The limnology and biology of the Dufek Massif, Transantarctic Mountains 82° South

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Abstract

Very little is known about the higher latitude inland biology of continental Antarctica. In this paper we describe the limnology and biology of the Dufek Massif, using a range of observational, microscopic and molecular methods. Here two dry valleys are home to some of the southernmost biota on Earth. Cyanobacteria were the dominant life forms, being found in lakes and ponds, in hypersaline brines, summer melt water, relict pond beds and in exposed terrestrial habitats. Their species diversity was the lowest yet observed in Antarctic lakes. Green algae, cercozoa and bacteria were present, but diatoms were absent except for a single valve; likely windblown. Mosses were absent and only one lichen specimen was found. The Metazoa included three microbivorous tardigrades (Acutuncus antarcticus, Diphascon sanae and Echiniscus (cf) pseudowendti) and bdelloid rotifer species, but no arthropods or nematodes. These simple faunal and floral communities are missing most of the elements normally present at lower latitudes in the Antarctic which is probably a result of the very harsh environmental conditions in the area.

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1. Introduction

The evolutionary history and geographical isolation of the Antarctic continent have produced a unique environment, inhabited by species adapted to its extreme conditions. However, very little is known about the higher latitude inland limnology and biology of continental Antarctica and the few data that do exist are largely based on opportunistic non-specialist collections made during geological field studies.

The southernmost aquatic systems studied to date are in the Brown Hills and Darwin Glacier region (~80°S), about 300 km south of McMurdo Sound (Vincent and Howard-Williams, 1994). Proglacial lakes are also known to be present as far south as 85°S in the Patuxent Range, and in the Mt. Heekin area of the Transantarctic Mountains.

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In the International Geophysical Year (1957) geologists discovered the Davis Valley and ‘Forlidas Valley’ (the name is unofficial) — unique, over-deepened dry valleys occupying an area of 53 km$^2$ in the northern Dufek Massif (Behrendt et al., 1974) (Figs. 1 and 2). Although less than 1% of the area of the McMurdo Dry Valleys, Davis Valley and Forlidas Valley are nevertheless the largest ice-free valley system found south of 80°S in the sector between 90°W and 90°E. They contain a near-unprecedented geomorphological record of glacial history of the ice sheet (Boyer, 1979), including evidence of up to seven distinct phases of glaciation (Hodgson et al., unpublished data). The area also contains a number of proglacial lakes and ponds and one land-locked pond, Forlidas Pond; the remnant of a once much larger proglacial lake (Fig. 2). The latter, to our knowledge, is the southernmost example of this type of water body. Despite the cold and dry climate of this region of continental Antarctica the presence of these lakes and ponds showed that liquid water is available which could potentially support a biota. More remarkable were reports from the International Geophysical Year of a ‘primitive leafy-type water plant’ (Neuburg et al., 1959) and a ‘strange pinkish plant that is somewhat leafy’ (Behrendt, 1998). This is a reference to the abundant cyanobacterial mats found there, which grow as foliose clumps and would resemble plants to a non biologist.

After nearly 50 years since these first observations we revisited the Davis Valley to describe and measure the limnology of the lakes and ponds and carry out the first biological inventory. The area is particularly interesting for biological studies as a result of the remarkable lack of human impacts, being only briefly visited as part of a traverse in the International Geophysical Year (1957), by the US Geological Survey in 1978–1979 and for 9 days by the authors in December 2003. The primary research objective was to determine whether, compared with lower latitude and coastal Antarctic studies (e.g. Convey and Stevens, 2007; Convey et al., 2008), the terrestrial and freshwater biota present at these high continental latitudes are post-glacial colonists or long-term survivors.

2. Site description

The Dufek Massif (Fig. 1) is a range of peaks in the Pensacola Mountains (part of the Transantarctic Mountain range), centred at 82°24' S 52°12' W. It is situated approximately mid-way between the Support Force Glacier and the Foundation Ice Stream, two of the major glaciers draining northwards from the Polar Plateau into the Ronne-Filchner Ice Shelf. Approximately 60 km to the southeast is the Forrestal Range (also part of the Pensacola Mountains), which is separated from the Dufek Massif by the Sallee Snowfield. The Ford Ice Piedmont separates the Dufek Massif from the Ronne and Filchner Ice Shelves, about 50 km to the northwest and 70 km to the northeast respectively. The nearest significant mountain chains are the Ellsworth Mountains 800 km to the west—north—west and the Shackleton Range 400 km to the north-east, both of which do not form part of the Transantarctic Mountains.

The total area of the Dufek Massif is 11,668 km$^2$ and its highest point is England Peak (2150 m). Its geology consists of a middle Jurassic differentiated stratiform mafic igneous complex overlain in the lower parts of the valleys by a glacial drape sorted into polygons by freeze thaw processes. The geology has been described by Behrendt et al. (1974), Ford (1976, 1990) and Ford et al. (1978) and remotely by Ferris et al. (1998). Cosmogenic isotope surface exposure age dating and geomorphological data point to a complex glaciological history with the repeated exposure of ice free surfaces for at least the last 1.6 million years (Hodgson et al., unpublished data). Meteorological studies are limited, but mean annual temperatures inferred from nearby ice boreholes lie between −24.96 °C, 32 km due north of Forlidas Pond on the Ford Ice Piedmont measured in December 1957 (Aughenbaugh et al., 1958), and −9 °C measured in December 1978 in the Enchanted Valley, 26 km to the south (Boyer, pers. comm.). Near surface winds in winter are predominantly from the west—north—west with modeled mean winter velocities of c. 10 m s$^{-1}$ (van Lipzig et al., 2004). Many geomorphological features related to wind erosion such as vents, facts and tafoni are present. Regionally, it has been identified as an ablation area comprising two ‘ablation types’ (van den Broeke et al., 2006). Type 1 includes erosion-driven ablation areas, caused by 1-D and/or 2-D divergence in the katabatic wind field where solid precipitation and sublimation are small but where divergence in the snowdrift transport can be considerable. Type 2 dominates in the Davis and Forlidas Valleys and includes sublimation-driven ablation areas occurring at the foot of steep topographic barriers, where temperature and wind speed are high and relative humidity low, with individual glacier valleys serving as gates for air drainage from the plateau to the Ronne-Filchner Ice Shelf. Strongest sublimation rates occur on these localized glaciers in the Transantarctic Mountains, where widespread blue ice areas are present (van den Broeke et al., 2006).
Fig. 1. Location map.
Combined, the Davis and Forlidas Valleys are approximately 7 km north to south and 7 km west to east (Figs. 2 and 3). Their northern extent in the Davis Valley is defined by the blue ice lobes that form part of the southern margin of the Ford Ice Piedmont (Fig. 4), and southern limit rises to escarpments breached by outlet glaciers, the largest of which is the Edge Glacier which extends approximately 4 km into the Davis Valley.
Valley from the Sallee Snowfield (Fig. 2). The western and eastern margins are enclosed by Forlidas Ridge and Wujek Ridge (Figs. 2 and 3).

Both valleys contain frozen and liquid water bodies. In Forlidas Valley there is Forlidas Pond (51°16′48″W, 82°27′28″S), a 90.3 m diameter, shallow pond (1.83 m deep) with a perennially frozen water column and evidence of an occasional freshwater moat. It is an isolated remnant of a formerly much more extensive proglacial lake, which had mid-late Holocene water levels up to 17.7 m above present delineated by an upper limit of salt efflorescence, an absence of well-developed frost-sorted polygons, and a series of lake terraces at 11.6 m, 8.61 m, 4.16 m and 1.25 m above the present water level (Fig. 5 and Hodgson et al., unpublished data). An ephemeral frozen melt water pond also occurs where the valley meets the Ford Ice Piedmont. A series of melt water ponds also occurs along the blue-ice margin of the northern Davis Valley at 51°05.5′W, 82°27.5′S and 51°07′W, 82°27.55′S (Fig. 6), whilst inland of this a number of relict pond beds mark the position of former proglacial ponds likely formed during periods of ice advance into the valley. Edge Lake (Fig. 2), a perennially frozen proglacial lake at the terminus of the Edge Glacier is surrounded by a series of 4-5 depositional proglacial lake ice-push shorelines cut into the valley side, particularly near the eastern side of the terminus of the Edge Glacier, indicating higher lake ice levels in the past. The surface of the lake has an uneven, slightly domed, topography suggesting that it has accumulated from successive surface melt water refreezing events, but experiences enhanced ablation at the lake margins. Seasonal melt water streams were observed on the eastern margin of the glacier during the field sampling campaign.

Incised dry stream channels and water erosion features are evident within the ice-free area. Some are fed by seasonal supraglacial melt water, but others appear to be relict features. The presence of liquid water at or near the surface of all the water bodies, and even the small glacial melt streams at the margin of the Edge Glacier, illustrates the ability of the relatively large areas of bare rock and soil to absorb solar radiation and emit heat causing local ice and snow melt.
Soils are not well-developed in the area and generally lack a significant organic component. Parker et al. (1982) collected a soil sample (S7) that was light brown in colour, resulting from gravel weathering predominantly to muscovite. The soil comprised sand (81%) with silt (14%) and clay (5%); a composition different from other sites in the Pensacola Mountains where the clay proportions of six samples ranged from 0.4% to 1.6%. The soil sample from the Davis Valley had a pH of 6.4 (Parker et al., 1982). Nitrate was the primary nitrogen ion and orthophosphate-P concentration was below the detection limit of 0.01 \( \mu \)g g\(^{-1}\). Microbial analyses of soil cultures showed that one pseudomyce- lium-forming yeast and measurable numbers of viable, aerobic heterotrophic microorganisms were present including Gram-negative rods, but these were not identified further (Parker et al., 1977, 1982).

On the grounds that the area contains some of the most southerly freshwater ponds known in Antarctica that contain plant life which would be threatened by possible contamination by human activity (Behrendt, 1998), Forlidas Pond and Davis Valley Ponds were designated as a Specially Protected Area (no. 23) and then an Antarctic Specially Protected Area (ASPA No. 119). ASPA No. 119 lies within Specially Reserved Area No. 1, proposed by the USA and adopted at the Antarctic Treaty Consultative Meeting XVI (held in Bonn, 1991; http://cep.ats.aq/cep/ apa/aspa/sites/aspa119/summary.html).

3. Methods

3.1. Surveying and environmental measurements

Topographic maps were compiled by the Mapping and Geographical Information Centre (BAS) at 1:50,000 scale using aerial photographs from the United States Geological Survey (Lassiter Station 1−16 and 1−17, 01.02.1958), GPS-surveyed ground control and differential geodetic GPS survey transects using a Trimble 5700 base station and a Magellan ProMark 10CM rover unit. Altitudes were referenced to the WGS84 reference ellipsoid, and included accurate photogrammetric height measurements of key landforms. Gemini Tinytag Plus data loggers were deployed to measure temperature and relative humidity at the sampling sites from 3 to 15 December 2003. Loggers were placed over snow and rock to measure the influence of advected radiation, with the sensors oriented to shield them from direct sunlight and data recorded at 30 min intervals. Lake water conductivity, temperature and oxygen saturation were measured using a SOLOMAT WP4007 water quality meter and 803PS Sonde. Water chemistry analyses followed the protocols described in Hodgson et al. (2009). Briefly, sodium, potassium, calcium, magnesium, iron, aluminium, manganese and silicon were determined by ICP-OES. Anions nitrate, chloride and sulphate were determined from direct analysis of aqueous solutions by Ion Chromatography. Total dissolved nitrogen was determined on a Shimadzu TNM-1 analyser equipped with a thermal conductivity detector DOC and TOC were determined by Shimadzu TOC-Vcph analyser with a detection limit of 0.5 mg/l. Nutrients phosphate and ammonium were determined by colorimetry.

3.2. Biological sampling

The biological sampling programme is summarised in Table 1. To minimise contamination we followed the protocols outlined in the Management Plan for Antarctic Specially Protected Area No. 119. All sampling equipment was scrubbed with Virkon multi-purpose disinfectant before use, and subsamples were collected in sterile WhirlPak bags or acid-washed bottles. Water samples were collected directly into acid-washed bottles (surface waters) or via a UWITEC water sampler (brine layer). Biological material was collected manually from lake benthic, littoral and catchment areas at each of the study sites. Samples for microscopic investigation were preserved in Lugol’s iodine solution or ethanol and those for molecular analysis were frozen.

The presence of mosses and lichens was assessed. Due to the extreme rarity of lichens in the area (and its protected status) we did not remove samples, but instead photographed them for later taxonomic study.

A combination of microscopic and molecular methods was used to identify the microbiota.
Microscopic methods included analyses of water samples in UWITEC plankton counting chambers, and analyses of natural samples and cultures. Representative samples obtained for microbial analyses included the hypersaline brine at the bottom of the Forlidas Pond (sample TM1), a cyanobacterial mat that was actively growing in the littoral zone (air bubbles on the mat surface and trapped under ice were taken as evidence of recent photosynthetic activity) situated under 15 cm of ice and 15 cm of water (TM2), and a sample of a red-orange foliose clump of terrestrial cyanobacterial mat located 20 m from the shoreline and probably corresponding to the “plant-like” organisms reported previously (Neuburg et al., 1959; Behrendt, 1998) (TM3).

Cyanobacteria were analysed by a combination of morphological and molecular methods, as described in detail by Fernández-Carazo et al. (unpublished data). Briefly, they involved the observation of cultures by microscopy with reference to the taxonomic works of Komárek and Anagnostidis (2005) and the diacritical morphological traits described by Taton et al. (2006a). DNA extraction methods were slightly modified from Taton et al. (2003). Following this, DGGE, construction of clone libraries and isolation and characterisation of the strains were carried out (Taton et al., 2003, 2006b).

Sequencing was performed by GIGA (http://www.giga.ulg.ac.be/) (Liége, Belgium) using an ABI 3730 xls DNA analyser (Applied Biosystems, Foster City, USA). A distance tree was constructed with the software

### Table 1

<table>
<thead>
<tr>
<th>Biological sampling program in the Davis and Forlidas Valleys: groups of taxa identified and the methods used.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
</tr>
<tr>
<td>Bryophyta</td>
</tr>
<tr>
<td>Lichens</td>
</tr>
<tr>
<td>Bacillariophyceae/Diatoms</td>
</tr>
<tr>
<td>Cyanobacteria</td>
</tr>
<tr>
<td>Chlorophyta/Green algae</td>
</tr>
<tr>
<td>Rhizaria/Cerezoa</td>
</tr>
<tr>
<td>Bacteria</td>
</tr>
<tr>
<td>Bacteria</td>
</tr>
<tr>
<td>Arthropods</td>
</tr>
<tr>
<td>Invertebrates</td>
</tr>
<tr>
<td>Tardigrades</td>
</tr>
<tr>
<td>Rotifers</td>
</tr>
<tr>
<td>Soil bacteria and algae</td>
</tr>
<tr>
<td>Avifauna</td>
</tr>
</tbody>
</table>

\(^a\) Previously published.
\(^b\) Tentative identification based on about 100 bases.
\(^c\) Analyses carried out on morphologically congruent samples from the Shackleton range.
\(^d\) Not considered as evidence of an extinct community.
package TREECON for Windows 1.3b (Van de Peer and De Wachter, 1997) by the neighbor-joining method (Saitou and Nei, 1987) using 379 positions covered by most sequences. The formula of Jukes and Cantor (1969) was used to correct for multiple mutations. The tree comprised DGGE bands, clones and strains’ sequences from Forlidas Pond samples as well as their three most similar strain sequences, and five uncultured sequences selected using Seqmatch from RDP (http://rdp.cme.msu.edu). A bootstrap analysis was performed involving the construction of 500 resampled trees. The OTUs were calculated using DOTUR (Schloss and Handelsman, 2004), with a threshold at 97.5% 16S rRNA similarity to define OTUs. In the case of OTU 16ST80, the sequence of sdG4 is exactly at the limit of similarity, and it was included in this OTU.

In order to study the cultivable bacterial diversity, isolates of the littoral sample (TM2) were grown on a selection of heterotrophic media (Peeters et al., unpublished data). The isolates were screened for duplicates and grouped by rep-PCR using primer (GTG)5 as described in Gevers et al. (2001). Representative isolates were identified by partial 16S rRNA sequencing using primer BKL 1 (Coenye et al., 1998). A fragment of approx. 400 to 450 bp from the gene sequencing using primer Euk1A-CHLO02r and Euk1A Euk516r as described in Gevers et al. (2001). Representative isolates were identified by partial 16S rRNA sequencing using primer BKL 1 (Coenye et al., 1999). A fragment of approx. 400 to 450 bp from the 5’ end was obtained and compared with the EMBL database using FASTA (http://www.ebi.ac.uk/Tools/fasta33) for preliminary identification.

To study the uncultivable diversity of bacteria, green algae and cercozoa, we first optimized the protocols to extract DNA from environmental samples by removing extracellular DNA (Corinaldesi et al., 2005) prior to bead-beating extraction. For the Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the bacteria, we followed the protocols as described in Van der Gucht et al. (2001). For the green algae, we used a nested PCR approach using the primer combinations Euk1A-CHLO02r and Euk1A Euk516r GC (Díez et al., 2004). These primers are known to also detect cercozoa (Zhu et al., 2005), hence these taxa were also included in the DGGE analysis. Excised bands were sequenced and identified after re-extraction and amplification. A nucleotide BLAST search (Altschul et al., 1997) was performed in order to obtain sequences that were most similar.

Samples for diatom analysis were prepared following a slightly modified protocol from Renberg (1990) and embedded in Naphrax®. The slides were screened for the presence of frustules at 1000× magnification using a Zeiss Axioplan II microscope.

Fauna were extracted using Baermann and Tullgren extractions on return of the collected substrata to the BAS Rothera Research Station, having been kept under field conditions in the intervening ~two week period. Tardigrades from the Baermann funnel extractions were permanently mounted on microscopes slides (using de Faures medium) for identification. Individual tardigrades were grouped into morphotypes under 400× magnification. A representative of each morphotype was mounted and examined under high power (1000×) magnification for detailed taxonomic identification. It was not possible to extract tardigrade DNA from ethanol preserved samples from the Dufek Massif, so our specimens were compared with DNA from morphologically congruent dried meiofaunal samples collected from Lake Lundström, in the adjacent Shackleton Range (400 km distant). Meiofauna were separated from the substrate by homogenising and centrifugation of samples using an Optiprep™ gradient solution (see Sands et al., 2008 for detailed methods). Specimens were lifted into individual tubes with 5 µL double distilled H2O and kept stored frozen (−80 °C). DNA was released from individual tardigrades by disrupting the tissue by a series of freeze thaws followed by a 15 min incubation at 95 °C in a 5% chelex solution. Three genes were amplified, a fragment of mitochondrial cytochrome c oxidase (CO1), the near complete small ribosomal sub unit (18S), and a fragment of the Wingless gene (Wnt). Details of the above protocol, including primers and amplification strategy, and results are given in Sands et al. (2008).

4. Results and discussion

4.1. Environmental measurements and observations

Microclimate temperatures over snow from 3 to 15 December 2003 ranged from a maximum of +12.8 °C to a minimum of −14.5 °C, with a mean over the period of −0.56 °C. Microclimate temperatures over rock ranged from a maximum of +16.0 °C to a minimum of −8.6 °C, with a mean over the period of +0.93 °C (Fig. 7). Relative humidity recorded over snow ranged from a maximum of 80.4% to a minimum of 5.6%, with a mean over the period of 38.7% (Fig. 7).

Being in an ablation area (van den Broeke et al., 2006) evaporation and sublimation dominate over precipitation. As a result Forlidas Pond has evaporated down to a small remnant of a once much larger proglacial lake (Fig. 5). The pond was frozen almost...
completely to its base, with a thin layer of hypersaline slush at the lake bottom. The depth of the pond was 1.83 and the thickness of the ice between 1.63 and 1.83 m. The conductivity of the hypersaline slush was 142.02 mS cm\(^{-1}\), approximately four times greater than seawater. The ionic order of the brine layer was Cl\(\ce{-}\)Na\(\ce{-}\)Mg\(\ce{-}\)SO4\(\ce{-}\)Ca-K and its temperature was \(-7.67\, ^\circ\text{C}\) (Table 2). At the margins of the pond, liquid water was present in a moat area under 10–15 cm of ice. At Forlidas Pond this moat water had a freshwater ion sum of 178 mg L\(^{-1}\) compared with the 111,942 mg L\(^{-1}\) of the brine layer (Table 2). The Davis Valley Ponds also had shallow freshwater littoral moats that, at the time of sampling, were either locally ice free, frozen with liquid water present under ice, or frozen to the bed. The surface morphology of the pond ice suggested that these moats could have been 1–2 m laterally more extensive during warmer years.

### 4.2. Flora

Visible biota was limited within the study area, and macroscopic vegetation appeared to be restricted to cyanobacterial mats, found both in lakes and terrestrial habitats, and a very sparse occurrence of small (~mm scale) yellow and black crustose lichens deep within crevices on larger boulders (Fig. 8), as previously observed by Neuburg et al. (1959). Often, only the black apothecia were visible. Through analyses of the photographs the species has been identified as *Lecidea cancriformis* Dodge & Baker; one of a few lichens which occurs in the severest environments of continental Antarctica, especially on far inland nunataks as

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Brine layer</th>
<th>Freshwater moat</th>
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<tbody>
<tr>
<td>Sample depth (m)</td>
<td>1.63</td>
<td>0.2</td>
</tr>
<tr>
<td>Conductivity (mS cm(^{-1}))</td>
<td>142.37</td>
<td>2.22</td>
</tr>
<tr>
<td>Temperature ((^\circ\text{C}))</td>
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<tr>
<td>pH</td>
<td>7.3</td>
<td>8.15</td>
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<tr>
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<td>Fe (mg L(^{-1}))</td>
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<tr>
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<td>K (mg L(^{-1}))</td>
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<td>NH4(\ce{-})N (mg L(^{-1}))</td>
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<tr>
<td>PO4(\ce{-})P (mg L(^{-1}))</td>
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<td>&lt;0.005</td>
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far as 86°S, and at high altitudes (Ovstedal and Smith, 2001, p. 220, plate 48). The British Antarctic Survey Plant Database also reports Blastenia succinea Dodge & Baker and Xanthoria elegans (Link.) Th. Fr. in samples from elsewhere in the Dufek Massif; although these have not been independently verified by us. Previous anecdotal reports of the possible occurrence of mosses within the area could not be substantiated, and it is probable that the rich cyanobacterial mat growth was previously mistaken for bryophytes by non-specialists. The cyanobacterial community is the most abundant biota and is present in at least three distinct environments:

(1) In the permanent water bodies; particularly in the moat of Forlidas Pond, at the bottom and littoral zones of the Davis Valley Ponds, and in the seasonally wetted perimeter of Edge Lake. These habitats were extensively covered by red-brown cyanobacterial mats (Fig. 9a). These were actively photosynthesizing, as evidenced by gas bubbles trapped against the lower ice surfaces, and bubbles incorporated into the ice. Because perennially ice covered lakes have elevated concentrations of dissolved O$_2$ gas, the microbial mats growing on the bottom can become buoyant and start to float off the bottom as ‘lift-off’ mats (cf. Doran et al., 2004, p. 480), or become incorporated into the base of the lake ice when it makes contact with the bed. In Forlidas Pond and the Davis Valley Ponds lift off mats frozen into the base of the lake ice eventually migrate up through the ice profile (cf. Adams et al., 1998). In the Davis Valley, this appeared to take place over several years with each summer marked by the development of a 2–3 cm melt-cavity formed by the upward progression of the clump thorough the lake ice due to preferential heating of its upper surface (Fig. 9b). These clumps eventually break out at the surface and are dispersed by wind onto the shoreline, or further afield. In the littoral zone of Forlidas Pond, melting and refreezing of the moat has resulted in cyanobacterial mats being incorporated under shoreline boulders. Fossil examples of this type of mat were also found buried under boulders between the present and previous (higher) shorelines. Cyanobacteria were also present in the hypersaline brine of Forlidas Pond as single cells and as small flakes. A strain corresponding to the morphology of Leptolyngbya antarctica was isolated from the saline slush of TM1 (Fernández-Carazo et al., unpublished data).

(2) In exposed terrestrial locations, particularly at the edge of larger rocks and within the boundary crevices of frost sorted polygons. These were generally very foliose in form, mid brown in colour, and best developed at the edge of larger rocks accumulated to depths of at least 10–15 cm (Fig. 9c and d). Nearly all clumps were completely dry on discovery, although those near to melting snow were damp and some had lower thalli that were often deep green in colour. Particularly good examples of this growth form were found in the mid valley floor of Forlidas Valley and in Davis Valley (near a large snow gully where it meets the second major terrace above Edge Lake).

(3) In a series of dry pond beds, two of up to 50 m diameter are present in the Davis Valley (Fig. 2), and have extensive areas of almost continuous cyanobacterial mat on the former pond floors (Fig. 9e). These pond beds and gullies occupy depressions and therefore may accumulate snow in winter, permitting the cyanobacteria to take advantage of the wet and protected environment within the snow patches (Cockell et al., 2002).

Analyses of the cyanobacterial molecular diversity in and around Forlidas Pond showed that the richness obtained in the current study was lower (3–5 OTUs per sample) than in Antarctic coastal lakes (4–12 OTUs per sample) (Fernández-Carazo et al., unpublished data). The spatial distribution of the cyanobacterial OTU’s showed that TM1 (from the hypersaline brine at the bottom of the pond) and TM2 (mats in the littoral zone) shared three OTUs, 16ST63, 16ST14 and 16ST49 (Table 1, Fig. 10). In addition,

![Fig. 8. Lichen Lecidea cancriformis in rock crevice (1 cm diameter lip balm container for scale).](image)
TM2 shared OTUs 16ST44, 16ST49 and 16ST80 with the terrestrial mat (TM3). These data support the idea that the cyanobacterial diversity is not limited to specific aquatic or terrestrial habitats, but that aquatic species are able to colonise the surrounding terrestrial niches, and vice versa (cf. Gordon et al., 2000). On the basis of the geomorphological evidence around Forlidas Pond of the former presence of a larger proglacial lake that evaporated, it is possible that the terrestrial cyanobacteria were once aquatic, but still survive on the now dry lake bed. Another hypothesis could be that the foliose clumps were made by submersed aquatic cyanobacteria that died subsequently, but whose undegraded DNA has contributed to the 16S rRNA survey for TM3.

Microscopic analyses revealed that no diatom communities were present. One solitary valve of the diatom *Pinnularia microstauron* (Ehr.) Cl. was detected but, as this is a common windblown subaerial diatom, we do not consider it as evidence of an extant...
community. A study of ponds in the Darwin Glacier region (79.9°S) reported a similar absence of diatoms (Vincent and Howard-Williams, 1994). Analysis of the DGGE bands in the environmental samples revealed only a single green algal sequence (Urospora sp.; E30) and two cercozoans (a Heteromitidae; E12.5 and a Paulinella sp.; E23). Bacteria identified by DGGE bands included Cyanobacteria (Nostocales, Oscillatoriales, Chroococcales, Gloeobacteriales), Bacteroidetes (Sphingobacteriales and Flavobacteriales), Firmicutes (Clostridiales) and Gammaproteobacteria (Pseudomonadales; Psychrobacter) (Tables 1 and 3).

Preliminary data based on partial 16S rRNA gene sequence analysis of the isolated bacteria in cultures revealed a large diversity. Of more than 330 isolates sequenced, 33% belonged to the Firmicutes (low %GC Gram-positive bacteria), 23% were Bacteroidetes, 25% were Alphaproteobacteria, 9% were Actinobacteria (high %GC Gram-positive bacteria) and 8% were Betaproteobacteria. Gammaproteobacteria (1.5%) and Deinococci (0.3%) were present in smaller numbers.

Viable yeast species have previously been recorded in the soil, along with the cyanobacterium Oscillatoria sp., and the algae Trebouxia sp. and Heterococcus sp. (Parker et al., 1982). Chasmolenticoidichnium cyanobacteria have been recorded in rocks on the west spur of Walker Peak at about 1070 m in the Dufek Massif (Friedman, 1977), although we found no evidence of their presence within the dry valley areas included in our survey, suggesting that endolithic organisms are not widespread here.

4.3. Fauna

The invertebrate fauna within the area was equally impoverished, with both the diversity and abundance of organisms being extremely limited compared with lower latitude and coastal Antarctic sites. A total of 50 Tullgren extractions of terrestrial cyanobacterial mat and soil substrates generated no arthropods. Although a negative result, this strongly suggests that these groups are absent from the area as there are no other obvious habitats which might be expected to harbour them. A total of 130 Baermann extractions revealed three species of tardigrade support are drawn as unresolved. The tree comprised the sequences of 7 DGGE bands, 8 clones and 4 strains from the Forlidas Pond samples (in bold italics) and their 3 most similar strain sequences and 5 uncultured sequences from rdpII (http://rdp.cme.msu.edu). The sequences found in Antarctic samples are in bold. E. coli sequence is the outgroup. The OTU numbers are indicated on the right. The evolutionary distance between two sequences is obtained by adding the lengths of the horizontal branches connecting them and using the scale bar (0.1 mutation per position).

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from two Classes: Echiniscus (cf) pseudowendti Dastych, 1984 (Heterotardigrada), Acutuncus antarcticus (Richters, 1904) and Diphascon sanae Dastych, Ryan and Watkins, 1990 (Eutardigrada) and a few unidentified bdelloid rotifers (Table 1). Tardigrades were commonly found (c. 40–60 per cc) while rotifers were rarer (c. 5–15 per cc); although not a quantitative extraction technique, these values are low in comparison with those obtained from similar extractions from other Antarctic locations. Surprisingly, the most productive sites for these organisms were not the aquatic environments of the permanent lakes, but the former pond beds in the Davis Valley. Acutuncus antarcticus is an Antarctic species that occurs in semi-permanent damp/wet habitats throughout the Antarctic continent and sub-Antarctic islands, but has not been reported from any of the close neighbor continents. Echiniscus (cf) pseudowendti and Diphascon sanae found in samples from Forlidas Pond are also endemic to the Antarctic, with restricted distributions. For example, Echiniscus (cf) pseudowendti has been found in the maritime regions of the Antarctic Peninsula, Dronning Maud Land (Heimemontjella) and Enderby Land (Thala Hills), and Diphascon sanae from Dronning Maud Land (Robertskollen), Enderby Land (Prince Charles Mountains and Mawson Station) and Ellsworth Land (for a discussion of the molecular data see Sands et al., 2008).

Avifauna was sparse. A single snow petrel (Paga-droma nivea) was noted flying around one of the peaks above Davis Valley.

4.4. Biological interactions with the physical environment

As “dry valley” ecosystems, the Davis and Forlidas Valleys share environmental features with the Dry Valleys of Victoria Land. However, they are 2100 km distant from them and 570 km further south (82°S vs. 77°S). The climate of the Dufek Massif is that of an Antarctic cold desert, experiencing limited precipitation and rapid ablation. This restricts the potential for metabolic activity and prevents growth for much of the year, possibly even requiring dormancy on multi-year timescales. However, summer microclimate temperatures measured during the field campaign were relatively high, ranging from −14.5 °C to +12.8 °C over snow and −8.6 °C to +16.0 °C over rock during our visit. The valleys also have many features related to wind erosion but some of these may be relatively ancient, as they occur mostly on rock surfaces above the glacial drift limits, whilst the foliose terrestrial cyanobacterial growth forms remain intact on the valley floor.

With these relatively harsh conditions and physical isolation, it is perhaps not surprising that the valleys appear to lack many of the components typical of the Victoria Land ecosystems (see Adams et al., 2006), including nematodes, arthropods and mosses, and that there is an extremely sparse development of lichens. Cyanobacteria are the dominant phototrophs, in common with other aquatic and terrestrial polar ecosystems (cf. Vincent, 2000), and benefit from a range of biochemical adaptations for survival in shallow water and terrestrial habitats (Hodgson et al., 2004). In the Dufek Massif their greatest biomass was found in the microbial mats that form in the benthic and littoral zones of lakes and ponds and in terrestrial habitats.

The abundance and macroscopic growth-form of the terrestrial cyanobacteria despite apparent limited water availability is something of a paradox. At the time of our visit there was very little snow within either valley bottom. However, water is required for the mats to be metabolically active and is likely derived from within snow patches or from snow melt focused into depressions and in the lee of boulders, with metabolically active periods being short and unpredictable. Alternatively, later in the season there may be periods of increased supraglacial melt water flowing off the local ice sheet and outlet glaciers, which could potentially provide a source. Although there was no implication of this process occurring during our visit, we located deep footprints from a previous visit (i.e. 20–45 years old), which indicated that some ground was waterlogged at that time.

The most productive terrestrial habitats appeared to be the dried areas of mat on the beds of the relict proglacial ponds (Fig. 9e) and in cracks, crevices, the lee side of boulders (Fig. 9c), and in the shallower parts of the ponds (Fig. 9a). This is a likely function of these environments either containing seasonally liquid water or accumulating snow cover in winter which persists into spring. Previous studies suggest that such under snow habitats can be biologically very active in Antarctica (Cockell et al., 2002), and they also have the advantage of protecting the biota from exposure to

Table 3

<table>
<thead>
<tr>
<th>Bacteria (uncultured diversity)</th>
<th>TM1</th>
<th>TM2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsequenced or unidentified DGGE bands</td>
<td>16</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gammaproteobacteria</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>22</strong></td>
<td><strong>18</strong></td>
<td><strong>32</strong></td>
</tr>
</tbody>
</table>

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wind abrasion. Extractions from samples taken from within these areas were found to yield the greatest numbers of rotifers and tardigrades.

Mats collected within the freshwater ponds did not generate larger numbers or diversity of invertebrates. Rotifers, at least, were present in clumps of mat that had travelled upwards through the pond ice — it is plausible that they have been present throughout the upwards journey, although colonisation while on the ice surface is also possible.

4.5. Species diversity and endemism

The very limited species diversity in the Forlidas and Davis Valleys supports the hypothesis that, in the more extreme and remote regions of continental Antarctica, species assemblages present are characterised by a low biodiversity, taxa tolerant of extreme cold and dry conditions, and a certain degree of endemism. For Antarctic microbial communities, molecular studies have shown that their composition includes both cosmopolitan OTUs, and also a greater number of Antarctic endemic species than has been estimated by traditional morphological methods (Taton et al., 2006a; DeWever et al., 2009). Our molecular data for cyanobacteria in Forlidas Valley show a depleted diversity, with only 3–5 OTUs per sample compared with the greater number of OTUs found in other regions of Antarctica (Taton et al., 2006a). This is likely a product of geographical isolation (cf. Vyverman et al., 2007; Verleyen et al., 2010) combined with multiple environmental stressors such as salinity and seasonal desiccation, and UV radiation (Bowman et al., 2000; Taton et al., 2006a). However some of the cyanobacteria, such as OTU 16ST63 from the brine of Forlidas Pond, are related to sequences from other hypersaline Antarctic lakes such as Rauer 8 in the Rauer Islands (for location see Hodgson et al., 2001) and Ace Lake in the Vestfold Hills (16ST23 in Taton et al., 2006a), but are also present in desert crusts on sand dunes in Israel (AM398947) and in saline lakes in Chile (EF633019). OTU 16ST07 only includes sequences from different sampling locations on the Antarctic continent and sequences from Tibetan glaciers, which could indicate that this OTU has particular adaptations to glacial conditions (Fig. 10). The six cyanobacterial OTUs are all found outside Antarctica, but in addition, they are all distributed in more than one location within the continent (Fig. 10). This is in agreement with previous studies where the cosmopolitan OTUs have been found to be more widespread on the continent than endemics. For example, Taton et al. (2003, 2006a, b) studied the molecular diversity in benthic cyanobacteria in lakes from different and geographically separated Antarctic biotopes, including Lake Fryxell (McMurdo Dry Valleys) and coastal lakes in the Prydz Bay region (East Antarctica). In addition, two melt water samples from Livingston Island (Antarctic Peninsula) have recently been studied (unpubl. data). Using clone libraries based on 16S rRNA sequences, 70% of OTUs in these studies were only found in Antarctica. This suggests a rather high degree of endemism, though the influence of geographic gaps in the database might be biasing the data. However, within these studies, a higher proportion of the cosmopolitan genotypes were found in multiple Antarctic regions (47%, compared to 16% for the apparently ‘endemic’ sequences). These cosmopolitan genotypes are likely to possess resistance capacities (Taton et al., 2006b) that will also be beneficial during dispersal to and between different Antarctic regions (Zakhia et al., 2007).

At Forlidas Pond the three OTUs found in the hypersaline brine (TM1) were also present in the littoral zone (TM2), three of the OTUs from the littoral zone were also found in the terrestrial sample (TM3) and one OTU (16ST49) was present in all three samples, suggesting that although the diversity is low some OTU’s have a wide environmental tolerance (Fernández-Carazo et al. unpublished data). Contrary to some of the observations in Wright and Burton (1981), who reviewed the biology of Antarctic saline lakes, the cold brine in Forlidas Pond does not appear to be incompatible with cyanobacterial growth, although salinity and freezing conditions are likely to limit metabolic processes as they do in some of the more saline ponds on the McMurdo Ice Shelf (cf. Vincent, 2000). The dominance of the cyanobacteria over the green algae agrees with other studies which have suggested that continental cyanobacteria are more resistant to freezing and desiccation regimes than sub-Antarctic taxa, and are more abundant than green algae whose membranes are poorly adapted to freeze-thaw processes (Šabacká and Elster, 1996).

Of the cultivated bacterial isolates characterised from sample TM2, 13.5% had less than 97% similarity to known sequences in the EMBL database, indicating that these taxa represent organisms that have not been reported previously and are potentially new to science. This observation is in line with previous reports (Brambilla et al., 2001; Van Trappen et al., 2002) and is not unexpected in view of the limited amount of studies using cultivation to study Antarctic bacterial diversity, and the estimate that only a small fraction of bacterial species have so far been described (Schloss and Handelsman, 2004).
The degree of endemism of many microbial groups in Antarctica is still debated (see Vyverman et al., 2010; this volume). A consistent problem faced by researchers interested in the possibility of microbial endemism is the paucity of Antarctic data relating to microbial diversity and distribution (Wynn-Williams, 1996). As a broad generalisation, microbiota are thought not to face the same dispersal limitations as do many larger organisms or their propagules. This has led to the development of the ‘global ubiquity hypothesis’ (Finlay, 2002), whereby their propagules. This has led to the development of the ‘global ubiquity hypothesis’ (Finlay, 2002), whereby their small size means that they can easily enter and remain in the air column and thereby reach all parts of the planet. This has also been rationalised in terms of ‘everything is everywhere, and the environment selects’, in other words that dispersal is not a limiting factor on species distribution, which is rather controlled by possession of appropriate adaptations to allow survival, development and reproduction under the conditions imposed by the ‘recipient’ environment. This is consistent with previous descriptions of a largely cosmopolitan microbial flora, for example as applied to classical morphological studies of the eukaryotic algae (Broady, 1996) and many of the diatom studies cited in Jones (1996).

However, some authors suggest that microbial endemism is still possible because of the long isolation of Antarctica from other parts of the world, the fact that dispersal processes which favour local species are more efficient than long distance dispersal processes and that there has probably been strong environmental selection for adaptive strategies (Vincent, 1988; Franzmann, 1996). The application of molecular biological techniques of identification has led recently to an increase in records of microbial diversity through sequence data (e.g. Adams et al., 2006), although an inherent weakness of these approaches, as with more classical culture techniques, is that sequence presence and detection does not automatically prove biological activity or functional significance within the ecosystem. In the absence of baseline microbial diversity data against which to compare, assessment of endemism, or indeed of post colonisation adaptation, remains difficult. However, Lawley et al. (2004) argued that there was circumstantial support for Antarctic endemism in diverse microbial groups based on very limited overlap in OTU composition between different locations in a comparative study based on the same soil habitat, and Boenigk et al. (2006) argued that there is evidence for considerable ecophysiological specialization within Antarctic strains of certain microbial taxa, only possible if these have been isolated for long periods of evolutionary time.

Few studies have attempted to assess directly the mechanism of dispersal employed by microbial communities in Antarctica, although representatives of the cyanobacteria have been recorded in simple aero-biological trapping studies at locations on the Antarctic Peninsula (Hughes et al., 2004; Pearce and Galand, 2008). However, the mechanisms of dispersal in Antarctica are unlikely to be different to those commonly recorded elsewhere, with the major difference for terrestrial biota relating to the paucity and isolation of suitable establishment sites. Thus, the major routes of dispersal are likely to be through transport in the air column, incidental attachment to other biota, transport in freshwater flows (on a local scale within Antarctica) and, more recently, human transportation (Frenot et al., 2005; Hughes et al., 2006; Convey, 2008).

In the Dufek Massif, we found no evidence of local endemic organisms isolated there for long periods of evolutionary time. This is consistent with the glacio-biological history which suggests the repeated exposure of ice free surfaces for about 1.6 million years (Hodgson et al., unpublished data) which is insufficient time for in-situ speciation, which for bacterial 16S rRNA genes, is c. 50 million years for 1% divergence. Instead, there are some groups of taxa present that are endemic to the Antarctic continent (tardigrades, a lichen and possibly some bacteria), and others which are cosmopolitan (for example none of the green algal, cercozoan or bacterial DGGE-bands are unique to Antarctica). For the cyanobacteria, only cosmopolitan cyanobacterial OTUs were found, and among the bacterial strains obtained in cultivation about 13.5% represent potentially new species some of which may be endemic. These results imply that the Dufek Massif has not functioned as a biological refuge over long timescales (cf. Convey et al., 2008, 2009). Instead it has been colonised in the Quaternary by a combination of Antarctic endemic and cosmopolitan taxa whose distribution, dispersal and establishment has been dependent upon life cycle characteristics (e.g., formation of resting spores and resistance to the extreme environmental conditions).

5. Conclusions

There has been recent recognition that levels of endemism and/or molecular evolutionary differentiation are considerably greater than previously appreciated across most of the groups of terrestrial biota (with the exception of bryophytes) that currently dominate Antarctic terrestrial communities (Convey and Stevens, 2007; Peat et al., 2007; Convey et al., 2008; Pugh and Convey, 2008). These are interpreted as supporting long-term terrestrial biological presence in Antarctica.
through glacial cycles and even in some cases back to the breakup of Gondwana. Furthermore, this includes considerable regionalisation within Antarctica itself (Chown and Convey, 2007; Pugh and Convey, 2008). Although we found no evidence in this study of regionalisation within the Dufek Massif, we did find a mix of Antarctic endemic and cosmopolitan species that have colonised ice free land there during the Quaternary period. We also found that the Dufek Massif contains some of the most reduced metazoan terrestrial and freshwater ecosystems known from Antarctica with autotrophs limited to cyanobacteria, plus a few green algae and lichens. This pristine and low diversity flora and fauna, only 800 km from the South Pole reinforces the importance of the area’s designation as an ASPA, and highlights a particular vulnerability to human impacts.

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