/ 651 nm for Pc, and standard

solutions for calibration. Results were converted to µg cm^{-2} using the total sample and aliquot weights.

Total EPS

For the total EPS determination, subsamples stored in eppendorf were extracted for 15 min at 20 °C in 1.5 ml of Na2EDTA (100mM). The extract was then centrifuged at 3,620 g, the supernatant was discarded and the resultant pellet was resuspended in ultrapure water following the method by Yallop et al. (2000). The carbohydrate content of the resuspension was estimated as glucose equivalents using the phenol–sulphuric acid method described by Dubois et al (1956). The absorbance was measured against a reagent blank at 485 nm and calibrated against glucose standard. Results were converted to glucose equivalents mg/cm^2 using the total sample and aliquot weights.

Organic matter

For determination of organic matter content, dried subsamples were carefully transferred to weighed crucibles, re-weighed and then combusted at 450 °C for 4 h in a muffle furnace. After it cooling down to room temperature in a desiccator, the ash was weighed on an electrobalance. Weight loss was taken to estimate the organic matter as loss of mass on ignition. Results were converted to mg/cm^2 using the total sample and aliquot weights (Sutherland & Hawes, 2008).

2.2.6 Benthic cyanobacterial mat composition

Initially, an aliquot from each mat sample was thawed and was directly observed under a microscope (BX51, Olympus) at up to 1000x magnification. For later observation, remaining subsamples were transferred to 50-ml plastic bottles containing the mineral nutrient medium MLA (Bolch & Blackburn, 1996) and kept at the natural conditions of laboratory (± 21°C) and moderate irradiance on the laboratory windowsill but out of direct sunlight. We did not isolate cyanobacterial morphospecies.

Cyanobacterial morphotypes were identified using a ten morphological criteria to describe the morphotypes (trichome shape, number of trichomes in sheath, presence or absence of terminal attenuation of trichome, calyptra on mature apical cell, shape of apical cell, presence or absence of constrictions at transverse walls, granules, branching and range in width of trichomes and range of cell length). Tentative identifications were made with reference to Komárek & Anagnostidis (1989); Komárek & Anagnostidis (2000); Komárek &

Anagnostidis (2005) and other literature sources (Broady & Kibblewhite, 1991; Taton et al., 2011; Strunecký et al., 2011).

Table 1. Locations of study sites and mat description. Ponds were grouped into four areas based on the geographic distance.

Area	Informal names of ponds	Location		Mat description			
		Lat.	Long.	Colour	Bilayer	cohesive	thickness
Upper Wright Valley	L26	77 33 03.2	160 43 24.0	1	1	2	1
	L09	77 33 02.4	160 44 26.2	2	2	3	2
	L15	77 33 13.1	160 43 01.2	3	1	3	2
	L16	77 32 29.0	160 45 20.0	1	1	1	2
	E9	77 31 35.1	160 46 16.7	2	1	1	1
	E4	77 31 21.3	160 44 17.9	2	1	1	1
Lower Wright Valley	LW2	77 26 43.9	162 41 30.3	1	1	3	2
	LW12	77 27 00.1	162 39 00.5	1	1	3	2
	LW13	77 27 02.0	162 38 52.5	1	2	3	2
	LW14	77 27 02.8	162 38 44.7	1	2	3	2
	LW15	77 27 03.1	162 38 35.3	1	2	3	2
	LW16	77 27 03.0	162 38 28.6	1	2	3	2
	LW18	77 27 02.7	162 38 19.3	1	1	2	2
Ross Island	OHP	77 51 20.9	166 41 25.7	1	1	2	2
	HP1	77 50 36.6	166 38 41.6	1	1	2	1
	HP2	77 50 41.8	166 38 36.8	1	2	3	2
	HP2	77 50 41.4	166 38 35.5	1	2	3	2
McMurdo IceShelf	FH	78 00.962	165 33.070	2	2	2	1
	NSCT	78 00.832	165 33.288	2	1	2	1
	P70	78 00.892	165 33.136	1	2	3	2
	Skua	78 00.798	165 33.113	2	2	3	2
	OGE	78 00.838	165 33.339	1	2	3	2
	P70E	78 00.949	165 33.082	2	1	2	1
	BRK	78 00.947	165 32.723	1	2	3	2
	Salt	78 00.963	165 32.734	4	1	2	2

Colour: 1 = orange; 2=brown; 3=black; 4=green
Bilayer: 1= no; 2= yes
Cohesive: 1=flake; 2=sheet; 3= mat
Thicknees: 1= thin; 2=thick

Table 2. Estimating distances (km) between the four working area. Distance measurements provided by Google Earth software.

	Ross Island	MIS	UWV	LWV
Ross Island	-	27	130	98
MIS	-	-	102	88
UWV	-	-	-	30

2.3 Statistical analysis of data

In order to understand the factors that might be involved in the distribution of the cyanobacteria community in Antarctic systems, the relationship between community composition and environmental factors was evaluated. Firstly, we tried to identify whether there are any physical and chemical environmental differences among the studied areas (all in the McMurdo Sound region). For testing statistically significant differences between the environmental parameters between the four areas we used the non-parametric Kruskal Wallis test ($p=0.05$), and to explore the major patterns of variation in the environmental dataset, we used Principal Component Analysis (PCA).

To evaluate whether the occurrence of cyanobacteria morphospecies is confined to a limited range of physical and chemical environmental properties we used the Canonical Correspondence Analysis (CCA). CCA incorporate the environmental data into the ordination axes, forming a linear combination of environmental variables that maximally separates the niches of the species (ter Braak & Verdonschot, 1995).

To perform the CCA, data were organized into two matrices: one morphospecies and other environmental variables. The first was formed by the presence and absence of morphorspecies at each pond. The ordination was tested for significance with a Monte Carlo test (998 runs) through PC-ORD software. Prior to multivariate analyses, all environmental factors were transformed by applying (x – mean/Standard deviation) to reduce or remove dataskewness.

Ponds HP1, HP3 and E9 were taken out of the CCA and PCA ordination by missing data of the variables introduced in these analyses. The morphotypes 16 and 18 were also left out of the multivariate analyses by the restrict occurrence on the ponds HP1 and E9.

To determinate the grouping of the sampling sites, based on morphospecies occurrences, we used the TWINSPAN program. The dendrogram from TWINSPAN is constructed by the classification of sites according to preferences of morphospecies

occurrences (based on the presence and/or absence of the morphospecies). The final result of this analysis consists in hierarchy divisions of the sampling units, with respective eigenvalues and indicators morphospecies for each grouping. Indicators species are those that tend to occur more on one side than the other division (Hill & Šmilauer, 2005).

3. Results

Twenty five ponds from four sub-regions in the McMurdo Sound region of Antarctica (Upper Wright Valley, Lower Wright Valley, McMurdo Ice Self and Ross Island) were studied by using microbial mats and water samples. The locations of each site and mat characteristics are shown in Table 1.

Physical and Chemical parameters

The ponds studied along four working areas are generally shallow, with most ponds <100 cm deep. However, when comparing the four areas, they differentiated significantly in relation to depth ($p= 0,03$), with deeper ponds were found in MIS area while shallower ponds were reported in UWV. The areas of the ponds in the study region are also not homogeneous. Larger areas were reported for UWV ponds, followed of LWV, and smaller sizes recorded for the MIS area.

In terms of pH, all ponds are alkaline, and they varied significantly between the four areas ($p= 0,003$). The higher pH values were in ponds from Ross Island and MIS, while lower values were found in UWV and LWV (Table 3). The same tendency was observed for dissolved oxygen. Higher concentrations of DO were detected in ponds from the coastal region while lowest values were found for those from inland areas.

Eletrical conductivity varied considerably between ponds from very dilute to saline, but the broad range of values encountered meant that there was no significant difference when comparing the four areas. Ponds from Ross Island tended towards the lowest values of conductivity while the greatest values were observed in ponds from Upper and Lower Wright Valleys (Table 3).

The ponds were differentiate relative to dissolved inorganic carbon (DIC) ($p= 0,05$) as well. The higher values of DIC were found in ponds spread in the UWV area but these were not strongly differentiated from those of the MIS and Ross Island area, while lower values were recorded at the LWV (Table 3).

Regarding Nitrogen and Phosphorus, the studied areas were consistently separated into two distinguished areas; one poor in nitrogen and rich in phosphorus while the other one poor in level of phosphorus and rich in nitrogen. Ponds from UWV and LWV displayed the greater values of both nitrate and ammonium, mainly nitrate level. Conversely, ponds distributed along coastal areas (MIS and Ross Island) showed very low levels of both nitrogen forms (NO_3-N and $NH4^{+)}$. On the other hand, higher phosphorus amounts (DOP and DRP) were recorded in ponds from MIS and Ross Island, with lower levels for those inland (Upper and Lower Wright Valley) (Table 3).

Dissolved organic nitrogen (DON) has not been detected in ponds from UWV (below limit of detection), but high values were observed in the MIS area.

Table 3. Records from field sampling: area, depth, conductivity, pH and dissolved oxygen (DO) and water chemistry.

Ponds	Group	Area (m^2)	Depth (cm)	Conductivity (mS/cm)	pH (units)	DO	DIC (mg/L)	NO_3 (µg/L)	NH_4 (µg/L)	DON (µg/L)	DRP (µg/L)	DOP (µg/L)
L9	1	551.54	55	42.6	8.32	11.85	7.00	2,000,000.00	410.00	BLD	0.1	1.00
L15	1	95.03	80	1.92	ND	ND	5.10	97,400.00	9.00	BLD	2	1.00
L16	1	2827.43	1	5.71	8.74	11.73	2.30	227,000.00	181.00	BLD	0.1	3.00
L26	1	283.52	45	40.23	8.48	11.48	11.20	1,800,000.00	5.00	BLD	0.1	2.00
E9	1	3318.30	1	0.13	8.745	11.73	0.04	3,690.00	52.00	BLD	0.1	1.00
E4	1	2827.43	1	2.05	7.93	12.40	0.45	40,900.00	39.00	BLD	2	0.10
LW2	2	490.87	60	38.9	8.05	12.27	2.70	75,600.00	20.00	BLD	0.1	4.00
LW12	2	415.47	100	19.95	8.11	12.43	1.50	17,400.00	36.00	1,264.00	0.1	11.00
LW13	2	213.82	50	12.46	8.77	13.94	1.00	73,900.00	208.00	BLD	0.1	4.0-0
LW14	2	103.86	40	7.44	8.33	13.44	1.80	3,040.00	16.00	824.00	6	6.00
LW15	2	143.13	50	3.789	8.37	12.70	1.30	1,520.00	115.00	385.00	2	3.00
LW16	2	240.52	80	0.32	9.69	13.35	0.15	827.00	36.00	217.00	0.1	2.00
LW18	2	78.53	60	0.35	9.93	12.00	0.04	34.00	5.00	149.00	0.1	3.00
OHP	3	490.87	30	0.75	9.8	14.22	3.00	1.00	10.00	1,099.00	9	30.00
HP 1	3	56.74	60	3.82	9.46	13.63	3.80	ND	ND	ND	ND	ND
HP 2	3	113.09	100	2.46	9.68	14.00	4.70	0.10	6.00	716.00	32	24.00
HP 3	3	15.90	50	0.72	10.5	19.32	1.60	ND	ND	ND	ND	ND
FH	4	44.17	30	0.86	10.03	15.30	4.27	1.00	9.00	518.00	32	14.00
Nostoc	4	19.63	30	1.217	10.15	17.05	2.58	1.00	14.00	868.00	13	23.00
P70	4	113.09	100	4.87	10.21	16.36	3.19	1.00	18.00	1,751.00	39	56.00
Skua	4	201.06	150	0.97	9.87	14.46	1.59	0.10	6.00	568.00	86	26.00
Orange	4	50.26	120	1.8	10.24	14.97	4.75	0.10	7.00	1,023.00	8	34.00
P70E	4	28.27	80	7.33	8.93	16.60	4.48	1.00	1.00	1,750.00	4	13.00
Brack	4	240.52	100	10.84	9.84	15.78	3.87	4.00	11.00	2,975.00	27	140.00
Salt	4	70.88	50	42.1	9.84	19.48	6.93	2.00	30.00	7,817.00	9	408.00

ND indicates not determined; BLD= Below limit of detection. DIC = Dissolved inorganic carbon; NO_3= nitrate; NH_4= ammonium
DON=Dissolved organic nitrogen; DRP = Dissolved reactive phosphorus; DOP= Dissolved organic phosphorus

Biological parameters

In order to better understand whether the microbial mats differ from each other, mat samples were analyzed in terms of pigments content, total EPS and organic matter. In general, mats showed significant variability of the pigments contents. The properties of these microbial mats differ significantly in terms of chlorophyll-a, phycoerythrin and phycocyanin contents. McMurdo Ice Self ponds showed the highest contents for both plankton and benthic chlorophyll-a, while the lowest values were found in Low Wright Valley site (Fig. 1 A-B). In terms of phycoerythrin and phycocyanin pigments, highest values were found in samples from LWV and lower content for the UWV ponds (Fig. 1 C-D). Differences in the content of benthic chlorophyll-a ($p= 0,0004$), phycoerythrin ($p= 0,01$) and phycocyanin ($p= 0,01$) were statistically significant between sites. A significant difference in the planktonic chlorophyll-a concentration in water samples was also observed ($p= 0,003$). On the other hand, the amount of organic matter and EPS did not differ significantly between sites.

Cyanobacterial morphotypes composition

The composition of the cyanobacteria community in all ponds was characterized by morphological description (see Chapter 1). Twenty nine cyanobacterial morphotypes belonging to three orders (Oscillatoriales (22), Nostocales (3) and Chrooccocales (4)) were identified in the field samples. The presence or absence of the morphotypes for each pond is shown in Table 5. There is a varying composition of cyanobacteria community in the mat samples from ponds from the four sites, and a pattern characterized by the occurrence of morphotypes belonging to Chrooccocales and Nostocales orders was identified. In contrast, most Oscillatoria morphospecies were widely distributed across sites

Morphotypes from Nostocales, attributable to the genera Nodularia, Nostoc and Calothrix were abundant in samples from MIS and Ross Island areas, while only one pond from UWV was dominated by filaments of Nostocales order (morphotype 25). Conversely, morphotype 1 (Borzia sp. Oscillatoriales) was entirely restricted to the ponds of the Upper and Lower Wright Valleys, where it occurred in almost all ponds and colonies of Chrooccocales strains were also rare in samples from coastal areas, but abundant in samples from inland sites.

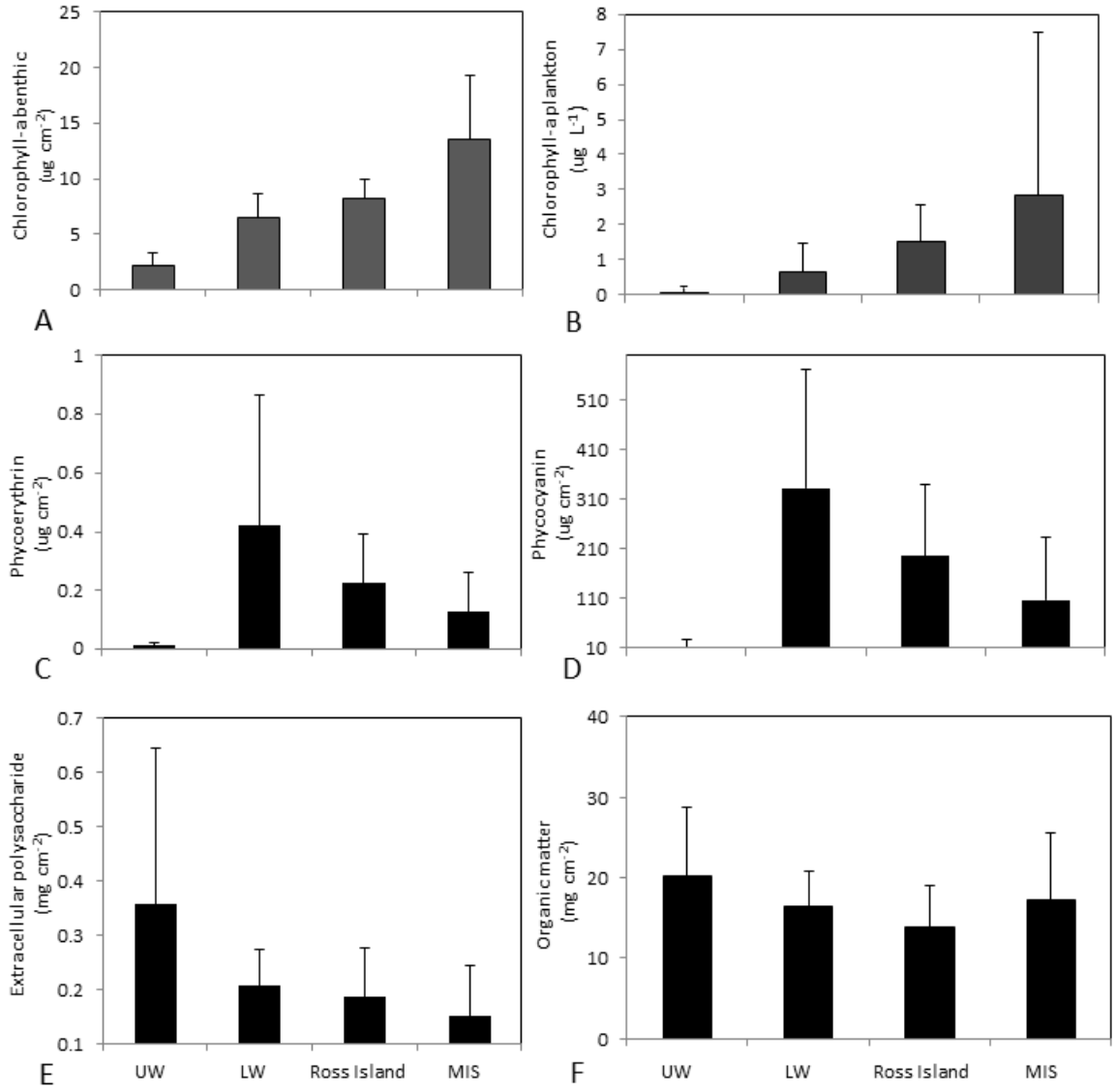

Figure 1. Contents of pigments, extracellular polysaccharide and organic matter in the microbial mat and water samples. (A) chorophyll-a benthic; (B) chorophyll-a plankton; (C) phycoerithrin; (D) phycocyanin; (E) extracellular polysaccharide and (F) organic matter. The values are mean (+SD) taken from sites sampled of the each studied area. UW = Upper Wright; LW = Lower Wright and MIS= McMurdo Ice Shelf.

In contrast to the restricted distribution of Nostocales, Chroococcales and Borzia, some of the morphotypes, including those belonging to the genus Leptolynbya, had wide distributions, occurring widely in all of the localities and in a wide range of environmental conditions. Overall, of the 29 morphotypes identified only nine were distributed across all regions, wheares seven were present at only one, 11 at two and two at three. The species richness of the four regions was similar, with 18 morphotypes recognized at UWV and MIS, 16 and 17 at LWV and Ross Island respectively.

In the absence of definitive DNA-based identifications, it is not possible to be certain of the identities that are assigned to each morphotype. However, for ease of understanding and comparison with the majority of existing literature on Antarctic cyanobacteria, here to forth we will refer to the morphotypes as "morphospecies", and use the names in column 3 of Table 4.

Table 4. Cyanobacterial morphotypes, their probable identities and abbreviation used in the ordination analyses

Morphotype	Abreviation	Designation based on literature
Oscillatorales		
1	Mph 1	Borzia sp.
2	Mph 2	cf. Kamvophorum
3	Mph 3	Geitlerinema sp.
4	Mph 4	Pseudanabaena sp.
5	Mph 5	cf. Pseudanabaena
6	Mph 6	cf. Pseudanabaena
7	Mph 7	Leptolyngbya sp.
8	Mph 8	Leptolyngbya sp.
9	Mph 9	cf. Leptolyngbya
10	Mph 10	Plectolynbya cf. hodgsonii
11	Mph 11	cf. Plectolynbya
12	Mph 12	Leptolyngbya (with resemblance to P. prestleyei)
13	Mph 13	cf. Schizothrix
14	Mph 14	Oscillatoria sp.
15	Mph 15	Oscillatoria sp.
16	Mph 16	cf. Oscillatoria
17	Mph 17	Oscillatoria sp.
18	Mph 18	Crinalium sp.
19	Mph 19	cf. Lyngybya
20	Mph 20	Phormidium cf. murrayi
21	Mph 21	Phormidium cf. autumnale
22	Mph 22	Phormidium cf. setchellianum
Nostocales		
23	Mph 23	Nodularia sp.
24	Mph 24	Nostoc sp.
25	Mph 25	Calothrix sp.
Chroococcales		
26	Mph 26	Chroococus sp.
27	Mph 27	cf. Aphanocapsa
28	Mph 28	cf. Aphanocapsa
29	Mph 29	cf. Aphanocapsa

Table 5. The distribution of Cyanobacteria in the McMurdo Sounds sampling regions. *Mph = Morphotype

	Upper Wright Valley						Lower Wright Valley							Ross Island				McMurdo Ice Self						
Mph*	L9	L15	L16	L26	E4	E9	LW2	LW12	LW13	LW14	LW15	LW16	LW18	OHP	HP1	HP2	HP3	NST C	SKUA	P70	OGE	P70-E	FH	BRK	Salt
1	x	x	x	x			x	x	x	x	x	x	x												
2																					x				
3								x		x		x	x				x	x			x		x	x	
4					x	x	x	x	x	x	x	x	x			x	x	x			x		x		
5	x			x			x		x			x		x								x			x
6	x	x	x	x	x			x	x	x	x	x	x	x							x	x			
7	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
8	x	x	x	x	x	x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	
9												x													
10									x	x			x											x	x
11		x	x																						
12		x				x	x	x		x	x	x	x			x	x	x		x	x	x	x	x	x
13					x	x		x																	
14																		x			x		x		
15																	x								
16																x	x								
17																					x		x		
18						x																			
19																x		x		x					
20					x			x		x	x	x		x		x		x	x	x	x	x	x	x	
21			x					x	x	x		x		x	x	x	x	x	x	x	x	x	x	x	x
22														x	x			x	x	x			x		
23																x		x	x		x		x	x	
24		x												x		x	x	x		x	x	x	x		
25		x																							
26		x										x													
27	x			x				x	x	x	x	x													
28	x	x											x			x									x
29					x												x								

Ponds contrasted with cyanobacteria occurrence

The relationship between ponds determined by dendrogram ordination based on morphotype distribution, first showed a division into two large groups (Eigenvalue: 0.34) (Figure 2). The first main group (A) was formed mostly by localities in the Lower and Upper Wright Valley, with the addition of only one pond (Salt) from McMurdo Ice Self (Figure 2). The second main group (B) was formed by localities strongly associated with coastal areas (McMurdo Ice Self and Ross Island). The indicator "morphospecies" of specific groups are shown in the Table 6.

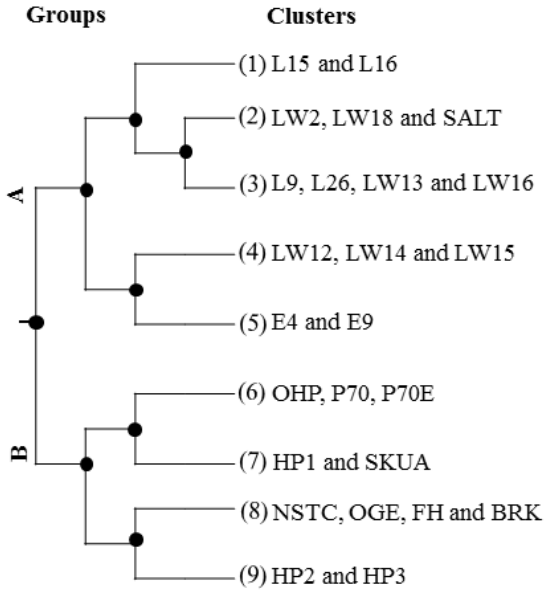

Figure 2. Dendrogram of the relationship between 25 areas along inland-coastal gradients in the McMurdo Sound region as produced by the divisive clustering analyses of 29 cyanobacteria morphotype.

Table 6. Determinant morphotype of the two main group formed by TWISPAN analysis. The indicator species of specific groups are indicated in bold type. Morphotypes with low occurrence do not appear in the table. * Morph = Morphotype

Group A
Morph. 1(Borzia sp.); morph. 5(cf. Pseudanabaena); morph. 6 (cf. Pseudanabaena); morph. 10 (Plectolynbya cf. hodgsonii); morph. 13(cf. Schizothrix); morph. 27 (cf. Aphanocapsa); morph. 28 (cf. Aphanocapsa)
Group B
Morph. 14 (Oscillatoria sp.); morph. 19 (cf. Lyngybya); morph. 20 (Phormidium cf. Murrayi); morph. 21(Phormidium cf. autumnale); **morph. sp22** (Phormidium cf. setchellianum); **morph. sp23** (Nodularia sp.); **morph. sp24** (Nostoc sp.)
Group non-preferentials
Morph.3 (Geitlerinema sp.); morph.4 (Pseudanabaena sp.); morph.7 (Leptolyngbya sp.); morph.8 (Leptolyngbya sp.); morph.12 (Leptolyngbya (with resemblance to P. prestleyei)

Ponds ordered by physical and chemical parameters

The ordination of ponds based on physical-chemical parameters by Principal Component analysis (PCA) is shown in figure 3 A-B. The first two main component captured 61 % of the total variance, 37% in the first axis and 24% in the second axis.

PCA ordination showed a single cluster of ponds from coastal areas (MIS and Ross Island), and a second cluster from the Lower Wright Valley. Within the coastal cluster, ponds aligned with axes representing the environmental variables depth, DRP and pH (fig. 3 A-B). Within the inland cluster (Upper and Lower Wright Valleys) ordering was mainly by ammonium content and pond area (fig. 3 A-B). Salt, L9 and L26 were characterized by strong association with conductivity, and separated as the most saline ponds (fig. 3 A-B).

Concentration of ammonium (NH_4+) was negatively correlated with dissolved reactive phosphorus (DRP) and showed a positive correlation with electrical conductivity and dissolved inorganic carbon (DIC). Depth, pH and dissolved oxygen were positively correlated to DRP.

Physical and chemical water parameters versus cyanobacteria distribution

A Canonical Correspondence Analysis (CCA) was used to evaluate both the relative contribution of the environmental variables to the ordination axes and to determine whether variance in the cyanobacterial community data could be explained by water parameter variables.

The CCA ordination reproduced the same pattern shown by PCA ordination in terms of pond clustering, except that Salt pond was more closely associated to those inland ponds from Upper and Lower Wright Valleys (fig.4 A-B). This finding is in agreement with the dendrogram based on the presence and absence of cyanobacteria morphospecies (fig.2).

The cumulative percentage of variance explained was 28% for first two axes together, which suggest that a high proportion of the total variance in the morphotype data was not explained. However the first two canonical axes were significant, as shown by a Monte Carlo test ($p = 0.006$).

Despite the low proportion of the variance explained, two morphospecies clusters appeared to be correlated to the environmental factors. A first cluster showed a positive association with depth, pH, dissolved oxygen and DRP. The second cluster situated on the

negative site of the CCA correlated with conductivity, NH_4^+ and pond area. Morphospecies that had the broadest distributions were placed on the center of the CCA diagram.

Nodularia and Nostoc respectively were positioned on the positive site of the CCA and strongly associated to high levels of dissolved reactive phosphorus and away from high concentrations of nitrate and ammonium. In the same cluster were placed all Oscillatoria (morphotype 17, 14, 16) for which the occurrence was restricted to the ponds from MIS and Ross Island. Although morphospecies belonging to Phormidium genus has been reported in the four working areas, it also was associated on the positive site of the CCA and thus towards high DRP and low nitrate.

Chrooccocales Aphanocapsa and Chroococus as well as Borzia were associated to high levels of nitrate, ammonium and conductivity on the negative site of axis 1. Additionally, morphospecies which were assigned to Pseudoanabaena and Calothrix genus were clustered on the negative site of the axis 1, as well.

Biological mat parameters versus cyanobacteria distribution

We also used the canonical ordination to evaluate the relative contribution of the mat bulk characteristics (chlorophyll, phycoerythrin, phycocyanin, organic matter and EPS content) to the ordination axes (fig. 5A-B).

The mat characteristic accounted a very low proportion (18% for the first two axes together) to the total variance in the morphospecies data, but did show significance by Monte Carlo test. Three clusters could be distinguished, a larger one associated to chlorophyll content (corresponding to the MIS area and its high chlorophyll), a second cluster associated to the pigments phycoerythrin and phycocyanin (corresponding to the Lower Wright Valley) and a third one loosely associated with organic matter, which grouped ponds from all of the four studied area. This result was expected since organic matter and EPS content did not show statistically significant results by Kruskal Wallis test.

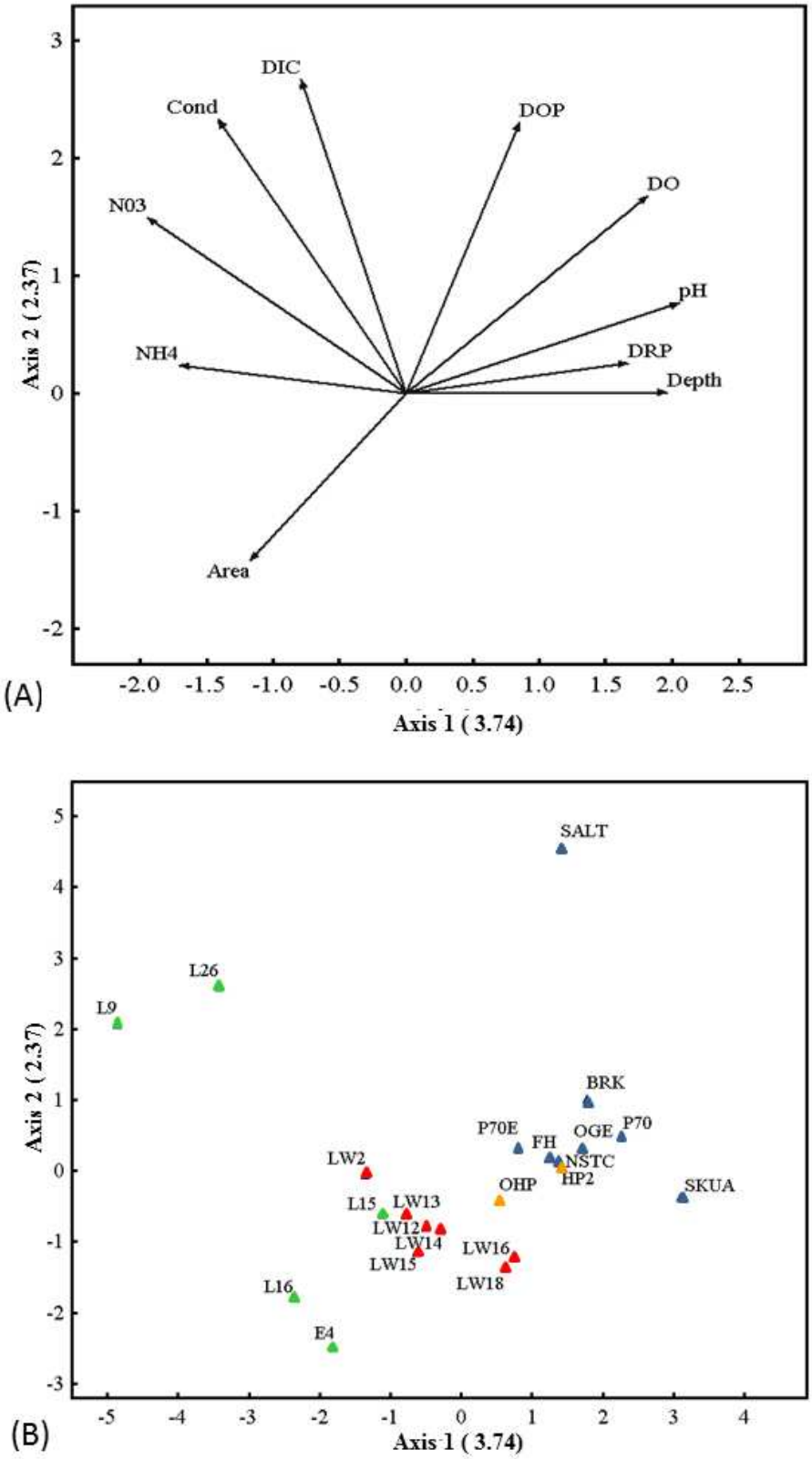

Figure 3. Principal component analysis (PCA) ordination biplot of physical-chemical variables of ponds. The symbols with different colors mean different groups of ponds: green and red colours represent ponds from Upper and Lower and Wright Valley, respectively; orange from Ross Island and blue from McMurdo Ice Self. NO_3= nitrate; NH_4= ammonium; Cond.= conductivity; DO= Dissolved oxygen; DIC = Dissolved inorganic carbon; DRP = Dissolved reactive phosphorus; DOP= Dissolved organic phosphorus.

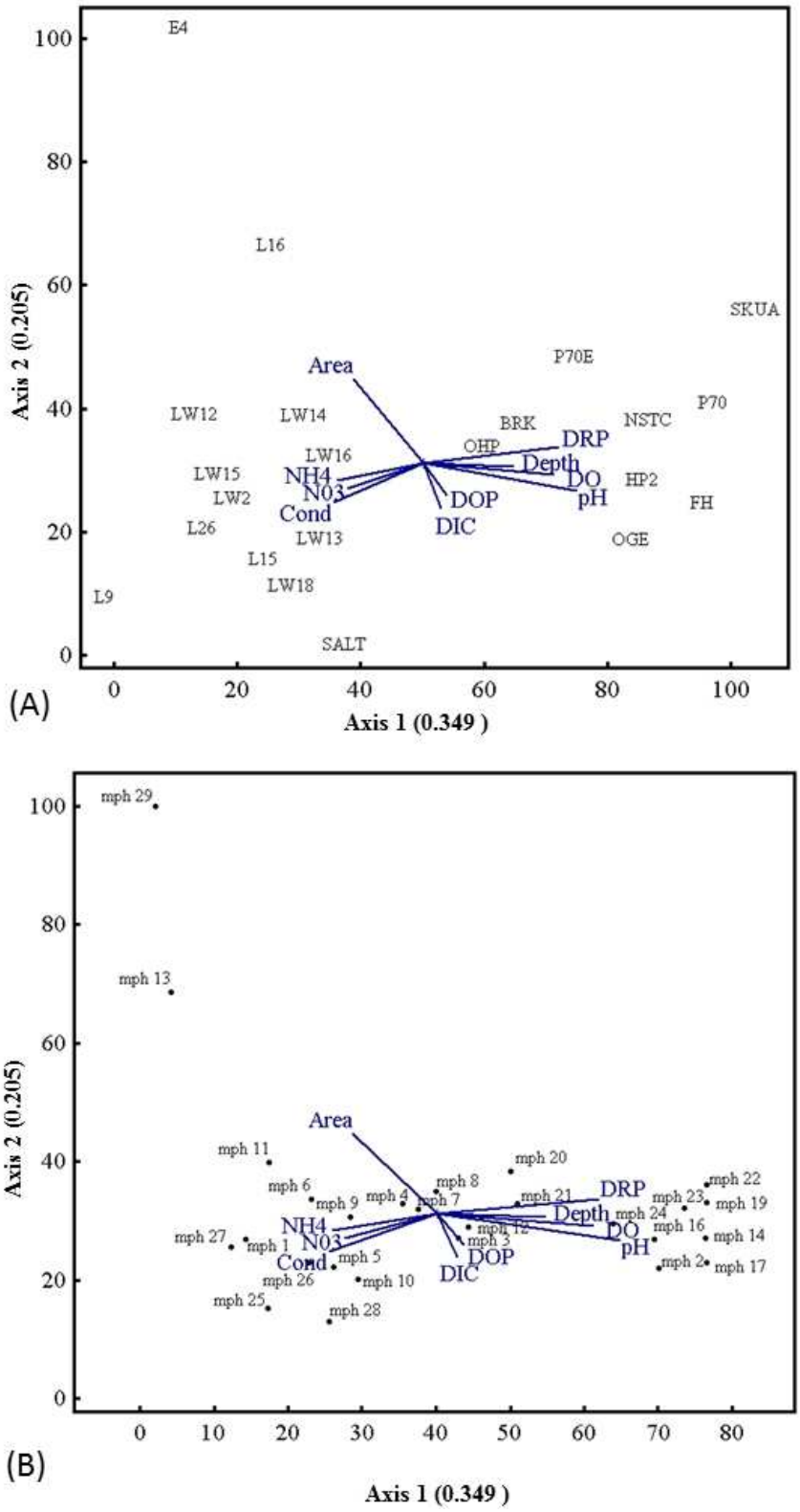

Figure 4. Canonical correspondence analysis (CCA) blipots showing the relationship between sites, presence-absence of cyanobacteria and environmental variables. (A) environmental variables and sites, (B) cyanobacterial morphotype and environmental variables. Morphotype abbreviation are shown in table 4. NO_3= nitrate; NH_4= ammonium; Cond = conductivity; DO= Dissolved oxygen; DIC = Dissolved inorganic carbon; DRP = Dissolved reactive phosphorus; DOP= Dissolved organic phosphorus.

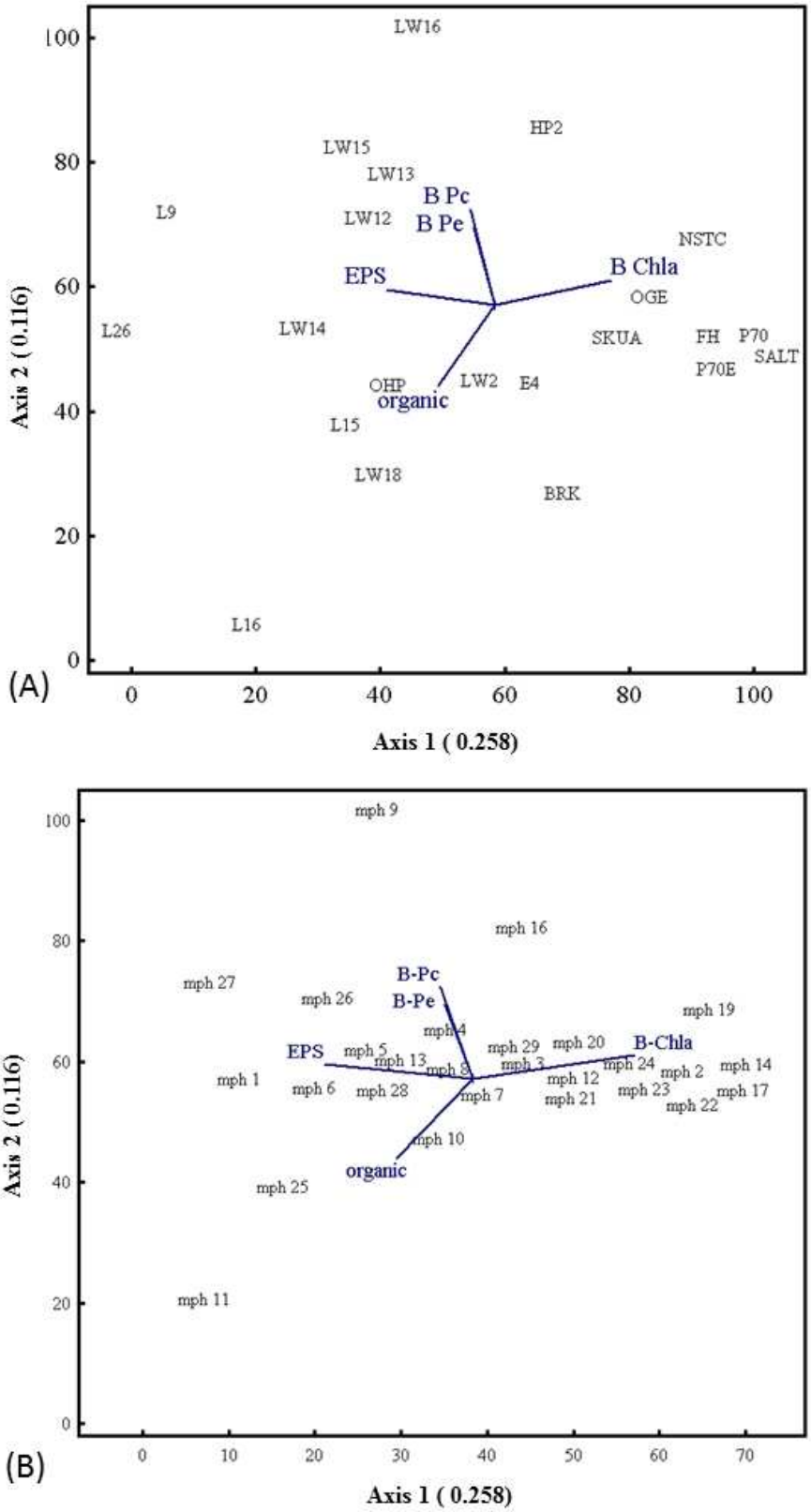

Figure 5. Canonical correspondence analysis (CCA) blipots showing the relationship between sites, presence-absence of cyanobacteria and biological variables of microbial mat. (A) microbial mat variable and site, (B) cyanobacterial morphotype and mat variable. Chla= Chlorophyll-a; B-Pc= Phycoerythrin; B-Pe=Phycocyanin. Morphotype abbreviation are shown in table 4.

4. Discussion

The study of microbial ecology, particularly cyanobacteria, including those from Antarctica is still at an early stage. The ecological preferences are unclear, as well as the relationship between the distribution of cyanobacteria morphospecies and environmental variables. Many studies lack good characterization of habitat and niches.

The kruskall-wallis test of environmental factors showed that the ponds of the four studied areas can be differentiated in terms of depth, pH, dissolved oxygen, DIC, concentration of nitrogen (nitrate and ammonium) and phosphorus (DRP and DOP), but overlapped with reference to other environmental variables. Additionally, the PCA ordination revealed that the ponds formed a clear gradient in physical-chemical variables that are primarily distinguished by variation in nitrogen and phosphorus levels, depth and pond area. Furthermore, they also can be distinguished by electrical conductivity, despite a great amplitude of results between ponds.

To date, very little has been reported about shallow water limnology of Upper and Lower Wright Valley, and this study is the first to describe some of these small inland ponds. Webster-Brown et al. (2010), studying Antarctic inland ponds from Darwin Glacier region also recorded very high concentration of nitrogen (nitrate and ammonium) and low levels of phosphorus in these areas, which suggests that inland ponds, distant from the coastal zone are generally nitrogen-rich and phosphorus-poor.

The chemical composition of ponds from MIS has been intensively studied (de Mora et al., 1994; Fernandez-Valiente et al., 2001; Mountfort et al., 2003; Jungblut et al., 2005; Hawes et al., 2011) and they include some of the ponds presently investigated. These authors also reported that melt waters on the McMurdo Ice Shelf exhibit a wide spectrum of chemical properties, with levels of electrical conductivity ranging over several orders of magnitude, including very high levels (de Mora et al., 1994). Also, high concentration of P and low N levels were earlier reported in the coastal ponds (Hawes et al., 2011) corroborating our results. A wide range of limnological information of ponds from Ross Island also can be found from previous studies (Goldman et al. 1972; Broady 1989; Schmidt et al., 1991). Schimidt et al. (1991) reported that nitrate concentrations are low or undetectable in the ponds from Ross Island throughout the year, showing that these ponds are very poor in nitrogen, consistent with the present study.

While pigments, EPS and organic matter cannot be only associated to cyanobacteria community, given other mat-dwelling microorganisms (e.g. microalgae) also contribute, the

studied pond systems can be differentiated in terms of pigments content as chlorophyll-a, phycoerythrin and phycocyanin. Mats from McMurdo Ice Shelf showed greater values of chlorophyll, followed by the mats from Ross Island and LWV, while the lowest concentrations were found in UWV samples. Conversely, mats from UWV and LWV showed the greater values of both phycoerythrin and phycocyanin. Whether this difference is due to environemtnal conditions, or to the taxonomic differences between the coastal and inland sites cannot be currently determined.

The TWINSPAN analysis and CCA ordination grouped the Salt pond nearby to those from Wright Valley areas. This fact can be related not only by the similarity of the cyanobacteria community composition found in these ponds, but also to high values of electrical conductivity found in the Salt pond, consistent with values detected in ponds from UWV and LWV. This provides some support for the view that the taxonomic composition is in part determined by the ability of taxa to tolerate extreme high conductivity.

Previous studies indicated that Antarctic benthic ecosystems have electrical conductivity as a key factor that regulates the species composition of microbial mat (Vincent & James, 1996; Roberts & McMinn, 1996). However, other similar studies from elsewhere in continental Antarctic (Larsemann Hills and Bølingen Islands), did not find any clear relationship between electrical conductivity and cyanobacteria morphotypes distribution, but showed that the mat structure and species turnover were primarily determinated by depth (Sabbe et al., 2004).

Although is well-known that there are differences in relative abundance of species in microbial mat composition, our concern is the difference in the distribution of morphospecies that occupy the same habitat and/or between habitats. Unfortunately the tendency for some environmental variables to correlate to sites meant that a balanced design, where similar ranges of environmental variables were found at each site was not achieved. Upper and Lower Wright Valleys, for example are occupied by ponds with high concentration of nitrate and ammonium, and low levels of phosphorus, while the coastal sites were the opposite.

From our work the CCA analysis revealed, even on the generic level, that differences in the cyanobacteria morphospecies distribution along the study area are linked to: (1) nitrogen levels and electrical conductivity (one side) and (2) to phosphorus and depth. We did, however, observe a particular pattern on the distribution of some morphospecies, involving representatives of the three orders of cyanobacteria identified in this work (Oscillatoriales, Nostocales and Chroococcales).

Few works have reported the presence of the genus Borzia in Antarctic systems. It has been previously identified by Hodgson et al., (2001) in hypersaline lakes from different biotopes in the Rauer Islands (eastern Antarctica). In addition, Broady (2005) documented the presence of Borzia in locations from southern Victoria Land. However, no report to this morphospecies is available for Antarctic Peninsula, suggesting confined distribution of this morphospecies in the drier, more saline part of continental Antarctic. The Chroococcales morphospecies, in addition to Leptolyngbya and Schizothrix genera have also been identified, by Hodgson et al. (2001), as dominant components in hypersaline lakes, being less abundant in lakes characterized as mesosaline and hyposaline. In the present study the presence of Chroococcales morphospecies was also closely associated to Leptolyngbya and they were abundant in mats from Upper and Lower Wright Valleys ponds, where high conductivity values prevailed. Schizothrix sp. was found by Taton et al. (2006) in eastern Antarctica sites (Vestfold Hills, Larsemann Hills and Shcherbinina Island) exclusively dwelling in oligosaline lakes, whereas in our study this morphospecie was recorded in ponds that exhibited either low (0.13 – 2.05 mS/cm) or high (19,95 mS/cm) electrical conductivity values. The salinity effects provide support for a deterministic separation of taxa specific to the more saline Wright Valley, though the absence of Borzia from hypersaline ponds at the MIS sites would suggest that dispersal may also play some role.

Nostoc sp. and Nodularia sp. were identified by Hodgson et al. (2001) as components of hyposaline lakes. Additionally, Taton et al. (2006) using a morphological approach, reported the presence of Nostoc sp. widely spread from oligosaline to hyposaline ponds, while Nodularia sp. was restricted to hyposaline lake and present at only one of the five geographic studied areas. We documented morphospecies belonging to Nostoc and Nodularia in ponds that had the conductivity range between 0.72 mS/cm to 7.33 mS/cm, and no very high conductivity ponds (>20 mS/cm) showed the presence of both genera, but all in low inorganic nitrogen environments. This distribution pattern is consistent with the potential advantage of fixing nitrogen in the low-N coastal ponds (Smith, 1983) and again supports a mechanistic distribution pattern.

The absence of nitrogen-fixing cyanobacterium at low levels of phosphorus and high concentration of nitrogen was also documented by Webster-Brown et al. (2010). It has been described by Chen et al. (2012) that Nostoc colony developments, growth and photosynthesis were restricted at low levels of phosphorus, suggesting that phosphorus may be an important factor limiting the productivity and distribution of Nostoc.

One pond (L15) that showed low levels of phosphorus was composed of mat dominated by heterocyst-forming cyanobacterium, represented by genus Calothrix sp. The restricted and dominant presence of Calothrix at only one of the studied sites also was identified by Simmons (1993) studying lakes from other regions of McMurdo Dry Valleys who argued that it could be related to high content of magnesium sulfate found in the water column. Hodgson et al. (2001) identified Calothrix sp. as constituent of microbial mat only in hyposaline lakes, whereas Taton et al. (2006) reported the presence of this morphospecie in lakes ranging between oligosaline to hyposaline at different locations. We found Calothrix at a pond with one of the lowest conductivity values in Upper Wright Valley.

In general, the cyanobacterial morphospecies identified in our work were similar to those from many terrestrial and stream environments in Antarctica (Broady & Kibblewhite, 1991; Komárek, 1999; Sabbe et al., 2004; Jungblut et al., 2005, Komárek et al., 2008; Quesada & Vincent, 2012; Fernández-Carazo et al., 2012; Corrêa, 2012). Although fewer of these studies have described the physical-chemical environmental properties, the similarity of the morphospecies mentioned indicates that these are widespread on the Antarctica continent from the coastal area to Antarctic Peninsula, with the possible exception of Borzia.

On the basis of the results obtained and those from literature, the variability of microbial mat composition across the study area, in terms of cyanobacteria morphospecies, cant be largely related to environmental variables, overlain on a very broad distribution of some taxa that clearly have wide environmental tolerances. The differences found in the mat diversity and the confined distribution of some morphospecies might also be related to factors such as dispersion (geographical location and distance from source), with some taxa accumulating in regions where higly saline, or low inorganic nitrogen waters are common. Few studies have included spatial measurements into the analyses to predict the influence of environmental factors on the benthic cyanobacteria composition. However, studies for aquatic bacteria have found good correlations of both distance (geographic proximity) and environmental properties with patterns of diversity (Reche et al., 2005; Yannarell & Triplett, 2005; Sommaruga & Casamayor, 2008).

The ability to colonize a new place can also pushes the limits of distribution of these microorganisms. All studied sites consist of hydraulically interdependent pond system, which may difficult the propagule dispersal, though Hawes et al. (1992) and Fountain & Lyons (2003) argued that mats which grow on the pond edge are subjected to drying, and can be blown away by Antarctic's strong winds. This fact may provide continual recruitment of these

organisms across wide areas (Fountain & Lyons, 2003). However, the dispersal potential of each taxa and its tolerance to thrive under conditions imposed by the colonized habitat might be limiting for the propagule establishment at new region or habitat. Hodgson et al. (2001) reported that the successful biota is defined both through its ability to survive and adapt to the local environmental conditions and through the limits on colonization and re-colonization resulting from biogeographical isolation.

5. Final remark

It is difficult to isolate which variables (directly or indirectly) affects cyanobacteria communities composition and how. Our data revealed that there is a clear gradient from coastal ponds (nitrogen-depleted and high phosphorus concentration) to inland ponds (high nitrogen concentration, high electrical conductivity and low phosphorus concentrations). Additionally, the results showed that the four Antarctic sub-regions are not homogeneous in terms of cyanobacterial diversity, though they do share many elements. In conclusion, our findings suggest that within the area bounded by our study sites, a metapopulation of cyanobacteria appears to exist, most of which are well dispersed, probably by wind. The association of some taxa with a specific site, where average conditions tend to favour those taxa, suggests that a deterministic mode might be acting on local cyanobacteria community composition. The presence of local taxa across a range of pond conductivities within that site may reflect the inevitability that efficient short-distance dispersal within sites may create more local specificity than across the whole McMurdo Sound region.

While this paradigm has not been broadly clarified, this present study provides an increased knowledge of Antarctic cyanobacteria ecology, as well as, provides the importance of the maintenance of these areas in pristine conditions to keep the biodiversity of regional Antarctic ecosystems. Future works comprising further exploration of other Antarctic regions, including genetic methods to unequivocal identification of species and spatial measurements can strengthen our understanding to clarify the forces that affect the cyanobacteria morphospecies distribution and, consequently the mat community composition in Antarctic systems.

6. Reference

Allison, S. D.[†‡] & Martiny, J. B. H. Resistance, resilience, and redundancy in microbial communities. PNAS 105 doi_10.1073_pnas.0801925105

Bolch, C. J. S. & Blackburn, S. I. 1996. Isolation and purification of Australian isolates of the toxic cyanobacterium Microcystis aeruginosa. Journal of Applied Phycology 8: 5-13.

Broady, P.A. 1989. Broadscale patterns in the distribution of aquatic and terrestrial vegetation at three ice-free regions on Ross Island, Antarctica. Hydrobiologia 172: 77-95.

Broady, P. A. & Kibblewhite, A. L. 1991. Morphological characterization of Oscillatoriales (Cyanobacteria) from Ross Island and southern Victoria Land, Antarctica. Antarctic Science 3: 35-45.

Broady, P. A. 2005.The distribution of terrestrial and hydro-terrestrial algal associations at three contrasting locations in southern Victoria Land, Antarctica. Algological Studies 118: 95-112.

Chen, Z.; Chen, S.; Lu, G. & Chen, X. 2012. Phosphorus limitation for the colony formation, growth and photosynthesis of an edible cyanobacterium Nostoc sphaeroides. Biotechnology Letters 34: 137-143.

Corrêa, D. M. 2012. Caracterização morfológica e molecular de cianobactérias da Antártica Marítima e potencial para biodegradação e bioindicação em ambientes contaminados por hidrocarbonetos. Tese (Doutorado em Botânica), Universidade Federal de Viçosa, MG.

de Mora, S.J.; Whitehead, R.F. & Gregory, M. 1994. The chemical composition of glacial melt water ponds and streams on the McMurdo Ice Shelf, Antarctica. Antarctic Science 6: 17-27.

Doran, P. T.; Mckay, C. P.; Fountain, A.G.; Nylen, T.; Mcknight, D. M.; Jaros, C. & Barrett, J. 2008. Hydrologic response to extreme warm and cold summers in the McMurdo Dry Valleys, East Antarctica. Antarctic Science 20: 499-509.

Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A. & Smith, F. 1956 Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry 28: 350-356.

Fernández-Carazo, R.; Namsaraev, Z.; Mano, Marie-Jose; Ertz, D. & Wilmotte, A. 2012. Cyanobacterial diversity for an anthropogenic impact assessment in the Sør Rondane Mountains area, Antarctic. Antarctic Science 24: 229-242.

Fernandez-Valiente, E.; Quesada, A.; Howard-Williams, C. & Hawes, I. 2001. N_2-fixation in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. Microbial Ecology 42: 338-349.

Fountain, A.G. & Lyons, W.B. 2003. Century-to millennial-scale climate change and ecosystem response in Taylor Valley, Antarctica. In: Greenland, D.; Goodin, D.G. & Smith, R.C. (eds). Climate variability and ecosystem response at Long-Term Ecological Research Sites. Oxford: Oxford University Press, 319-340p.

Foreman, C. M.; Wolf, G. F. & Priscu, J,C. 2004. Impact of Episodic Warming Events on the Physical, Chemical and Biological Relationships of Lakes in the McMurdo Dry Valleys, Antarctica. Aquatic Geochemistry 10: 239-268.

Goldman, C.R.; Manson, D.T. & Wood, B.J. B. 1972. Comparative study of the limnology of two small lakes on Ross Island, Antarctica. Antarctic. Research Series 20: l-50.

Hawes, I.; Howard-Williams, C. & Vincent, W. F.. 1992. Desiccation and recovery of antarctic cyanobacterial mats Polar Biology12, 587-594.

Hawes, I.; Howard-Williams, C. & Pridmore, R. D. 1993. Environmental controls on microbial biomass in the ponds of the McMurdo Ice Shelf, Antarctica. Archiv fuer Hydrobiologie 127: 271-287.

Hawes, I.; Schwarz, A.-M.; Smith, R. & Howard-Williams, C. 1999a. Environmental conditions during freezing, and response of microbial mats in ponds of the McMurdo Ice Shelf, Antarctica. Antarctic Science 11, 198-208.

Hawes, I.; Moorhead, D.; Sutherland, D.; Schmeling, J. & Schwarz, A.-M. 2001. Benthic primary production in two perennially ice-covered Antarctic lakes: patterns of biomass accumulation with a model of community metabolism. Antarctic Science 13: 18-27.

Hawes, I.; Safi, K.; Webster-Brown, J.; Sorrell, B. & Arscott, D. 2011. Summer-winter transitions in Antarctic ponds II: Biological responses. Antarctic Science 23: 243-254.

Hawes, I.; Howard-Williams, C. & Sorrell, B. 2013. Variability in ecosystem properties in the ponds of the McMurdo Ice Shelf, Southern Victoria Land, Antarctica on decadal timescales. Antarctic Science. doi:10.1017/S0954102013000576.

Hill, M.O. & Šmilauer, P. 2005. TWINSPAN for Windows version 2.3. Centre for Ecology and Hydrology & University of South Bohemia, Huntingdon & Ceske Budejovice. 29pg.

Hodgson D.A., Vyverman W. & Sabbe K. 2001. Limnology and biology of saline lakes in the Rauer Islands, Eastern Antarctica. Antarctic Science 13: 255-270.

Howard-Williams, C.; Schwarz, A-M. J.; Hawes, I. & Priscu, J. C. 1998. Optical properties of the McMurdo Dry Valley lakes, Antarctica. Antarctica Research Series 72:189-204.

Howard-Williams, C. & Hawes, I. 2007. Ecological processes in Antarctic inland waters: interactions between physical processes and the nitrogen cycle. Antarctic Science 19: 205–217.

Hubbell S.P. 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press, Princeton, NJ.

Izaguirre, I.; Vinocur, A. & Tell, G. 1993. Temporal and spatial variations of phytoplankton from Boeckella Lake (Hope Bay, Antarctic Peninsula). Antarctic Science 5: 137-141.

Jones, C. M. & Hallin, S. 2010. Ecological and evolutionary factors underlying global and local assembly of denitrifier communities. ISME J 4: 633-641.

Jungblut A.-D.; Hawes I.; Mountfort, D.; Hitzfeld, B.; Dietrich, D.R.; Burns, B.P. & Neilan, B.A. 2005. Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environmental Microbiology 7: 519-529.

Kraft, N.J.B.; Cornwell, W. K.; Webb, C.O. & Ackerly, D. D. 2007. Trait evolution, community assembly, and the phylogenetic structure of ecological communities. The American Naturalist 170: 271-283.

Komárek, J. & Anagnostidis, K. 1989. Modern approach to the classification system of cyanophytes. 4 - Nostocales. Archiv für Hydrobiologie Supplement 56: 247-345.

Komárek J. 1999. Diversity of cyanoprokaryotes (cyanobacteria) of King George Island, maritime Antarctica - a survey. Archives of Microbiology 94: 181-193.

Komárek, J. & Anagnostidis, K. 2000. Cyanoprokaryota. 1. Teil, Chroococcales. In Ettl, H., Gartner, G., Heynig, H. & Molllenhauer, D. (eds), Süsswasserflora von Mitteleuropa vol 19. Gustav Fisher, Jena: 548.

Komárek, J. & Anagnostidis, K. 2005. Cyanoprokaryota. 2. Teil, Oscillatoriales. In Budel, B., Krienitz, L., Gartner, G. & Schagerl, M. (eds), Süßwasserflora von mitteleuropa, Band 19/2 Spektrum Akademischer Verlag, Elsevier GmbH. München, German.

Komárek, J.; Elster, J. & Komárek, O. 2008. Diversity of the cyanobacterial microflora of the northern part of James Ross Island, NW Weddell Sea, Antarctica. Polar Biology 31:853-865.

Leibold, M. A.; Holyoak, M.; Mouquet, N.; Amarasekare, P. J.; Chase, M. & Hoopes, M. F. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters 7: 601-613.

Llames, M. E. and Vinocur, A. Phytoplankton structure and dynamics in a volcanic lake in Deception Island (South Shetland Islands, Antarctica). Polar Biology 30: 849-857.

Margules, C. R. & Pressey, R. L. 2000. Systematic conservation planning. Nature 405: 243-253.

Mataloni, G.; Tell, G. & Wynn-Williams, D.D. 2000. Structure and diversity of soil algal communities from Cierva Point (Antarctic Peninsula). Polar Biology 23: 205-211.

Mountfort, D. O.; Kaspar, H. F.; Asher, R. A. & Sutherland, D. 2003. Influences of pond geochemistry, temperature and freeze-thaw on terminal anaerobic processes occurring in sediments of six ponds of the McMurdo Ice Shelf, near Bratina Island, Antarctica. Applied and Environmental Microbiology 69: 583-592.

Quesada, A. & Vincent, W. F. 2012. Cyanobacteria in the Cryosphere: snow, ice and extreme cold. In: Whitton, B.A. (ed) Ecology of cyanobacteria II: Their Diversity in Space and Time. Springer, Dordrecht, 387-399pp.

Quesada, A.; Fernández-Valiente, E.; Hawes, I. & Howard-Williams, C. 2008. Benthic primary production in polar lakes and rivers. In: Vincent, W.F. & Laybourn-Parry, J. (eds). Polar lakes and rivers - Arctic and Antarctic aquatic ecosystems. Oxford: Oxford University Press, 179-196.

Reche I.; Pulido-Villena E.; Morales-Baquero R. & Casamayor E.O. 2005. Does ecosystem size determine aquatic bacterial richness? Ecology 86: 1715-1722.

Roberts D. & McMinn A. 1996. Relationships between surface sediment diatom assemblages and water chemistry gradients in saline lakes of the Vestfold Hills, Antarctica. Antarctic Science 8: 331-341.

Sabbe, K.; Hodgson, D.A.; Verleyen, E.; Taton, A.; Wilmotte, A., Vanhoutte, K. & Vyverman, W. 2004. Salinity, depth and the structure and composition of microbial mats in continental Antarctic lakes. Freshwater Biology 49: 296-319.

Smith, V.H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221: 669-671.

Schmidt, S.; Moskal, W.; de MORA, S.J.; Howard-Williams, C. & Vincent, W.F. 1991. Limnological properties of Antarctic ponds during winter freezing. Antarctic Science 3: 379-388.

Simmons, G. M.; Vestal, J. R. & Wharton, R. A. 1993. Environmental regulators of microbial activity in continental Antarctic lakes. Antarctica Research Series 59:165-95.

Sloan, W. T.; Lun, M.; Wooddcock, S.; Head, I.M.; Nee, S. & Curtis, T. P. 2006. Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environmental Microbiology 8: 732-740.

Sommaruga, R. & Casamayor, E. O. 2009. Bacterial "cosmopolitanism" and importance of local environmental factors for community composition in remote high-altitude lakes: Freshwater Biology 54: 994-1005.

Strunecký, O.; Elster, J. & Komárek, J. 2011. Taxonomic revision of the freshwater cyanobacterium „Phormidium" murrayi = Wilmottia murrayi. Fottea 11: 57-71.

Sutherland, D. L. & Hawes, I. 2008. Annual growth layers as proxies of past growth conditions for benthic microbial mats in a perennially ice-covered Antarctic lake. FEMS Microbiology, Ecology 67: 279-292.

Sutherland, D. L. 2009. Microbial mat communities in response to recent changes in the physiochemical environment of the meltwater ponds on the McMurdo Ice Shelf, Antarctica. Polar Biology 32:1023-1032.

Taton, A.; Grubisic, S.; Brambilla, E.; de Wit R. & Wilmotte A. 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Applied and Environmental Microbiology 69: 5157-5169.

Taton, A.; Grubisic, S.; Balthasart, P.; Hodgson, D.A.; Laybourn-Parry, J. & Wilmotte, A. 2006. Biogeographical distributionand ecological ranges of benthic cyanobacteria in East Antarctic lakes. FEMS Microbiology, Ecology 57: 272-289.

Taton, A.; Wilmotte, A.; Šmarda, J.; Elster, J.; Komárek, J. 2011. Plectolyngbya hodgsonii: a novel filamentous cyanobacterium from Antarctic lakes. Polar Biology 34: 181-191.

ter Braak, C. J.F. & Verdonschot, P. F. M. 1995. Canonical correspondece analysis and related multivariate methods in aquatic ecology. Aquatic Sciences 57: 255-289.

Terauds, A.; Chown, S. L.; Morgan, F.; Peat, H. J.; Watts, D. J.; Keys, H.; Convey, P. & Bergstrom, D.M. 2012. Conservation biogeography of the Antarctic. Diversity and Distribution 18: 726-741.

Vincent, W.F. 1987. Microbial ecosystems of Antarctica. Cambridge: Cambridge University Press, 304p.

Vincent, W. F. & James, M. R. 1996. Biodiversity in extreme aquatic environments: lakes, ponds and streams of the Ross Sea Sector, Antarctica. Biodiversity and Conservation 5: 1451-1471.

Vincent, W. F. 2000. Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. Antarct. Sci. 12:374-385.

Webster-Brown, J.; Gall, M.; Gibson, J.; Wood, S. & Hawes, I. 2010.The biogeochemistry of meltwater habitats in the Darwin Glacier region (80°S), Victoria Land, Antarctica. Antarctic Science 22: 646-661.

Wilson, D. S. 1992. Complex interactions in metacommunities, with implications for biodiversity and higher levels of selection. Ecology 73: 1984-2000.

Wynn-Wllliams, D.D. 1996. Antarctic microbial diversity: the basis of polar ecosystem processes. Biodiversity and Conservation 5: 1271-1293.

Yallop, M. L.; Paterson, D. M. & Wellsbury, P. 2000. Interrelationships between Rates of Microbial Production, Exopolymer Production, Microbial Biomass, and Sediment Stability in Biofilms of Intertidal Sediments. Microbial Ecology 39: 116-127.

Yannarell, A. C. & E. W. Triplett. 2005. Geographic and environmental sources of variation in lake bacterial community composition. Applied and Environmental Microbiology 71: 227-23.

CHAPTER 3

A comparison of the mat-building ability of five strains of Antarctic cyanobacteria

1. Introduction

Cyanobacteria grow in association with surfaces in the vast majority of terrestrial biotopes (Wood et al., 2008; Macedo et al., 2009; Jungblut et al., 2010; Quesada & Vincent, 2012). The growth of these microorganisms on surfaces, incorporating sediment and detrital particles leads to microbial mat formation, in wet, humid and arid areas (Reid et al., 2000; Decho, 2000; Kurtz et al., 2005). The framework of these microbial mats is often mostly a tangled matrix of cyanobacterial trichomes, but they also contain consortia of other organisms including algae, fungi and a variety of heterotrophic and chemoautotrophic microorganisms (Ludwig et al., 2006; Vu, et al., 2009). Microbial mats are often found in extreme environments (Stahl, 1995), and while this is in part due to the absence of bioturbation due to the absence of higher organisms from such habitats, there is little doubt that advantages accrue from the mat morphology (Pearl & Pinckney, 1996; Geddes, 2010).

An important processs during the early development of the mat, and which is essential to the adhesion of the subsequent colonizers, is the production of extracellular polymeric substances – EPS (Rickard et al., 2003; Vu, et al., 2009). These exopolymers act as a site for co-aggregation and allow the attachment and stabilization of the cells on the colonized surface (Sutherland, 2001; Rickard et al., 2003). As well, it facilitates the spatial arrangement of different species within the mat (Allison et al., 1998).

EPS in microbial mat is also directly related to minimizing environmental constraints on the microorganisms (Sutherland, 2001; Tamaru et al., 2005; Decho et al., 2005). EPS provides a barrier between the organism and its immediate contact with the environment, and gives protection against factors such as desiccation, freezing and ultraviolet irradiation (De Phillipis & Vincenzini, 1998; Tamaru et al., 2005).

Properties such as productivity, stability, and the ability to resist disturbance are often thought to emerge as a benefit of the interactions of organisms within microbial mats, and particularly with the presence of EPS (Schink, 2002; Velicer, 2003; Little et al., 2008). Thus, the production of expolysaccharide and the consequent arrangement of organisms into the matrix of mucilage may be seen as a cooperation between these microorganisms which enhances survival under adverse conditions (Rickard et al., 2003; García-Meza et al., 2005). The interactions among mat-forming microorganisms are, however, unclear, and whether they cooperate or compete with each other is still an active question in microbiology (Mitri & Foster, 2013). Which organisms benefit most from, or are most active in the production of the mat morphology are not always certain.

On the Antarctic continent, where the extreme climate places severe limitations on terrestrial life, cyanobacteria grow in association with other microorganisms forming a range of complex mats, across a variety of aquatic and semi-aquatic habitats (Quesada et al., 2008; Andersen et al., 2011). They may grow associated with mosses, rock, glaciers and ice-shelf, streams, in the edge of ponds or at the bottom of lakes (Stal, 2000; Pizarro et al., 2004; Andersen et al., 2011; Quesada & Vincent, 2012). The great variety of microbial ecosystems that existent in Antarctica is an extraordinary opportunity for research on microbial evolution, and of singular importance to evolutionary-ecology approach (Vincent, 2000).

Investigation of the production of microbial mat in Antarctic microbes, including the production of expolysaccharide, chlorophyll content and organic matter, may help to clarify some of the questions around the species-specific and non-specific issues pertaining to mat formation. In this work we assess the production of microbial mats role by individual strains and mixed cultures of cyanobacteria isolated from Antarctic mats, focusing on the chlorophyll-a, exopolysaccharide and organic matter production. Our goal is to understand the different phenotypes of mats produced by different strains and mixes of strains, with a focus on identifying the organism key to development of mat morphology.

2. Material and Methods

2.1. Acquisition and acclimation of the cyanobacteria strains

The cyanobacterial strains are described in Martineau et al. (2013) and were obtained from Cawthron Institute, Nelson, New Zealand, and all were isolated from microbial mats collected from the McMurdo Dry Valley region of Antarctica. To obtain enough biomass the strains were placed in plastic container (4.5 cm diameter and 5.5 tall) and cultivated in MLA culture medium (Bolch & Blackburn, 1996). All cultures were maintained in an incubator at 35±10 µmol photons m^{-2} s^{-1}; 12:12 h light:dark; at 15 ±1°C. Six strains which grew well under these conditions, were selected to set up this experiment: CYN50 (Phormidium cf. autumnale); CYN68 and CYN65 (strains belonging to genus Leptolyngbya); CYN66 (strain belonging to genus Microcoleus); CYN67 (strain from Chroococcales order, cf. Aphanocapsa) and, CYN72 (strain representative of the Nostoc genus). Although the identifications of these organisms are tentative, for clarity they are hereafter referred to, respectively, as Phormidium, Leptolyngya A, Leptolyngbya B, Microcoleus, Aphanocapsa and Nostoc.

2.2 Experimental Design

In order to explore the contribution of each cyanobacterial strain to mat formation, we prepared cultures containing different combinations of isolates, and individual cultures of each isolate (Table 1). Thirteen cultures were established, comprising seven mixed and six single-species cultures. The mixed cultures were based around a core group of three isolates, Phormidium, Leptolynghbya A and Microcoleus that were expected to be important mat formers (Culture 1). Mixed cultures 2-4 then removed one of these three from the core mix, and cultures 5-6 each added an extra isolate.

Table 1. Experimental design. In addition to the mixed cultures below, each strain was grown in isolation.

Culture	Phormidium	Microcoleus	Leptolyngbya a	Nostoc	Leptolyngbya b	Aphanocapsa
1	x	x	x			
2	x		x			
3		x	x			
4	x	x				
5	x	x	x	x		
6	x	x	x		x	
7	x	x	x			x
8			x			
9	x					
10		x				
11				x		
12						x
13					x	

The initial biomass used to setup the experiment was standardized using an in vivo fluorometer (Turner designs Aquafluor). Stock cultures were diluted to read between 170-223 $\mu g\ L^{-1}$, and the same volume of each was then added to each culture. Triplicates of each culture were raised in 50 ml sterile plastic containers to which was added with 10 g of sterile sand and 25 ml of MLA culture medium. The cultures were kept under the same microclimatic conditions as established during the period of acclimation, using a completely randomized design with three replicates for each culture. The culture medium was changed every fifteen days using pipettes. The experiment was carried on for three months.

2.3 Measurement of developed mat

At the end of the experiment, the resulting mats from the mix culture were carefully lifted from the sediment and transferred to petri dishes for subsequent analysis. A small part of each was used for microscope observation and the remainder was freeze-dried and

weighed. Weighed subsamples were then taken for assessment of chlorophyll-a, exopolysaccharide and organic matter production.

2.3.1 Chlorophyll-a

For chlorophyll-a determination, subsamples in 1.8-ml Eppendorf tubes were extracted into 1 ml of 90% acetone. Samples were left overnight in the fridge, and then centrifuged at 1250 g for 15 min. 0.5 ml of supernatant was diluted x5 with fresh 90% acetone, and the fluorescence of chlorophyll-a in the supernatant was read at 663 nm in a Turner Designs Aquafluor fluorometer, without acidification. Chlorophyll-a concentration was calculated based on calibrations using standard solutions of chlorophyll-a and the results were converted to µg/total sample using the total sample and aliquot weights.

2.3.2 Total EPS

For the total extracellular polymeric substances determination, subsamples in 1.8-ml Eppendorf tubes were extracted for 15 min at 20 °C in 1.5 ml of Na_2EDTA (100mM). The extract was then centrifuged at 3,620 g, the supernatant discarded and the resultant pellet resuspended in ultrapure water following the method of Yallop et al. (2000). The carbohydrate content of the resuspension was estimated as glucose equivalents using the phenol–sulphuric acid method described by Dubois et al. (1956). The absorbance was measured against a reagent blank at 485 nm and calibrated against glucose standard. Results were converted to glucose equivalents, as mg/sample, using the total sample and aliquot weights.

2.3.3 Organic matter

For determination of organic matter content, subsamples dried were carefully transferred to weighed crucibles, re-weighed then combusted at 450 °C for 4 h in a muffle furnace. After it had reached room temperature in a desiccator, the ash was weighed on an electrobalance. Weight loss was taken to estimate the organic matter as loss of mass on ignition. Results were converted to mg/sample, using the total sample and aliquot weights (Sutherland & Hawes, 2008).

2.4 Statistical Analyses

All data were analyzed by ANOVA (significance level p=0.05) and the means compared by the Scott Knott test, using the statistical analyses package SISVAR (Fereira, 1998).

3. Results

3.1 Visual characterization of the culture development

Culture 1, composed of the strains Phormidium, Microcoleus and Leptolyngbya A, exhibited at the end of the experiment a consistent mat formation (Fig. 1A). The mats were predominantly dark olive green, thick and it presented a large bubble within the mat. On microscopic examination, all the three strains were present in the mat, however, Phormidium was present in highest proportion (Fig. 2A).

Culture 2, made from Leptolyngbya A and Phormidium in the absence of the isolate Microcoleus, also showed a solid mat formation (Fig. 1B). The mat color was the mostly olive green but parts isolated around of the mat showed bright green color. The presence of bubbles within mat was observed again. Although both of the strains were present in the mat formation, Phormidium was predominantly observed under microscopic characterization (Fig. 2B).

Culture 3 made in the absence of Phormidium and including Leptolyngbya A and Microcoleus, showed mat formation, however, these mats were looser comparing to culture 1 and 2 (Fig. 1C). The mat exhibited mainly a bright blue color and bubble formation was not observed. When observed under microscope, the strain Microcoleus was more abundant than Leptolyngbya A (Fig. 2C).

Although one of the three replicates did not show the same visual growth pattern, the culture 4 composed of the strains Phormidium and Microcoleus developed a firm mat and presented an essentially dark olive green color (Fig. 1D). Bubbles were present within the mat. Under microscopic observation both of the strains were abundant (Fig. 2D).

The three replicates of culture 5, which added the morphotype Nostoc to the core isolates, built a visible and consistent mat (Fig. 1E). The mats were soft, thick and exhibited a mix of olive-green and bright-green color. Microscopic observation recorded the presence of Phormidium, Microcoleus and Nostoc composing the mat formation at the end of the experiment (Fig 2E).

Culture 6 prepared from the core isolates plus the second Leptolyngbya morphotype (Leptolyngbya B) developed a mat formation (Fig.1F). However, two of the three replicates were almost colorless at the end of the experiment. The strains Phormidium and Microcoleus were dominant under microscopic observation (Fig. 2F). Strains belonging to Leptolyngbya

also were found in the mat formation, however, it was not possible to distinguish the Leptolyngbya B from Leptolyngbya A.

In order to evaluate the contribution of strain belonging to the order Chroococcales to microbial mat formation we added to the base mix the strain Aphanocapsa as culture 7. At the end of the experiment the three replicates prepared from the culture 7 showed a consistent mat formation (Fig. 1G). From the microscopic observation, strains Phormidium, Microcoleus and Aphanocapsa were visible distinguished and spread observed in the mat sample (Fig. 2G). Unfortunately at the end of the experiment we found that the Aphanocapsa culture had been contaminated with an unknown Leptolyngbya strain. Care was therefore taken in interpretation of the results from this mix.

All the individual cultures displayed mat formation, however, they were visually differentiated from each other. Leptolyngbya A, developed a thin and loose mat, predominantly blue-green in color (Fig. 1H). No bubbles were detected. All the three replicates made from Phormidium developed a visible and cohesive mat, showing dark olive green color and the presence of bubbles within the mat (Fig. 1I). Microcoleus developed mats, but these were thin and almost colorless at the end of the experiment (Fig. 1J). Nostoc did not show a consistent mat formation (Fig. 1K), and showed visibly low biomass at the end of the experiment. Aphanocapsa was rejected from our experiment, once we observed at the end of the experiment that this culture was contaminated by cyanobacterial trichome. Culture 13 made from Leptolyngbya B did not form a consistent mat (Fig. 1L). At the end of the experiment the three replicates presented only a very thin and fragile mat.

Table 2 shows a summarized description of the mat formation from individual cultures of each isolate. Photomicrographs showing of the morphotypes Leptolyngbya A, Phormidium, Microcoleus, Nostoc and Leptolyngbya B are shown in the figures 2 H-M.

Table 2. Characteristic of the built mat from individual cultures of each isolate at the end of the experiment.

Isolate	Thickness		Degree of cohesion		Presence of bubbles	colour
	Thin	thick	cohesive	loose		
Leptolynbya A	x			x		1
Phormidium cf. autumnale		x	x		x	2
Microcoleus sp.	x		x			3
Nostoc sp.	x			x		4
Leptolyngbya B	x			x		1

1= blue-green; 2=black-olive-green; 3=black-green; 4=olive-green

Figure 1. Photographic record of the mat formation by combinations of isolates and individual cultures of each isolate. Ph= Phormidium; Mc= Microcoleus; LpA= Leptolyngbya A; Nt= Nostoc; LpB= Leptolyngbya B. Isolated Aphanocapsa was rejected from the experiment.

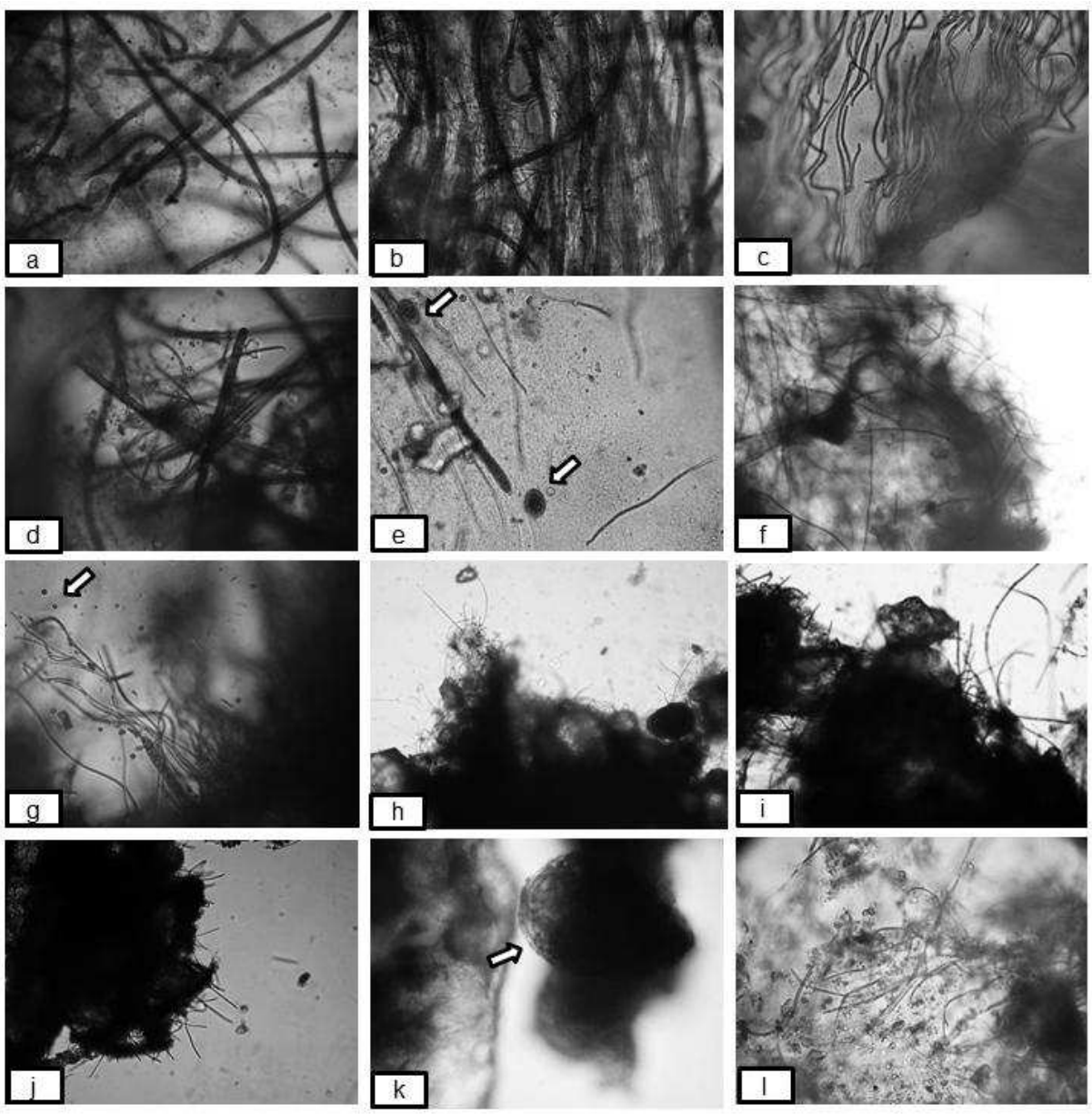

Figure 2. Photomicrograph record of the mat formation by combinations of isolates (**a** – **g**) and individual cultures (**h** –**l**). (**a**) culture 1, combination of isolates Phormidium, Microcoleus and Leptolyngbya A; (**b**) culture 2, combination of isolates Phormidium and Leptolyngbya A; (**c**) culture 3, comprising isolates Microcoleus and Leptolyngbya A; (**d**) culture 4, containing isolates Phormidium and Microcoleus; (**e**) culture 5, combination of isolates Phormidium, Microcoleus, Leptolyngbya A plus Nostoc (arrow showing the presence of small Nostoc colonies); (**f**) culture 6, containing isolates Phormidium, Microcoleus, Leptolyngbya A plus Leptolyngbya B; (**g**) culture 7, combination of isolates Phormidium, Microcoleus, Leptolyngbya A added of Aphanocapsa (arrow showing the presence of tiny cell of Chroococcales); (**h**) culture 8, isolated Leptolyngbya A; (**i**) culture 9, isolated Phormidium; (**j**) culture 10, isolated Microcoleus; (**k**) culture 11, isolated Nostoc (arrow showing the presence of small Nostoc colonies); (**l**) culture 13, isolated Leptolyngbya B. Culture 12 was rejected from the experiment.

3.2 Chlorophyll-a production

Culture 1, comprising the morphotypes Phormidium, Microcoleus and Leptolyngbya A showed highest values of chlorophyll when compared to all other cultures, though this difference was not statistically different ($p = 0.32$). The lowest values of chlorophyll content were detected in the culture 3 in the absence of the Phormidium morphotype (Fig. 3).

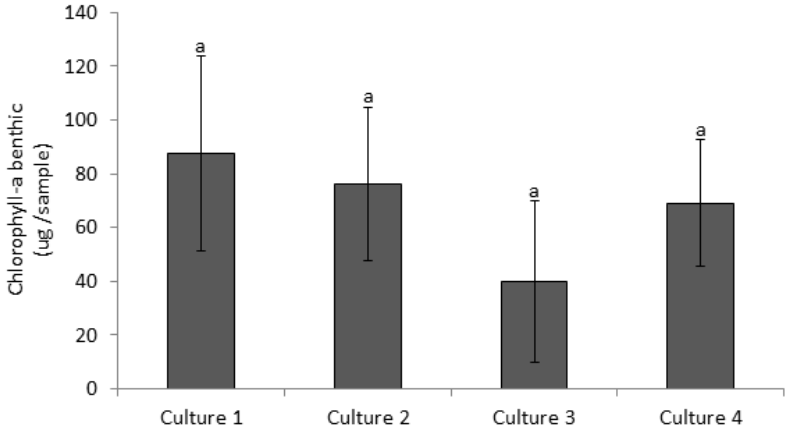

Figure 3. Contents of chlorophyll-a of the built mat from cultures 1, 2, 3 and 4. Culture 1 = Phormidium + Leptolyngbya A + Microcoleus; culture 2= Phormidium + Leptolyngbya A; culture 3= Leptolyngbya A + Microcoleus; culture 4= Phormidium + Microcoleus. Values were determined by mean (n=3) of the total biomass. Coefficient of variation (%) = 43.94. Letters indicate ANOVA groupings at $p<0.05$.

The content of chlorophyll also did not show significant difference ($p = 0.51$) when we added Nostoc or the second morphotype of Leptolyngya (Leptolyngbya B) to the core culture mix (Fig. 4).

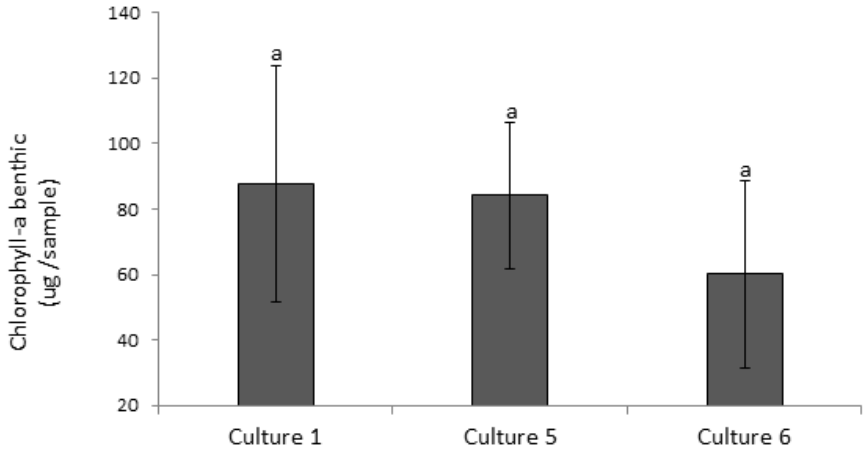

Figure 4. Contents of chlorophyll-a of the built mat from cultures 1, 5 and 6. Culture 1 = Phormidium + Leptolyngbya A + Microcoleus; Culture 5= Phormidium + Leptolyngbya A + Microcoleus + Nostoc; Culture 6= Phormidium + Leptolyngbya A + Microcoleus + Leptolyngbya B. Values were determined by mean (n=3) of the total biomass. Coefficient of variation (%) = 38.41. Letters indicate ANOVA groupings at $p<0.05$.

Despite the content of chlorophyll-a not showing statistically different values between different combinations of isolates, it showed significant variation ($p = 0.04$) between individual strain cultures (Fig. 5). The higher values of chlorophyll content were detected in the Phormidium culture 9, while the lowest values were observed in the culture 11 made from Nostoc.

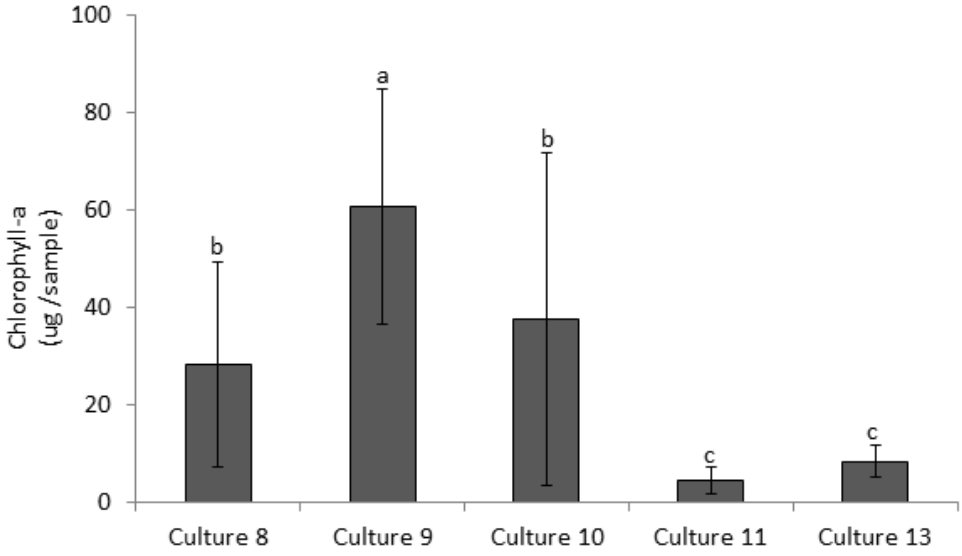

Figure 5. Contents of chlorophyll a of the built mat from cultures 8-13. Culture 8 = Leptolyngbya A; culture 9= Phormidium; culture 10= Microcoleus; Culture 11= Nostoc; Culture 13= Leptolyngbya B. Values were determined by mean (n=3) of the total biomass. Coefficient of variation (%) = 65.66. Letters indicate ANOVA groupings at $p<0.05$.

3.3 EPS production

The contents of extracellular polysaccharide did not differ significantly between the combination of isolates ($p = 0.29$). Although, the EPS content displayed a reduction when the morphotype Phormidium was taken out from the culture 3 and an increase was observed in the culture 4 in the presence of morphotype Phormidium and Microcoleus (Fig. 6)

As chlorophyll content, the values of EPS content were not significantly differentiated ($p = 0.17$) in the presence of Nostoc (culture 5) and Leptolyngbya B (culture 6) (Fig.7).

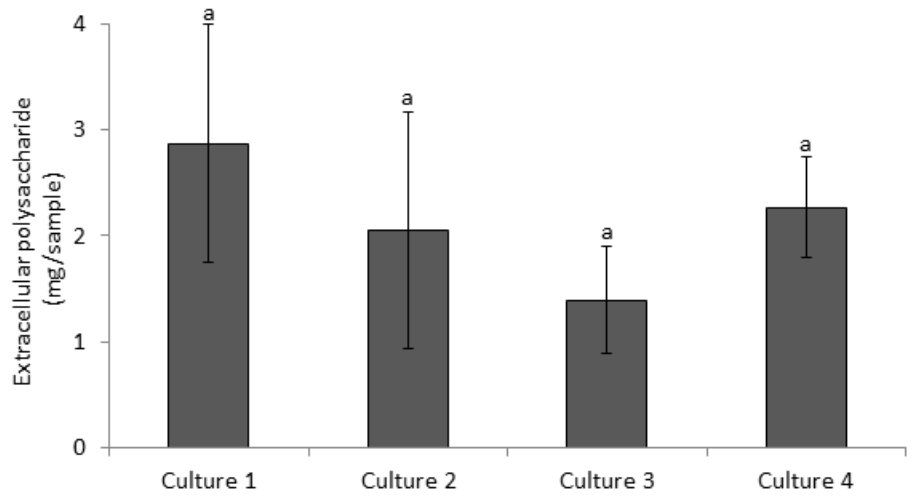

Figure 6. Contents of EPS of the built mat from cultures 1, 2, 3 and 4. Culture 1 = Phormidium + Leptolyngbya A + Microcoleus; culture 2= Phormidium + Leptolyngbya A; culture 3= Leptolyngbya A + Microcoleus; culture 4= Phormidium + Microcoleus. Values were determined by mean (n=3) of the total biomass. Coefficient of variation (%) = 40.43. Letters indicate ANOVA groupings at $p<0.05$.

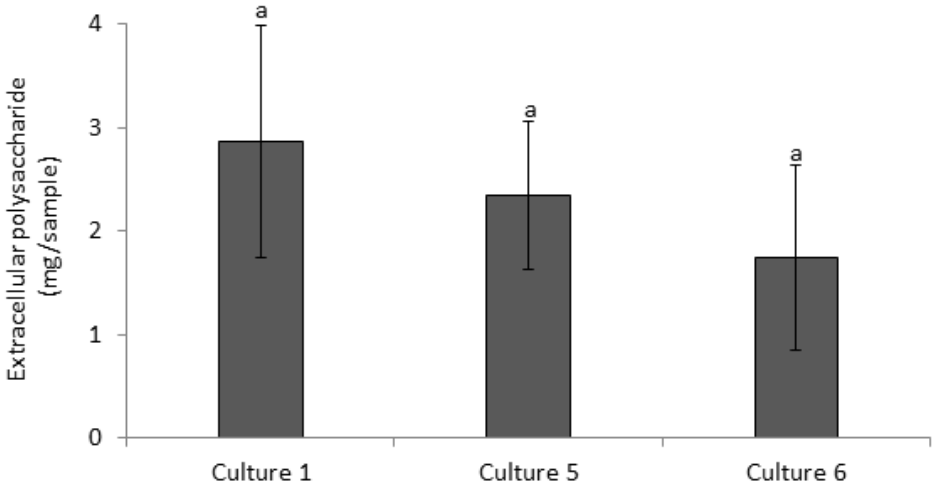

Figure 7. Contents of EPS of the built mat from cultures 1, 5 and 6. Culture 1 = Phormidium + Leptolyngbya A + Microcoleus; Culture 5= Phormidium + Leptolyngbya A +Microcoleus+ Nostoc; CYN 72; Culture 6= Phormidium + Leptolyngbya A + Microcoleus + Leptolyngbya B. Values were determined by mean (n=3) of the total biomass. Coefficient of variation (%) = 31.94. Letters indicate ANOVA groupings at $p<0.05$.

In terms of individual cultures, the contents of EPS were statistically different ($p = 0,0008$). The significantly higher values were found in culture 9 which comprised the Phormidium morphotype while culture 8, 10, 11 and 13 all showed lower values (Fig. 8).

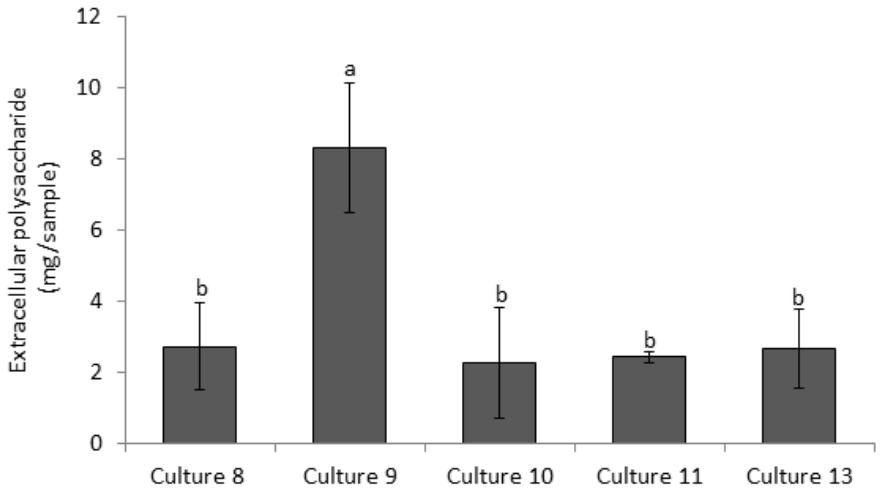

Figure 8. Contents of EPS of the built mat from cultures 8-13. Culture 8 = Leptolyngbya A; culture 9= Phormidium; culture 10= Microcoleus; Culture 11= Nostoc; Culture 13= Leptolyngbya B. Values were determined by mean (n=3) of the total biomass. Coefficient of variation (%) = 35.34. Letters indicate ANOVA groupings at $p<0.05$.

3.4 Organic matter production

When we compared cultures 1, 2, 3 and 4, the production of organic matter was not significantly differentiated at any combination of isolates ($p = 0.28$) (Fig. 9). The same was observed ($p=0.33$) when the morphotypes Nostoc and Leptolyngbya B were added to the core culture mix (culture 1) (Fig. 10) .

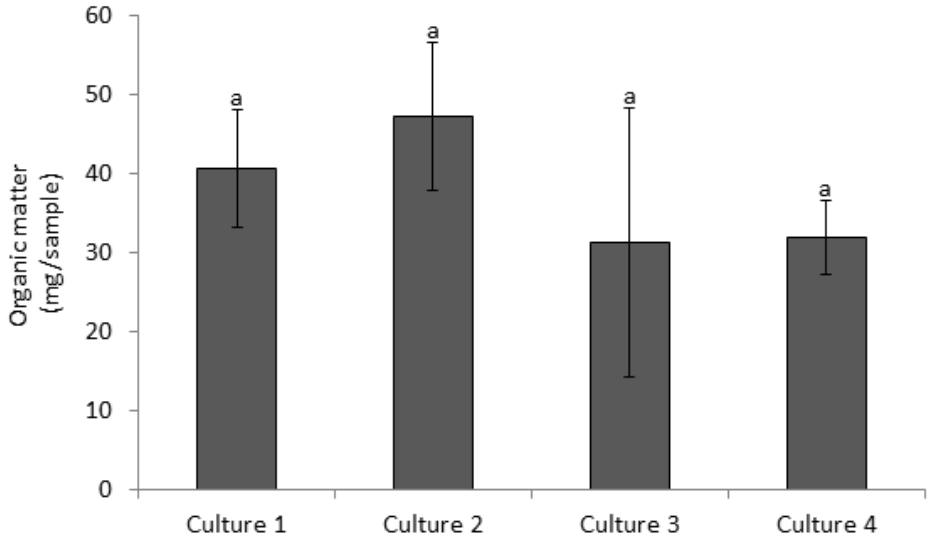

Figure 9. Contents of organic matter of the built mat from cultures 1, 2, 3 and 4. Culture 1 = Phormidium + Leptolyngbya A + Microcoleus; culture 2= Phormidium + Leptolyngbya A; culture 3= Leptolyngbya A + Microcoleus; culture 4= Phormidium + Microcoleus. Values were determined by mean (n=3) of the total biomass. Coefficient of variation (%) = 28.17.

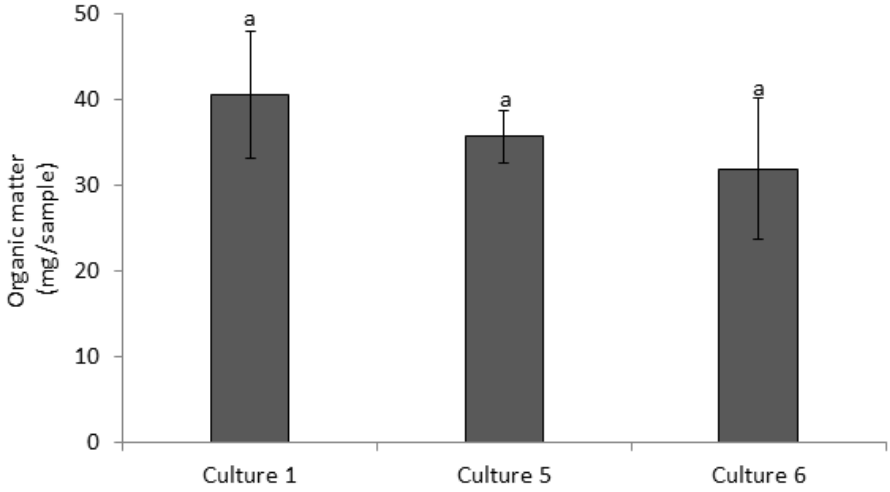

Figure 10. Content of organic matter of the built mat from cultures 1, 5 and 6. Culture 1 = Phormidium + Leptolyngbya A + Microcoleus; Culture 5= Phormidium + Leptolyngbya A +Microcoleus+ Nostoc; Culture 6= Phormidium + Leptolyngbya A + Microcoleus + Leptolyngbya B. Values were determined by mean (n=3) of the total biomass. Coefficient of variation (%) = 18.77.

The production of organic matter by individual cultures also did not show statistical significance from each other (p = 0.19). Highest values of organic matter content were, however, detected in the culture 9 and lower values to culture 13 (Fig. 11).

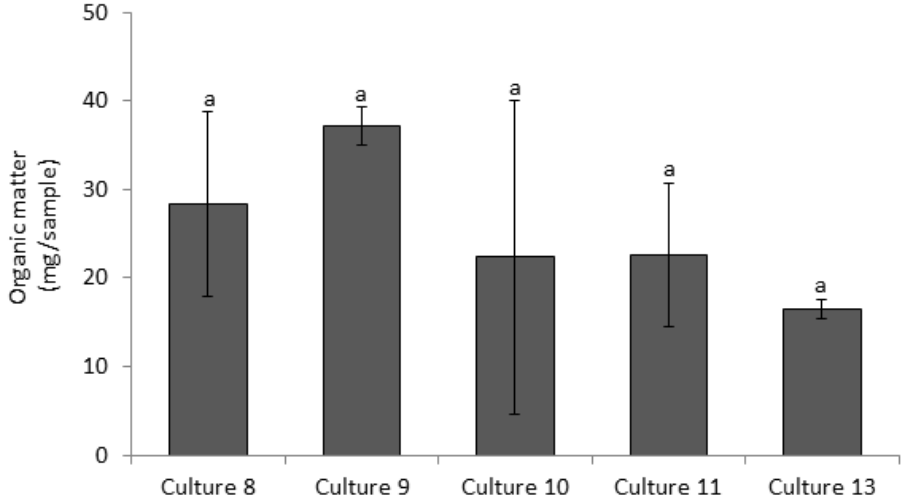

Figure 11. Figure 10. Content of organic matter of the built mat from cultures 8-13. Culture 8 = Leptolyngbya A; culture 9= Phormidium; culture 10= Microcoleus; Culture 11= Nostoc; Culture 13= Leptolyngbya B. Values were determined by mean (n=3) of the total biomass. Coefficient of variation (%) = 39.07.

4. Discussion

At the end of our experiment, it was clear both visually and through quantitative analysis that the mats that had developed as a result of the various mixes of isolates showed variety in morphology and absolute growth, which could to a large extent to be associated to the mix of cultures used (Fig. 1).

Although the mats growth was evaluated under controlled light and temperature, the cultivation condition is still an important concern which must be carefully considered in the judgement of the mats developments. The optimal culture condition for Antarctic microorganisms has not been fully understood yet, and is not likely to be the same for all strains. Cultivation conditions could be affecting not only the mat growth but also the outcome of the interactions, since these microorganisms may require different growth conditions. Our results are thus specific to the growth conditions and different outcomes may occur under other, or natural, conditions

In our experiment, for example, we have used sifted sand samples to standardize the sand grains, however, features of sediments may also act on the mat formation. Such features as mineral composition, grain size, and shape may cause implication on the development and mat shape. Large sizes of sediment grains result in larger interstitial pores and a deeper penetration of light (Decho et al., 2003). Also, chemical features of the sediment such as presence of humic organic matter may play implications on the culture medium and consequently on the mat formation. Additionally, the presence of heterotrophic bacteria attached on the morphospecie trichome (once we did not work with axenic strains) or from the environment can play negative effects on cyanobacterial growth. Heterotrophic bacteria may take advantage of the favorable conditions as available nutrient in the culture medium, reaching high cell numbers and then compete for space and nutrient, as observed by Svercel et al. (2011).

We have analyzed the content of chlorophyll a and organic matter as a parameter of assessment of the mat productivity. As well as, the EPS content, which is a good character to evaluate the potential of the mat-forming strains, once the growth of a biofilm is directly related to attachment supported by the production of EPS (Vu et al., 2009). EPS has been reported to act as intercellular “cement” during biofilm formation (Rickard et al., 2003). Although we have not found statistical difference in terms of chlorophyll-a, EPS and organic matter production between the different combinations of isolates, the results showed that there

is a trend of variation relating to presence and/or absence of the morphotype Phormidium (Phormidium cf. atumnale). The absence of the strains Phormidium tended to lead to a reduction of this pigment in mixed cultures (Fig. 3). Additionally, when we evaluated the production of chlorophyll in individual cultures, Phormidium strain showed the best values of chlorophyll content.

We observed the same trend of data when we estimated the effects of the presence and/or absence of the isolates on the production of EPS and organic matter. A decrease of EPS content and organic matter production was detected in the absence of Phormidium and in the individual strain cultures Phormidium showed the greatest EPS production. An isolate of Phormidium was indicated as a good candidate for production of EPS by Nicolaus et al. (1999) as well.

No significant difference in the chlorophyll, EPS and organic matter content was found when strains Nostoc and Leptolyngbya B were added respectively to culture 1 (Figs 4, 7 and 10). This was unexpected, as a strain belonging to the genus Nostoc has been often considerate as a potential source of exopolysaccharide (De Philippis et al., 2000; Tamaru et al., 2005; Yu, 2011) and thin cyanobacterial filaments (mainly Leptolyngbya sp.) is reported to form a dense network among the sediments and among other cyanobacterial filaments (Ríos et al., 2004), which is favorable to mat establishment.

The data provide no clear evidence that increased species diversity in mats increases biomass, chlorophyll or EPS production. Indeed, neither species compostion or species diversity appeared to strongly influence production of organic material or EPS in cultures.

In the present study, all strains and consequently mat formation were compared under the same cultivation conditions, where these were approximated to the Antarctic regime in terms of light period (24 hours) and temperature (summer season). While the variabilities in the mat development found in our experiment do not necessarily mimic the real life, the results showed that different morphospecies display different performance on the mat building processes. However, it is clear that a variety of Antarctic isolates can form mats, and that while some perform better than others, there was no strong evidence that mixed cultures performed better than the best single isolates, and that to a large extent different strains were able to "substitute" for each other in forming a mat.

From the five strains studied, Phormidium (Phormdium cf. autumnale) showed the best performance and important component to the mat building development, displaying greater production of EPS, organic matter and Chl-a contents. The presence of the

morphospecies belonging to the genus Phormidium (especially Phormidium autumnale) has been documented in many studies that focus on the Antarctic microflora from the vast range of Antarctic habitats (Komárek, 1999; Mataloni et al., 2000; Tanton et al., 2003; Jungblut et al., 2005). Furthermore, Phormidium sp. has been often considerate as a microorganism dominant in field microbial mats (Komárek, 1999). These findings in addition to our results might suggest that Phormidium is an important component not only to mat formation but also to the total Antarctic biomass.

5. Concluding remarks

Taken together our results suggested that there are clearly some challenges in growing mats under artificial conditions. Although we do not have yet a full enough understanding of the role and/or effect of each morphospecie on the mat formation, it may turn out from future works. Therefore, from the present experiment some concern can be addressed to improve next steps:

1. A previous analysis of the sediment composition used to set up the experiment will be important to obtain more information and control about the influence of the substratum on the mat building;

2. Implementation of aeration system may also bring an improvement to the cultivation conditions which could allow better development of mat;

3. Increase the characterization of the mat formation including more biochemical analyses;

4. Finally, a larger number of replicate would be essential to assess the variability of the mat development and additionally to dilute the effects of the experiment manipulation.

Implications

The study on EPS, chlorophyll, organic matter production and consequently culture combination evaluated in this work provide not only further information on cyanobacteria mat formation but it is also useful for other implications, such as biotechnology processes. There is an increasing interest in selection of mat-forming strains, mainly due to the wide composition and diversity of extracellular expolysaccharide that these microorganisms produce. Cyanobacteria have been showed as a potential to industrial sector application, as food, feed, agriculture purposes, biofuel production, pharmaceutical and bioremediation.

Therefore, research that focuses on understanding the processes of microbial mat formation and its production is also a good deal for the application of these in biotechnology.

6. References

Allison, D.G., Ruiz, B., SanJose, C., Jaspe, A. & Gilbert, P. 1998. Extracellular products as mediators of the formation and detachment of Pseudomonas fluorescens biofilms. FEMS Microbiology Letters 167: 179-184.

Andersen, D. T., Sumner, D. Y., Hawes, I., Webster-Brown, J. & Mckay, C. P. 2011. Discovery of large conical stromatolites in Lake Untersee, Antarctica. Geobiology 9: 280-293.

Bolch, C. J. S.& S. I. Blackburn, 1996. Isolation and purification of Australian isolates of the toxic cyanobacterium Microcystis aeruginosa. Journal of Applied Phycology 8: 5-13.

De Phillipis, R. & Vincenzini, M. 1998. Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiology Reviews 22: 151-175.

De Philippis, R. Ena, A., Paperi. R, Sili, C. & Vincenzini, M. 2000. Assessment of the potential of Nostoc strains from the Pasteur Culture Collection for the production of polysaccharides of applied interest. Journal of Applied Phycology 12: 401-407.

Decho, A.W. 2000. Exopolymer microdomains as a structuring agent for heterogeneity within microbial biofilms, In: R.E. Riding and S.M. Awramik (eds.) Microbial Sediments. Springer, Berlin, 9-15 pp.

Decho, A. W., Kawaguchi, T., Allison, M. A., Louchard, E. M., Reid, R. P, Stephens, F. C., Voss, K. J., Wheatcroft, R. A. & Taylor. B. B. 2003. Sediment properties influencing upwelling spectral reflectance signatures: The "biofilm gel effect. Limnology and Oceanography 48: 431-443.

Decho, A. W., Visscher, P. T. & Reid, R. P. 2005. Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. Palaeogeography, Palaeoclimatology, Palaeoecology 219: 71-86.

Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A. & Smith, F. 1956 Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry 28: 350-356.

Ferreira, D. F. Sisvar - sistema de análise de variância para dados balanceados. Lavras: UFLA, 1998. 19p.

García-Meza, J.V.; Barrangue, C. & Admiraal, W. 2005. Biofilm formation by algae as a mechanism for surviving on mine tailings. Environmental Toxicology and Chemistry 24: 573-581.

Huang, W.J., Lai, C.H. & Cheng, Y.L. 2007. Evaluation of extracellular products and mutagenicity in cyanobacteria cultures separated from a eutrophic reservoir. Science of the Total Environment 377: 214-223.

Jungblut, A.D., Hawes I., Mountfort, D., Hitzfeld, B., Dietrich, D.R., Burns, B.P. & Neilan, B.A. 2005. Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environmental Microbiology 7: 519-529.

Jungblut, A, D., Lovejoy, C. & Vincent, W. 2010. Global distribution of cyanobacterial ecotypes in the cold biosphere. ISME - International Society for Microbial Ecology Journal 4: 191-202.

Komárek J. 1999. Diversity of cyanoprokaryotes (cyanobacteria) of King George Island, maritime Antarctica - a survey. Archives of Microbiology 94: 181-193.

Kurtz Jr, H.D., Cox, R. & Reisch, C. 2005. A microcosm system for the study of cryptoendolithic microbial biofilms from desert ecosystems. Biofilms 2: 145-152.

Li, P., Liu, Z. & Xu, R. 2001. Chemical characterisation of the released polysaccharide from the cyanobacterium Aphanothece halophytica GR02. Jornaul Applied Phycolology 13: 71-77.

Little, A. E. F, Robinson, C.J, Peterson SB, Raffa K.F., Handelsman, J. 2008. Rules of engagement: interspecies interactions that regulate microbial communities. Annual Review of Microbiology 62: 375-401.

Ludwig, R., Pringault, O., De Wit, R., De Beer, D. & Jonkers, H.M. 2006. Limitation of oxygenic photosynthesis and oxygen consumption by phosphate and organic nitrogen in a hypersaline microbial mat: a microsensor study. FEMS Microbiology, Ecology 57: 9-17.

Macedo, M. F., Miller, A. Z., Dionísio, A. & Saiz-Jimenez, C. 2009. Biodiversity of cyanobacteria and green algae on monuments in the Mediterranean Basin: an overview Microbiology 155: 3476-3490.

Martineau, E.; Wood, S.A.; Miller, M.R.; Jungblut, A. D.; Hawes, I.; Webster-Brown, J. & Packer, M.A. 2013. Characterisation of Antartic cyanobacteria and comparison with New Zealand strains. Hydrobiologia 711: 139-154.

Mataloni, G., Tell, G. & Wynn-Williams, D.D. 2000. Structure and diversity of soil algal communities from Cierva Point (Antarctic Peninsula). Polar Biology 23: 205-211.

Mitri, S., & Foster, K. R. 2003. The Genotypic View of Social Interactions in Microbial Communities. Annual Review of Genetics 47: 247-273.

Nicolaus, B., Panico, A. , Lama, L., Romano, I., Manca, M.C., De Giulio, A. & Gambacorta, A. 1999. Chemical composition and production of exopolysaccharides from representative members of heterocystous and non-heterocystous cyanobacteria. Phytochemistry 52: 639-647.

Otero, A. &Vincenzini, M. 2003. Extracellular polysaccharide synthesis by Nostoc strains as affected by N source and light intensity. Journal of Biotechnology 102: 143-152.

Pearl, H. W. & Pinckney, J. L. 1996. A mini-review of microbial consortia:Their roles in aquatic production and biogeochemical cycling. Microbial Ecology 31: 225-247.

Pereira, S., Zille, A., Micheletti, E., Moradas-Ferreira, P., De Philippis, R. & Tamagnini, P. 2009. Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiology Reviews 33: 917-941.

Pizarro, H.; Allende, L. & Bonaventura, S. M. 2004. Littoral epilithon of lentic water bodies at Hope Bay, Antarctic Peninsula: biomass variables in relation to environmental conditions. Hydrobiologia 529: 237-250.

Quesada, A.; Fernández-Valiente, E.; Hawes, I. & Howard-Williams, C. 2008. Benthic primary production in polar lakes and rivers. In Polar Lakes and Rivers - Arctic and Antarctic Aquatic Ecosystems. (eds) Vincent,W.F. & Laybourn-Parry J. Oxford University Press, Oxford, UK, pp. 179-196.

Quesada, A. & Vincent, W. F. 2012. Cyanobacteria in the cryosphere: snow, ice and extreme cold, in Ecology of Cyanobacteria II: Their Diversity in Space and Time (ed. B.A. Whitton), Springer, Dordrecht, 387-399 pp.

Reid, R. P.; Visscher, P. T.; Decho, A.W.; Stolz, J. F.; Bebout, B. M.; Dupraz, C.; Macintyre, H. W.; Paerl, H. W.; Pinckney, J. L.; Prufert-Bebout, L.; Steppe, T. F. & DesMarais, D. J. 2000. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. Nature 406: 989-992.

Rickard, A. H.; Gilbert, P.; High, N. J.; Kolenbrander, P. E. & Handley, P. S. 2003. Bacterial coaggregation: an integral process in the development of multi-species biofilms. Trends in Microbiology 11: 94-100.

Ríos, A, Ascaso,C., Wierzchos,J., Fernández-Valiente, E. & Quesada, A. 2004. Microstructural Characterization of Cyanobacterial Mats from the McMurdo Ice Shelf, Antarctica. Applied and Environmental Microbiology 70: 569-580.

Schink B. 2002. Synergistic interactions in the microbial world. Antonie Van Leeuwenhoek 81: 257-261.

Stal, L.J. 2000. Cyanobacterial mats and stromatolites.The Ecology of Cyanobacteria: Their Diversity in Time and Space (Whitton BA & Potts M, eds), pp. 61-120. Kluwer Academic Press, Dordrecht.

Su, C., Zhenming, C. & Weidong, Lu. 2007. Optimization of medium and cultivation conditions for enhanced exopolysaccharide yield by marine Cyanothece sp. 113. Chinese Journal of Oceanology and Limnology 25: 411-417.

Sutherland, I. W. 2001. Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147: 3-9.

Sutherland, D. L. & Hawes, I. 2008. Annual growth layers as proxies of past growth conditions for benthic microbial mats in a perennially ice-covered Antarctic lake. FEMS Microbiology, Ecology 67: 279-292.

Tamaru, Y., Takani, Y., Yoshida, T. & Sakamoto, T. 2005. Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium Nostoc commune. Applied and Environmental Microbiology 71: 7327-7333.

Svercel, M., Saladin, B., van Moorsel, S.J., Wolf, S. & Bagheri, H. C. 2011. Antagonistic interactions between filamentous heterotrophs and the cyanobacterium Nostoc muscorum. BMC Research Notes, 4:357 < http://www.biomedcentral.com/1756-0500/4/357>.

Velicer, G.J. 2003. Social strife in the microbial world. Trends in Microbiology 11: 330-337.

Vincent W.F. 2000. Cyanobacterial dominance in the polar regions, p 321–340. In Whitton, B. A & Potts, M, (eds), The ecology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Vu, B., Chen, M. Crawford, R.J. & Ivanova, E. P. 2009. Bacterial Extracellular Polysaccharides Involved in Biofilm Formation. Molecules 14: 2535-2554.

Yallop, M.L., Paterson, D.M, Wellsbury, P. 2000. Interrelationships between Rates of Microbial Production, Exopolymer Production, Microbial Biomass, and Sediment Stability in Biofilms of Intertidal Sediments. Microbial Ecology 39: 116-127.

Yu, H. 2011. Effect of mixed carbon substrate on exopolysaccharide production of cyanobacterium Nostoc flagelliforme in mixotrophic cultures. Journal of Applied Phycology 24: 669-673.

Wynn-Williams, D.D. 1996. Antarctic microbial diversity: the basis of polar ecosystem process. Biodiversity and Conservation 5: 1271-1293.

Wood, S.A., Rueckert, A., Cowan, D. A. and Cary, S.C. 2008. Sources of edaphic cyanobacterial diversity in the Dry Valleys of Eastern Antarctica. ISME - International Society for Microbial Ecology Journal 2: 308-320.

CONCLUSIONS AND PERSPECTIVES

The resuts presented in this thesis, provided further insight into cyanobacteria identities, diversity and distribution along Antarctic sub-regions.

A wide range of limnological information from coastal and inland ponds is also available through this work, including ponds never previously sampled.

The results showed that the community composition of cyanobacteria in the microbial mat changes with the sites locations and that there is a clear gradient from coastal ponds to inland ponds linked mainly to nitrogen and phosphorus concentrations.

The relative roles of deterministic and stochastic processes can be acting together in determining the distribution and composition of cyanobacterial assemblages in Antarctic systems.

Different morphospecies presented different performance on the mat building processes. Phormidium (Phormidium cf. autumnale) showed greater contribution to mat formation, displaying higher production of EPS and organic matter and Chl-a contents when compared to the other four studied strains.

Many issues still need to be clarified in terms of cyanobacteria taxonomy and its ecological preference. The strengths and limitations of this thesis were considered and suggestions for further research into higher characterization are presented in this work.

These results may be helpful for design positive measures for preserving local and/or regional biodiversity of Antarctica and, predict likely threats to biodiversity, as well as, optimize the values of these Antarctic ecosystems.