RESEARCH ARTICLE

The Pyramid Trough Wetland: environmental and biological diversity in a newly created Antarctic protected area

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Abstract

The Pyramid Trough (Lat 78°S) has recently gained protection under the Antarctic Treaty system, owing to its wetland values. Here, we describe the microbial diversity of this system, with emphasis on cyanobacteria, and evaluate environment–biota relationships. Geochemistry separates ponds along hydrological gradients receiving recent inflows of dilute meltwater, from a second group that is rarely inundated and where chemistry is dominated by evaporation. Cyanobacteria-based microbial mats dominated the biota throughout. Mats were characterized by light-microscopy, pigment analysis, automated ribosomal intergenic spacer analysis and 16S rRNA gene clone libraries. A total of 17 morphotypes and 21 ribotypes were identified, mostly *Oscillatoriales* and several taxa that are usually rare in continental Antarctica, including *Chroococcales* and scytomin-rich *Calothrix/Dichothrix*, were abundant. There was a general decline in cyanobacterial diversity with increasing conductivity, but weak support for either differences in community composition between the two groups of ponds or sorting of taxa along the hydrological gradients with the pond groups. This implies a broad environmental tolerance and a prevalence of neutral assembly mechanisms in cyanobacterial communities of Antarctic wetland ecosystems.

Introduction

Continental Antarctica is a cold desert, and there are severe limitations on where and when liquid water can occur. Water bodies are rare, and all are ice bound or fully frozen in winter and many shallow surface water features, such as streams and ponds, are liquid for only a few weeks or months each summer. Water balances of such systems are acutely vulnerable to climate. During autumn, winter and spring, they are frozen and lose water via ablation, and they are dependent on warm temperatures and continuous insolation in summer to generate sufficient melt to replenish these losses (McKnight *et al.*, 1999; Howard-Williams & Hawes, 2007). Climatic variability, which sets the balance between melting and freezing, results in substantial differences in water balance over time (Fountain *et al.*, 1999; Doran *et al.*, 2002; Howard-Williams *et al.*, 2010).

Despite the precarious climatic regime that governs their existence, inland aquatic systems are distributed widely in the ice-free coastal regions of continental Antarctica. Although rare on an areal basis, they can be foci of biomass, productivity and diversity in otherwise barren landscapes (Vincent & James, 1996; Quesada *et al.*, 2008). Life in these water bodies is essentially microbial, and where substrate stability permits, streams can develop complex and extensive microbial accumulations dominated by Cyanobacteria and eukaryotic algae (Vincent, 2000). Ponds are also typically dominated by conspicuous microbial mats that can reach very high biomass, completely covering the bases of ponds (Hawes *et al.*, 1993; Vincent & James, 1996) with likely net export of organic carbon to terrestrial ecosystems (e.g. Moorhead *et al.*, 2003; Moorhead, 2007; Hawes *et al.*, 2008).

Codes of conduct for living and working in the Antarctic Dry Valleys implemented by national Antarctic agencies recognize the biological significance of surface water features and advocate minimal disturbance to these systems. Beyond this, specific protection for designated areas

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within the valleys rarely addresses the values and requirements of shallow surface waters and the microbial ecosystems that they support. In 2009–2010, this was partially redressed by the creation of a Restricted Zone in the Pyramid Trough in the McMurdo Dry Valleys in Southern Victoria Land in recognition of its particular abundance and variety of surface water features and microbial communities.

Our goal in this investigation was to assess the biological and environmental diversity of the Pyramid Trough wetland. This region is a polar wetland, within which we expect that habitat variability over small spatial scales will be largely driven by hydrological variability, and according to wetland paradigms, we hypothesize biological diversity along hydrologically induced environmental gradients. A competing hypothesis is that the physical and dispersal limitations to the pool of species within continental Antarctica will have led to sufficient ecological equivalence that neutral rather than niche-driven processes dominate community assembly (Rosindell *et al.*, 2011). We focus on the conspicuous cyanobacterial mats that dominate biomass in many elements of the Pyramid Trough wetland and are widely recognized as characteristic of Antarctic inland waters (Vincent, 2000).

Materials and methods

Study site and sampling

The Pyramid Trough (78°18′S, 163°15′E) is a steep-sided valley bounded by the Koettlitz Glacier, the Royal Society Mountains and the Bulwark (Fig. 1a and b). A stream, the Upper Alph River, originating at the glacier runs down the valley and spreads out over the flat valley floor as a series of stream channels, seeps and wet soil. On the northern side of the stream are a number of ponds of varying size and, at the time of our sampling, ice covered. Stream channels suggest that several of these ponds are connected to the stream system at various states of flow, but at the time of sampling the stream flowed only into Cleopatra Pond (all pond names are unofficial), and from there into the proglacial Trough Lake. From Trough Lake, which was not sampled, water enters the main Alph River and flows down to Walcott Bay (Howard-Williams *et al.*, 1986).

We selected six of the ponds for sampling, aiming to cover a spread of size and apparent microbial diversity. Ponds were variable in shape, ranging from 10 to > 100 m in maximum linear dimension. These are identified in Fig. 1b and were sampled on the 12 January 2010. On the northern side of the Pyramid Trough, there are a number of perched valleys, which have their own drainage systems. Streams from these valleys appear to have flowed at some times down into ponds on the valley floor, although at the time of sampling this was not the case. Two ponds in one of these perched valleys were sampled on 15 January 2010.

Ponds were either entirely or partially thawed, allowing samples of pond water and microbial mat to be taken from the pond edges. Temperature, pH and conductivity were then measured *in situ* using a freshly calibrated HACH HQ40d meter. The conductivity probe was lowered in the water column to ascertain the presence of chemical stratification, using an extendable pole to lower the probe through the deepest profile of the pond. Stratification was observed in only one pond, Tut Pond. Water

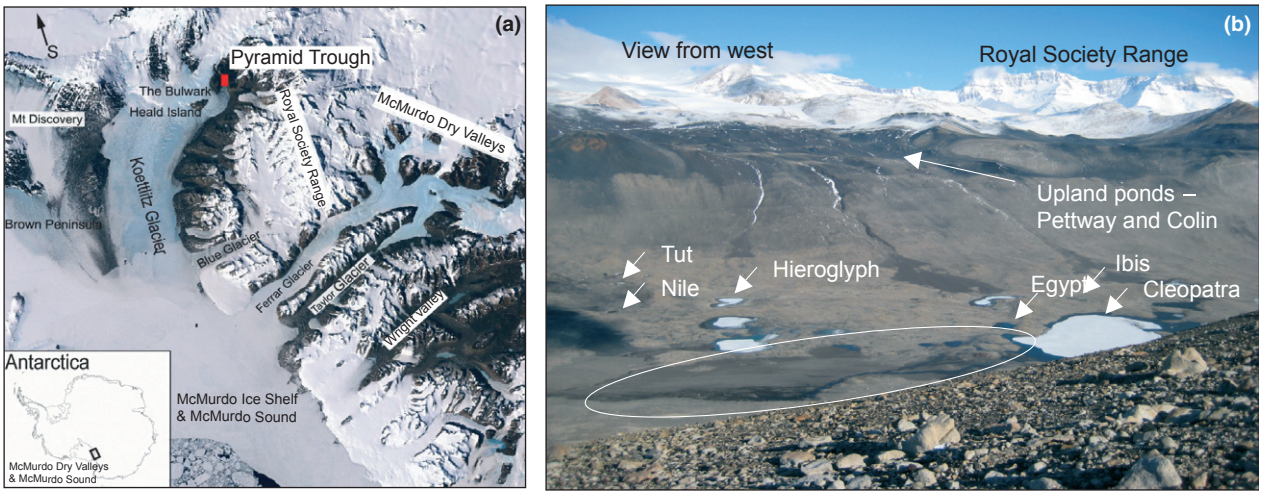

Fig. 1. (a) Location of Pyramid Trough (78°N, red square) in the McMurdo Dry Valley Region, Antarctica based on Landsat Image Mosaic of Antarctic (LIMA, USGS); (b) image acquired 12 January 2009 showing the ponds Nile, Tut, Egypt, Ibis, Cleopatra, Hieroglyph, Pettway and Collins in Pyramid Trough, Antarctica. The white circled area is a series of stream channels, seeps and wet soil.

samples were then taken and analysed for dissolved inorganic and organic carbon, major ions and nutrients, using methods described by Webster-Brown *et al.* (2010) (see Supporting information, Data S1). To assist with interpretation of chemical data, we also collected approximately 1 L of Koettlitz Glacier ice into clean plastic bags, subsequently melted in an acid-rinsed HDPE tray for later analysis of nutrients and major ions.

Macroscopic morphology of the cyanobacterial mats was noted where visible. Microbial mats were collected from near the edge of the ponds, using sterile equipment and gloves, into, sterile Falcon tubes (15 mL) and frozen for latter molecular and morphological analysis. Samples were taken and wrapped in foil and frozen for pigment analysis. Loss of samples during transport meant that only samples from Pettway, Colin, Egypt Nile and Tut ponds could be analysed. Finally, samples of salt precipitates from the immediate margins of the ponds were taken and returned to New Zealand for identification.

Identification, enumeration and benthic community structure analysis

The dominant cyanobacterial genera in microbial mats or sediment samples were identified and cells measured using an Olympus light microscope (BX51, Olympus) at 400–1000× magnification. Taxa were assigned based on Komárek & Anagnostidis (1989, 2000, 2005).

Pigments for high-performance liquid chromatography (HPLC) analysis of pigments were extracted from freeze-dried material by grinding in 95% acetone. A Dionex HPLC system, with PDA-100 diode array detector (300–800 nm), separated pigments according to the chromatographic method of (Zapata *et al.*, 2000). Full details of the HPLC methodology are given in Webster-Brown *et al.* (2010).

Automated ribosomal intergenic spacer analysis (ARISA) was used to assess cyanobacterial community structure in benthic samples. DNA was extracted from 100 mg of benthic mat using a MoBio Power SoilTM kit (Carlsbad, CA). ARISA PCR, using cyanobacteria-specific primers, followed the protocol described in Wood *et al.*, (2008). Amplicon lengths were resolved on a Megabase 500 series capillary sequencer (Amersham Pharmacia, Sunnyvale).

Cyanobacterial-specific PCR, cloning, restriction fragment length polymorphism analysis (RFLP) and sequencing

Based on the difference in water chemistry and morphological analysis, five ponds were selected for 16S rRNA gene sequence analysis. PCR amplification of cyanobacterial 16S rRNA gene was performed using 1 unit of DNA Taq polymerase (Bioline, Tauton, MA) in a 20-μL reaction as described in Jungblut *et al.* (2010). PCR products were purified (Quiagen, Germany) and cloned using the Stragegene clone kit (Stragagene, Cedar Creek, TX) following the manufactures instructions. Correct-sized amplicons for the samples Egypt, Cleopatra and Pettway were subjected to RFLP screening using the enzymes AluI and HpaII as described in Jungblut *et al.* (2010). At least two clones for each unique RFLP pattern were sequenced. For Tut and Ibis, correct-sized amplicons were directly sequenced without prior RFLP screening. Sequencing was carried using the vector-specific T7 universal forward primer (single read) at the Natural History Museum sequencing facility using Applied biosystems 3730xl DNA analyser (Applied Biosystems, Foster City, CA).

Diversity calculations and phylogenetic analysis

Sequences were checked for chimeras using Bellerophon (Huber *et al.*, 2004), and they were excluded from further analysis. Sequences were edited using 4Peaks (Version 1.7.2) and aligned using Clustal X (version 2.0.9). Manual editing was carried with MacClade 4.08 (Maddison & Maddison, 2002). For each ribotype, a closest match based on a BLASTN search (Altschul *et al.*, 1990) to GenBank was determined and if the closest match was an uncultured clone, the closest isolated strain was also included. Individual ribotypes or operational taxonomic units (OTU) were defined as groups of sequences that were at least 97% similar using DOTUR (Schloss & Handelsman, 2005). Library coverage, Chao1 and Ace nonparametric richness estimates and rarefaction curves were calculated using DOTUR (Schloss & Handelsman, 2005).

Phylogenetic trees were constructed using maximum likelihood with RAXML-HPC2 on TG (7.2.8; CIPRES Science Gateway V 3.0, Stamatakis *et al.*, 2008). The GTR-CAT substitution model was used for the bootstrapping phase and GTRGAMMA for the final tree inference. A best-scoring ML tree was obtained with 1000 bootstraps. The 11-bp insertion (5′ AGTTGTGAAAG-3′, Nadeau *et al.*, 2001) was removed from the alignment for the phylogenetic analysis. One representative for each ribotype from each site was included in the phylogenetic analysis. Reference sequences were from GenBank, and for each ribotype, the closest match based on a BLASTN search (Altschul *et al.*, 1990) of GenBank was selected as a reference sequence. If the closest match was an uncultured clone, the closest isolated strain was also included. For comparison, we also searched for 16S rRNA gene sequences from isolates of the identified morphotypes

and environmental 16S rRNA gene sequence data from other Antarctic and Arctic sites. The 16S rRNA gene sequences are available under GenBank accession numbers JN857976–JN858011.

Statistical analysis of community structure data

High-performance liquid chromatography pigment, ARISA and clone library data were analysed based on Bray-Curtis similarities using the PRIMER 6 software package (PRIMER-E, Ltd, UK). Nonmetric multi-dimensional scaling (MDS) was undertaken with 100 random restarts, and results were plotted in two dimensions. Agglomerative, hierarchical clustering of the Bray-Curtis similarities was undertaken using the CLUSTER function of PRIMER 6. Where appropriate, we used simple linear regression of MDS scores against measured variables to determine which were most closely associated. All regression analysis was undertaken using SIGMASTAT 3.5 (Systat Software Inc.)

Results

Physical and chemical properties

Surface waters of all ponds and the mainstream of the Upper Alph River were alkaline, although there was a considerable range of pH and conductivity amongst the ponds (Table 1). Similarities between Cleopatra Pond and the Upper Alph River are consistent with the stream flowing directly into that pond. With the exception of Tut (up to 8300 μS cm^{-1} in the base of this stratified pond), the highest conductivities were associated with the smaller ponds. There was no apparent relationship between dissolved salt content and pH. Observed precipitated salts were predominantly calcium carbonate, calcium sulphate and sodium sulphate, with minor halite (possibly formed during preparation of the salt for analysis).

Major ion composition varied between ponds (Table 2), and ternary relationships (Fig. 2) indicate Na is the dominant cation in all ponds, and Cl the dominant anion in all except Cleopatra (HCO_3 dominated) and Ibis (Cl-SO_4 dominated). Two axes of variation were evident in anion data that allow separation of the data into two groups. The first group, all of relatively low conductivity, showed an increase in Cl/HCO_3 as conductivity increased, from the Upper Alph River, through Cleopatra, Egypt and to Tut Ponds. Colin Pond fell onto this same anion axis, but did not form part of the conductivity trend. The second group of ponds, made up of Ibis, Hieroglyph, Nile and Pettway, all had a low-proportional HCO_3 and high conductivity (> 2000 μS cm^{-1}) relative to the ponds of group 1, indicating a higher degree of evaporation in their history and likely prior carbonate ion removal. In the cation data, group 2 ponds showed a trend of increasing Cl/SO_4 and Na(+K)/Ca ratios with increasing conductivity. Ion concentrations in the ice of the Koettlitz Glacier were very low, always close to detection limits (and with a relatively high error), and this sample was an outlier to the general relationships.

The existence of two distinct subgroups of ponds was also supported by stable isotope data (Table 2), with group 2 ponds showing greater enrichment in the heavier ^{18}O and deuterium isotopes, consistent with a history of evaporation. Group one ponds showed less effect of evaporation, particularly Cleopatra Pond, consistent with its position as receiving the flow of the Upper Alph River. Group 2 also all had remarkably high NO_3–N concentrations (Table 3), reaching a maximum in Pettway of 76.8 g m^{-3}. Amongst group 2 ponds, NO_3–N correlated closely with chloride (r^2 = 0.99), as did dissolved organic nitrogen (DON) and dissolved organic carbon (DOC), suggesting that all were in part concentrated by evaporation. Of the other nutrients, dissolved reactive and organic phosphorus (DRP and DOP, respectively) were commonly at or below the detection limit of 1 μg L^{-1}, and NH_4–N was variable but not unusually high.

Table 1. Properties of eight ponds in the Pyramid Trough and the Upper Alph River. MLD is maximum linear dimension. Depths are approximate and based on observations from the pond edges. Tut pond was stratified, with a conductivity 8300 μS cm^{-1} at a depth of > 0.8 m

Pond/River	Conductivity (μS cm^{-1})	pH	Temperature (°C)	MLD (m)	Ice cover (%)	Depth (m)
Upper Alph	65	8.22	2.2	–	0	
Cleopatra	115	8.73	6.8	> 100	90	3.0
Colin	201	10.45	3.2	85	95	2.0
Egypt	319	8.59	8.3	55	0	0.8
Tut (surface)	1143	10.05	8.6	14	0	1.0
Hieroglyph	2440	8.65	6	75	80	1.0
Nile	5430	8.47	8.9	22	0	0.6
Ibis	5900	8.35	9.3	33	0	0.4
Pettway	32 700	9.01	4.6	18	0	0.4

Table 2. Concentrations of major ions and stable isotopes in eight ponds and one stream (Upper Alph River) in the Pyramid Trough, together with ice from the Koettlitz Glacier. nd indicates no data, and DL indicated detection limit. All ions are expressed as mmol L^{-1} and isotopes as ‰

Pond	Ca	Mg	K	Na	Cl	SO_4	HCO_3	δD	δO^{18}
Koettlitz ice	0.01	< DL	< DL	0.01	0.01	0.01	nd	−272	−34.7
Upper Alph	0.11	0.02	0.02	0.11	0.04	0.08	0.53	nd	nd
Cleopatra	0.22	0.09	0.07	0.43	0.17	0.17	0.33	−276	−34.5
Egypt	0.22	0.25	0.27	1.78	0.83	0.59	0.46	−226	−24.0
Colin	0.34	0.17	0.08	0.65	0.89	0.33	0.3	−159	−15.9
Tut (surface)	0.22	0.79	0.69	8.7	4.09	1.93	1.09	−176	−16.5
Hieroglyph	3.28	2.71	1.51	10.87	12.3	7.08	0.38	−156	−14.7
Ibis	12.5	6.63	4.28	26.1	25.4	28.1	0.17	−151	−11.1
Nile	6.25	6.50	3.13	26.5	34.0	14.0	2.77	−172	−15.8
Pettway	19.0	47.1	24.4	221	262	56.2	0.13	−109	−5.2

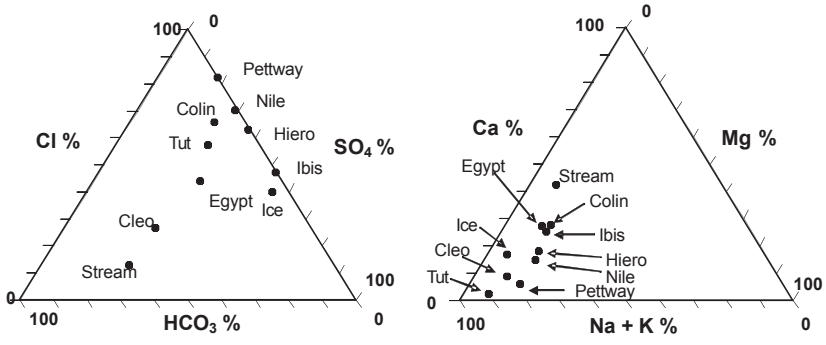

Fig. 2. Ternary diagram's of major ion concentrations as mole% of total anion or cation concentration, where 'Ice' is the Koettlitz Glacier ice, and 'Stream' is the Upper Alph River. Only the surface water for stratified Tut pond is plotted here.

Table 3. Concentrations of major nutrients in eight ponds in the Pyramid Trough

Pond	DRP (mg m^{-3})	NH_4–N (mg m^{-3})	NO_3–N (mg m^{-3})	DOP (mg m^{-3})	DON (mg m^{-3})	DOC (g m^{-3})
Cleopatra	1	45	2	1	69	0.7
Colin	2	20	461	1	135	1.7
Egypt	< 1	< 1	5	1	181	1.9
Tut	2	1	< 1	4	559	4.8
Hieroglyph	< 1	27	10 400	< 1	473	2.3
Nile	3	8	20 000	< 1	692	2.3
Ibis	1	12	15 000	1	1490	4.5
Pettway	2	44	76 800	6	3660	12

Biological diversity

Benthic communities were represented by a variety of encrusting and cohesive microbial mats. Tut, Nile, Heiroglyph, Ibis, Colin and Egypt Ponds were predominantly orange brown on the surface, although lower layers of mats were olive green. These mats were variously developed from thick and continuous (Tut), sometimes with small pinnacles (Egypt) to sparse and mainly between small stones and gravels (Hieroglyph and Ibis). A cohesive brown mat was seen at Cleopatra Pond, although around the edge a black, encrusting mat prevailed. Where benthic samples could be obtained from deeper in ponds, they were invariably thicker and better developed than those from the pond margins. Marginal communities in Tut contained gelatinous balls characteristic of *Nostoc*.

In addition to morphotypes, HPLC and ARISA signatures, as well as rRNA clone libraries provided information on composition of mat communities from the various ponds. Of the 17 morphotypes of *Cyanobacteria* identified from microscopic analysis (Table S1), none occurred in all ponds and only one (*Leptolyngbya* 1–1.2 μm – Fig. S1) in most ponds. Nearly a third occurred in only one pond and half in only one or two.

A total of 396 clones were analysed from five samples: Cleopatra (84 clones), Egypt (68 clones), Tut (77 clones), Ibis (75 clones) and Pettway Ponds (92 clones); 21 OTUs ($\geq$ 97% similarity) were identified, but none of these

ribotypes occurred in all ponds (Table S2 and Fig. 3). Only one ribotype was detected in four of five samples [PTcy10 (99% uncultured Antarctic cyanobacterium clone Fr121 (AY151728); 98% Cyanobacterium cCLA-17 (HQ230228))], which grouped within *Oscillatoriales* cf. *Leptolyngbya*. The remaining ribotypes were found in three or fewer samples, and approximately half were detected at one site.

Amongst the ribotypes, community coverage ranged between 95.2% (Cleopatra) and 98.9% in Pettway (Table S2). Rarefraction curves had not fully reached asymptote and nonparametric statistics richness estimations were slightly higher than the 16S rRNA gene sequence diversity (Fig. S2). The majority of ribotypes were most similar to other Arctic and Antarctic environmental sequences in the genera *Leptolyngbya*, *Phormidium, Oscillatoria*, *Microcoleus*, *Limnothrix* and *Pseudanabaena* spp. (Table 4), which was largely confirmed by phylogenetic analysis (Fig. S3a and b). Oscillatorian diversity is likely higher than reflected by the OTU definition of $\geq$ 97% similarity as sequences combined within PTcy03 (99% uncultured cyanobacterium clone (B91206D); *Phormidium autumnale* SAG 5.90 (EF654081), *Microcoleus vaginatus* CSU-U-KK1 (EF667962) and PTcy12 (99% uncultured bacterium clone ANTLV2_C09; 100% uncultured cyanobacterium clone RD010; 98% *Limnothrix redekei* CCAP 1443/1 (AJ580007*);* 98% *Pseudanabaena* sp. 1tu24s9 (AB039019) clustered in varying position in the phylogenetic tree.

Morphotype and ribotype diversity was highest in the least saline ponds, such that a simple linear regression of richness verses conductivity yielded a significant slopes in both cases ($r^2 > 0.83$, $P < 0.05$). Group 1 (low-conductivity, low-nitrate) ponds, Tut, Egypt and Cleopatra Ponds, had similar, but not identical, clusters of morphotypes, particularly the nitrogen-fixing *Nostoc* and *Dichothrix* (Fig. S1). Consistent with this, three ribotypes grouping within the *Nostocales* were detected only in these ponds. PTcy16 (93% *Calothrix* sp. PCC 7714, AF132779) dominated Cleopatra cyanobacterial 16S rRNA gene assemblages and also grouped closest to a *Calothrix* cluster. PTcy17 [98% *Tolypothrix distorta* SAG 93.79 (GQ287651)] formed a cluster with *Tolypothrix* spp. and *Coleodesmium* sp. ANTL52.B5 (AY493590) and was also only identified in Cleopatra mats. Egypt and Tut had both the ribotype PTcy18 [99% *Nostoc* sp. ANT.L52B.8 (AY493593)] belonging to the genus *Nostoc*.

Three ribotypes grouping within *Chroococcales* were detected and made up < 25% of the total cyanobacterial 16S rRNA gene assemblages (Fig. 3, Table 4). The exception was in Pettway Pond, the most chemically extreme, which had a unique assemblage based on both ribotype and morphotype. Here, PTcy19 (98% uncultured bacterium clone WP3_52 [JN122665), 91% *Synechococcus* sp. UH7 (AF448074)] was the most commonly detected ribotype, while coccoid genera also dominated morphotypes. PTcy19 clustered with several environmental sequences from the Great Salt Plains of Oklahoma (I.R. Caton & M.A. Schneegurt, unpublished data), but a confident assignment on genus was not possible. Nile, Hieroglyph and Ibis, the rest of the group 2 (high nitrate) ponds, shared few OTUs or morphotypes. In the Upper Alph River, *Leptolyngbya* 1–1.2 μm was common, as were morphotypes attributable to *Schizothrix, Oscillatoria, Dichothrix, Gloeocapsa* and *Chamaesiphon*.

Clustering based on HPLC and ARISA fingerprinting grouped samples differently, although both showed that samples from different parts of the same pond could be more disparate than samples from different ponds (Fig. S4). Linear regression showed that scytonemin was

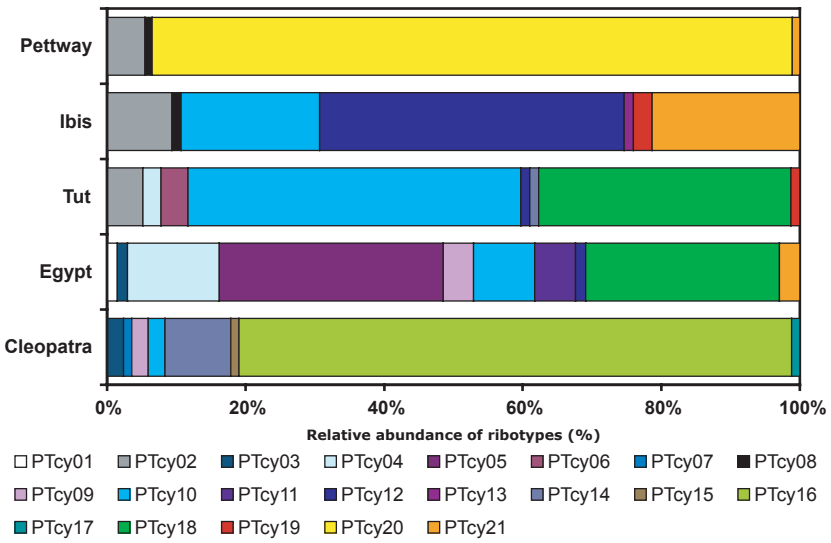

Fig. 3. Relative abundance of ribotypes based on cyanobacterial-specific 16S rRNA gene clone libraries from benthic cyanobacterial communities of Pettway, Ibis, Tut, Egypt and Cleopatra Ponds in Pyramid Trough, Antarctica.

Table 4. Ribotypes defined from the five clone libraries of Pyramid Trough microbial mats

Ribotypes OTUs	Similarity (%)	Accession number	Highest match (Blastn)	Similarity (%)	Accession number	Highest cultured match (BLASTN)
PTcy01	99	AY151722	Uncultured Antarctic cyanobacterium clone BGC-Fr054	99	AY493585	*Phormidium priestleyi* ANT.LPR2.6
PTcy02	99–100	–	–	98–100	AY493589	*Leptolynbya antarctica* ANT.LAC6.1
PTcy03		–	–	99	EF654081	*Phormidium autumnale* SAG 35.90
		99	Uncultured cyanobacterium clone B91206D	99	EF667962	*Microcoleus vaginatus* CSU-U-KK1
PTcy04	99–100	FJ977150	Uncultured cyanobacterium clone WHL72	99	AY493607	*Leptolyngbya antarctica* ANT.L18.1
PTcy05	–	99–100	Uncultured cyanobacterium clone H-B02	98–99	GQ859651	*Phormidium* sp. PMC301.07
PTcy06	–	–	–	99–100	AY493590	*Leptolyngbya antarctica* ANT.BFI.1
PTcy07	99	HQ622720	Uncultured bacterium clone IC4002	98	AY493581	*Phormidium priestleyi* ANT.L66.1
PTcy08	98	HQ327233	Uncultured bacterium clone TP-Snow-96	97	AY493581	*Phormidium priestleyi* ANT.L66.1
PTcy09	100	DQ181681	Uncultured cyanobacterium clone RJ088	97	AY493576	*Leptolyngbya frigida* ANT.L53B.2
PTcy10	99	AY151728	Uncultured Antarctic cyanobacterium clone Fr121	98	HQ230228	Cyanobacterium cCLA-17
PTcy11	100	FJ977133	Uncultured cyanobacterium clone MIS92	99	HQ201392	*Oscillatoria* sp. KNUA009
PTcy12	99	DQ521509	Uncultured bacterium clone ANTLV2_C09	98	AJ580007	*Limnothrix redekei* CCAP 1443/1
	100	DQ181672	Uncultured cyanobacterium clone RD010	98	AB039019	*Pseudanabaena* sp. 1tu24s9
PTcy13	99	DQ181677	Uncultured cyanobacterium clone RD107	93	AB039019	*Pseudanabaena* PCC7403
PTcy14	99	HQ189035	Uncultured cyanobacterium clone B108204C	98	HQ230229	Cyanobacterium cCLB-2
PTcy15	99	DQ181675	Uncultured cyanobacterium clone RD069	91/92	FJ933259	*Leptolyngbya* sp. FYG
PTcy16	97	EU753646	Uncultured cyanobacterium clone D1E09	93	AJ133164	*Calothrix* sp. PCC 7714
PTcy17	–	–	–	98	GQ287651	*Tolypothrix distorta* SAG 93.79
PTcy18	–	–	–	99	AY493593	*Nostoc* sp. ANT.L52B.8
PTcy19	99	DQ181740	Uncultured cyanobacterium clone H-A07	97	AF448080	*Synechococcus* sp. PCC 7502
PTcy20	98	JN122665	Uncultured bacterium clone WP3_52	91	AF448074	*Synechococcus* sp. UH7
PTcy21	99	FJ977127	Uncultured cyanobacterium clone B10805H	98	AY170472	*Chamaesiphon subglobosus* PCC 7430

primarily associated with MDS axis 1 ($r^2 = 0.88$) of the HPLC data, and the isolation of the Cleopatra inflow and Tut edge samples was owing to very high concentrations of this photoprotective, sheath-associated pigment. Neither HPLC nor ARISA convincingly separated samples from groups 1 and 2, although unlike with HPLC, ARISA yielded a weak but significant correlation between MDS axis 1 score and ln(conductivity) ($r^2 = 0.56$, $P = 0.03$).

Discussion

Limnology of Pyramid Trough

Based on their water chemistry, the ponds in the Pyramid Trough region can be divided into two main groups, and within each group, a gradient is evident. The first group is characterized on a more recent melt history, with low

NO_3–N concentrations, a high proportion of HCO_3 and lighter water isotopic signatures. Within this group, a gradient of increasing conductivity corresponds to increasing Cl/HCO_3, consistent with the removal of carbonate salts. Calcite ($CaCO_3$) was observed at the margin of Tut pond; a salt known to precipitate early in the evaporation sequence (Healy *et al.*, 2006). Group 2 appears to contain 'older' water, characterized by high NO_3 and low proportion of HCO_3 and a heavier isotopic signature, all indicating a greater degree of evaporation in these ponds. The increasing conductivity gradient in group 2 was attended by increasing Cl/SO_4 and Na(+K)/Ca, consistent with the precipitation of Ca sulphate salts. Salts precipitating around the margin of group 2 ponds were observed to be primarily gypsum ($CaSO_4$) with minor mirabilite (Na_2SO_4); sulphate salts typically precipitated in the later stages of pond evaporation (e.g. Healy *et al.*, 2006).

Chemical divergence of the two groups of ponds appears to follow classical wetland paradigms in that it is owing to shifts in the balance of evaporation and inundation. Group 1 ponds are likely to be receiving the highest loading of recent meltwater. During our sampling, Cleopatra Pond was receiving dilute ice-melt flowing in from the Upper Alph River, and whilst Egypt Pond was not connected, it was sufficiently close to the river to suggest that at times of exceptionally high discharge, it may receive such flow. Tut Pond, the third member of group 1 was, however, remote from direct inflow, residing in a closed basin but was stratified, so only part of its bulk chemistry is reflected in the surface layer plotted in Fig. 2. If mixed, it seems likely (from the isotopic signature for example) that Tut would have plotted as a group 2 pond on the ternary diagrams. All group 2 ponds have a closed basin catchment and evaporative evolution of the water in them, coupled perhaps with periodic redissolution of peripheral salts explains their higher conductivity and Cl–SO_4 anion dominance. Colin Pond provides an instructive outlier, which contains high NO_3, consistent with its upland location, but was also relatively dilute in other ions with HCO_3 a significant anion and an isotopic signature that suggested a significant meltwater loading. High nitrate concentrations have been noted previously in inland ponds in this part of Antarctica (Vincent & Howard-Williams, 1994; Webster-Brown *et al.*, 2010) and have been ascribed to accumulation of nitrates produced by upper atmospheric processes.

Environment–biota relationships in Pyramid Trough

As in many other Antarctic freshwater ecosystems (e.g. Sabbe *et al.*, 2004), mat-forming Cyanobacteria were the most conspicuous member of the benthic biota of Pyramid Trough wetland. The cyanobacterial taxonomic diversity identified by microscopy and 16S rRNA gene sequence clone libraries were similar (17 vs. 21 'taxa'), and there was a general agreement in the types of organism the two methods returned. As is frequently the case in Antarctica (Quesada *et al.*, 2008), filamentous members of the *Oscillatoriales* were abundant in the mat communities, although the abundance and diversity of *Chroococcales* and *Nostocales* featured here are striking. There were several morphotypes that were not recovered in the 16S rRNA gene surveys, particularly within the *Chroococcales*. These differences could be due to differential PCR amplification, over-representation of multi-cellular filamentous morphotypes and incomplete screening of the cyanobacterial 16S rRNA gene diversity. This highlights the need for a polyphasic approach when analysing cyanobacterial diversity (Taton *et al.*, 2003). Deep coverage sampling using high-throughput sequencing of cyanobacterial diversity at many sites, (Sogin *et al.*, 2006), would have improved resolution of the diverse assemblages and provided a better quantitative representation of community composition.

Differences in community composition between the two groups of ponds are evident, with some taxa, *Schizothrix, Calothrix/Dichothrix* occurring in all group 1 ponds but not in group 2. The presence of organisms from the *Dichothrix-Calothrix* complex in group 1 ponds also explains the high concentrations of scytomenin, as these taxa have a prevalence of producing the dark sheath pigment used to screening excess irradiance including UV (Quesada *et al.*, 1999). Similarly, high abundance of *Calothrix* has also been reported from James Ross Island in maritime Antarctica (Komárek *et al.*, 2012) and some streams in the Dry Valleys, including areas close to Pyramid Trough (Howard-Williams *et al.*, 1986, 1989). A relationship between overall salt content and cyanobacterial richness and community composition was observed to different degrees with ARISA, light-microscopy analysis and 16S rRNA gene sequence clone libraries. Conductivity has been previously reported to be of importance in shaping cyanobacterial assemblages in Antarctic terrestrial aquatic ecosystems including Laresman Hills, Prydz Bay and Ross Ice Shelf regions (Broady & Kibblewhite, 1991; Sabbe *et al.*, 2004; Jungblut *et al.*, 2005; Taton *et al.*, 2006; Verleyen *et al.*, 2010). Several taxa occur in only one or a few ponds such as *Chroococcales* ribotype Ptcy20 in Pettway, which interestingly clustered with environmental sequences from a hypersaline ecosystems. However, while this extreme habitat was distinctive, there was no clear evidence of taxa separating out systematically along the environmental gradients that we have identified. Sabbe *et al.* (2004) and Sutherland (2009) reported a similar lack of clear separation of cyanobacteria along conductance gradients in Antarctic lake systems.

With a few exceptions, the cyanobacterial community did not separate along environmental gradients, as might be expected if community structure was linked with environmental preference. The notable exceptions were the nitrogen fixers that were most abundant in the low-nitrate ponds. The more chaotic assembly seen in both morphotypes and ribotypes is more consistent with that expected from neutral rather than niche-driven dynamics. Neutral processes (Hubbell, 2008) tend to dominate community assembly when sympatric species show a degree of ecological convergence that minimizes the effects of niche-driven processes, and dispersal, chance and juvenile survival play significant roles in determining composition. Reasons why neutral processes may be important in Antarctic microbial communities, mostly relate to their common need for a high degree of tolerance of multiple stressors.

Our sampling was restricted to a single time-point at the peak of the summer growth season. Such a sampling strategy is not new to comparative limnology in Antarctica (e.g. Sabbe *et al.*, 2004) and poses a number of limitations on interpretation. These include the possibility of species succession, although unlikely in the short summer growth periods and high biomass communities, and the failure to fully document the dynamics of the environment. We suggest that the latter is more serious, and information has recently emerged on the conditions in Antarctic ponds outside the relatively benign summer period, which confirms that organisms must tolerate a cascade of stressors during the summer–winter transitions that are never experienced in summer (Hawes *et al.*, 2011a, b). These include exposure to high conductivities even in relatively dilute ponds, and it seems likely that a broad environmental tolerance may be a more important attribute for polar cyanobacteria than adaptation to one point on an environmental gradient. Vincent (2000) suggested that selection pressures would favour these multi-stress-tolerant taxa in the Antarctic cyanobacteria, and we suggest that selection for stress tolerance results in a degree of ecological convergence amongst Antarctic cyanobacteria.

Viewed within another limnological continuum, polar ponds are an unusual subset of temporary waters, one in which water is withdrawn by freezing rather than drying. Within temporary waters, increasingly stressful habitats (infrequent, short, unpredictable inundations) tend to result in dominance by increasingly stress-tolerant taxa, forming simple ecosystems with few trophic linkages (de Meester *et al.*, 2005; Vanschoenwinkel *et al.*, 2009). The microbial mats meet this description, and again, selection for stress tolerance may have led to a degree of ecological convergence that makes sorting along environmental gradients unlikely to dominate local biogeography.

In summary, our data show that the ponds of the Pyramid Trough capture a range of habitat diversity, with two distinct groups of ponds present and distinct gradients within each group. These different groups appear to relate to different predominant sources of water and salts. However, biological variables did not fall out clearly along the same lines as the habitats. Differences between communities in the ponds could not be related to habitat differences or the gradients in variables between ponds and groups of ponds. Taxa present appear to be a mix of those that can tolerate a broad, overlapping range of habitats, suggesting a prevalence of neutral assembly rules in this high-stress environment. These taxa present include both those representative of the wider Antarctic region area and others that are rare. The inclusion of this area in that part of the McMurdo Dry Valley Antarctic Specially Managed Area that receives an enhanced degree of protection from human activities, and the apparent importance of numerous varied habitats for optimizing appears fully justified for the purposes of protecting regional diversity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.

Fig. S1. Photomicrographs of Cyanobacteria in ponds of Pyramid Trough: (a and d) *Dichothrix* sp., Cleopatra Pond; (b) *Gloeocapsa* sp., Cleopatra Pond; (c) *Chroococcus* sp., Cleopatra Pond; (e) *Leptolyngbya* sp., Cleopatra Pond; (f) *Leptolyngbya* sp., Nile Pond; (g and h) *Nostoc* sp., Egypt Pond; (I) unknown *Chroococcales*, Egypt Pond; (J) unknown *Nostocales*, Egypt Pond; (K) *Schizothrix* sp., Ibis Pond; (L) *Cyanothece* sp., Peltway Pond; scale bar is 10 μm.

Fig. S2. Rarefraction analysis of clones recovered from 16S rRNA gene clone libraries from Pyramid Trough microbial mats.

Fig. S3. (a, b) Phylogenetic analysis of the identified cyanobacterial 16S rRNA gene ribotypes from microbial mats in Cleopatra, Ibis, Egypt, Tut and Pettway Ponds, Pyramid Trough, Antarctica.

Fig. S4. Two-dimensional nonmetric multi dimensional scaling (MDS) ordination based on Bray-Curtis similaritis of (a) HPLC pigment markers and (b) cyanobacteria-specific ARISA of benthic microbial mats from multiple sample sites in Pyramid Trough ponds and samples enclosed by green line cluster at 40% similarity; and (c) presence-absence data of cyanobacterial 16S rRNA gene sequence libraries, samples enclosed by full line cluster at 40% similarity and dotted line at 20% similarity, respectively.

Table S1. Morphotypes of cyanobacteria identified from marginal mats from the eight ponds examined in Pyramid Trough.

Table S2. Diversity indices, coverage, number of clones and OTUs for the five 16S rRNA gene cyanobacterial clone library assemblages; nd, no data; *N*, number of sequences; lci, lower confidence interval; hci, higher confidence interval.

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