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An Update on Sedimentary Pigments in Victoria Land Lakes (East Antarctica)

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Abstract

Antarctic ice-free areas contain lakes and ponds that have interesting limnological features and are of wide global significance as early warning indicators of climatic and environmental change. However, most limnological and paleolimnological studies in continental Antarctica are limited to certain regions. There are several ice-free areas in Victoria Land that have not yet been studied well. There is therefore a need to extend limnological studies in space and time to understand how different geological and climatic features affect the composition and biological activity of freshwater communities. With the aim of contributing to a better limnological characterization of Victoria Land, this paper reports data on sedimentary pigments (used to identify the main algal taxa) obtained through a methodology that is more sensitive and selective than that of previous studies. Analyses were extended to 48 water bodies in ice-free areas with differing lithology, latitude, and altitude, and with different morphometry and physical, chemical, and biological characteristics in order to identify environmental factors affecting the distribution and composition of freshwater autotrophic communities. A wider knowledge of lakes in a limnologically important region of Antarctica was obtained. Cyanophyta was found to be the most important algal group, followed by Chlorophyta and Bacillariophyta, whereas latitude and altitude are the main factors affecting pigment distribution.

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Introduction

Antarctic ice-free areas contain several lakes and ponds that have interesting limnological features and are of wide global significance, especially as refugia for unique species and as early warning indicators of climatic and environmental changes (Vincent and Laybourn-Parry, 2008). However, most limnological and paleolimnological studies in continental Antarctica are limited to certain regions such as the Larsemann Hills (e.g., Hodgson et al., 2001, 2006; Squier et al., 2004, 2005; Verleyen et al., 2004) and Vestfold Hills (Fulford-Smith and Sikes, 1996; Laybourn-Parry et al., 2002; Henshaw and Laybourn-Parry, 2002). Limnological research in ice-free areas of Victoria Land (from 79°S in Darwin Glacier to 71°S at Cape Adare) began about 50 years ago, focusing especially on the McMurdo Dry Valleys (76.50°–78.00°S and 160.00°–165.00°E) (e.g., Priscu, 1998). The latter is the largest ice-free area in Antarctica (4800 km²), with characteristics typical of cold deserts (precipitation <100 mm water equivalent/yr, mean annual temperatures of –16 to –21 °C and humidity <50%). As shown by the results of short-term (e.g., Green et al., 1989; Matsumoto, 1993; Webster et al., 1994; Spiegel and Priscu, 1998) and long-term investigations (e.g., Green and Friedmann, 1993; Priscu, 1998), streams and lakes in the McMurdo Dry Valleys are the sites of high biological activity during summer. However, there is a need to extend limnological studies in space and time in order to understand how different geological and climatic features throughout Victoria Land affect the composition and biological activity of freshwater communities. Although most lakes in the region have a perennial ice cover (3–5 m thick) with reduced radiant energy, gas exchanges, and productivity, several water bodies in the coastal ice-free areas have no ice cover during summer and contain simple truncated food webs with abundant

bacteria, algae, ciliated and flagellated protozoans, and rotifers (Laybourn-Parry, 2009). The benthos is dominated by extensive microbial mats (mainly consisting of filamentous cyanobacteria, pennate diatoms, and green algae) of different morphology probably determined by local environmental conditions (Wharton et al., 1983).

Limnological surveys in northern Victoria Land were first completed in the 1990s, after the establishment of the Italian “Mario Zucchelli” Antarctic Station (MZS) at Terra Nova Bay (Ross Sea). The main morphometric, hydrochemical, and edaphic features of several lakes and ponds located near MZS were reported by Fanzutti et al. (1989), Guilizzoni et al. (1992), Libera (1993), and Baudo et al. (2000). More recently, the physicochemical characteristics of lacustrine waters and sediments were reported by Borghini and Bargagli (2004, 2005), Borghini et al. (2007, 2008), and Malandrino et al. (2009). In general, these studies show that the water chemistry largely reflects that of seawater; additional and alternative sources of solutes were only found for NO₃[–], SO₄^{2–}, K⁺, and Ca²⁺. Ion concentrations are highly variable in space and time, and maximum values are usually measured in water samples collected at the end of summer from lakes with no ice cover (i.e. those with enhanced drainage and water evaporation; Borghini et al., 2008). Sedimentary concentrations of total carbon (TC), total nitrogen (TN), and S are very low, except in water bodies affected by the presence of seabirds. Biological investigations have been performed in lakes at Edmonson Point, Gondwana, Inexpressible Is., Tarn Flat, and the Northern Foothills (e.g., Broady, 1987; Guilizzoni et al., 1989; Andreoli et al., 1992, 1996; Fumanti et al., 1992, 1995, 1997; La Rocca et al., 1996; Cavacini, 1999; Cavacini and Fumanti, 2005), and microscopy-based studies have reported more than 100 taxa (not all at the species level). Phytoplankton communities are

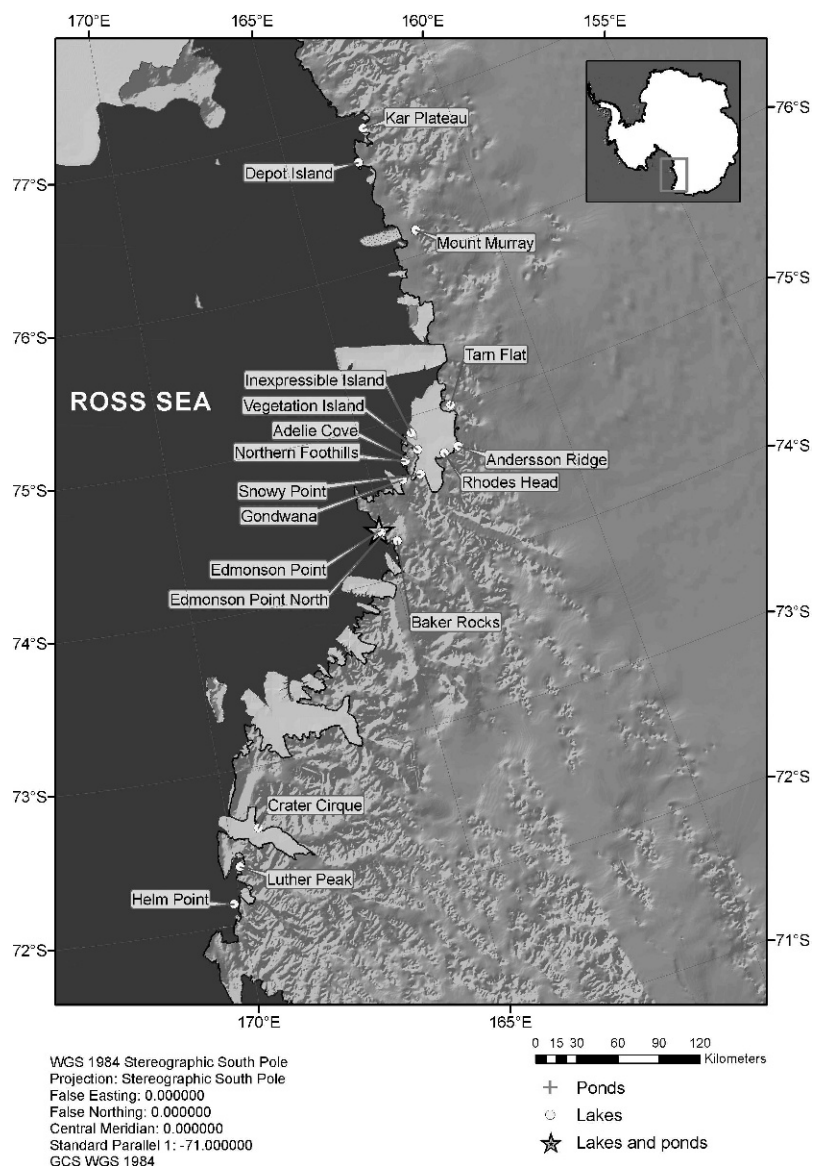


FIGURE 1. Study area and sampling sites (from the Antarctic Digital Database [SCAR 1993–2006] for the coastline and the Antarctic Geospatial Information Center (AGIC) of the National Science Foundation for the digital ground model, modified).

mainly characterized by Cyanophyta, Cryptophyta, Cryophyta, Chlorophyta and Bacillariophyta. The most abundant genera in microbial mats are *Oscillatoria*, *Nostoc*, *Leptolyngbya*, *Chroococcus*, *Phormidium*, *Calothrix*, *Schizothrix* (Cyanophyta), *Navicula*, *Luticola*, *Hantzschia*, *Diademsis*, *Pinnularia* (Bacillariophyta), and *Kentrosphaera*, *Pleurococcus*, *Binuclearia* (Chlorophyta). Although microscopy studies are very informative, they require time-consuming sample preparation and counting, and the identification of small cells is often problematic. Recent studies on pigments from lacustrine sediments and microbial mats collected in East Antarctica (Hodgson et al., 2001, 2004a, 2005; Squier et al., 2002, 2005; Verleyen et al., 2005) show that this approach allows the identification of temporal changes in the photoautotrophic community composition and paleoenvironmental conditions. We therefore began a general survey on the water geochemistry of and photosynthetic pigments in lakes and ponds of Victoria Land with the aim of creating a database of present-day physical, chemical, and biological characteristics for future assessment of possible variations due to local climatic and/or environmental changes. Preliminary results (Borghini et al., 2007) showed that despite the low taxonomic resolution, high performance liquid chromatography (HPLC) analysis of sedimentary pigments is a valuable tool for

identifying the dominant taxa in Antarctic freshwater ecosystems. With the aim of contributing to a better characterization of sedimentary pigments in Victoria Land, this paper reports data obtained using an HPLC methodology that is more sensitive and selective than that used in previous studies. Analyses were completed on 48 water bodies with different morphometry and physical, chemical, and biological characteristics and located in ice-free areas of differing lithology, latitude, and altitude in order to identify environmental factors affecting the distribution and composition of freshwater autotrophic communities.

Materials and Methods

Field surveys and sampling were performed during four Italian Antarctic Expeditions (austral summer 2001/2002–2005/2006) in lakes and ponds located in 19 ice-free areas between Kar Plateau (76.91°S, 162.54°E) and Helm Point (72.13°S, 170.15°E; Fig. 1). All lakes were endorheic (with no surface outflow), wet-based (not frozen to their bed in winter), and proglacial (i.e. they formed as a result of glacial retreat after the Last Glacial Maximum, when Victoria Land coasts were covered by a marine-based ice sheet). Their main characteristics are summa-

TABLE 1

Location of sampled lakes, their catchment lithology, distance from the sea (m), altitude a.s.l. (m), estimated lake surface (m²), presence of birds and ice-cover (at the time of sampling).

Site	Lake/pond	Latitude (°S)	Longitude (°E)	Catchment lithology	Distance from the sea (m)	Altitude (m)	Estimated surface (m ²)	Presence of birds	Ice cover
Kar Plateau	Lake	76.91065	162.54123	Granitic	500	160	2900	Few	No ice
Depot Is.	Lake	76.70270	162.96872	Granitic	150	10	2000	Few	Partial
Mt. Murray	Lake A	76.16020	162.00270	Granitic	300	250	5800	No birds	No ice
	Lake B	76.16120	161.99807	Granitic	500	256	4600	No birds	No ice
	Lake C	76.16327	161.99303	Granitic	700	262	6200	No birds	No ice
	Lake D	76.17053	161.96810	Granitic	1000	270	4100	No birds	No ice
	Lake E	76.16238	161.99727	Granitic	500	125	3900	No birds	No ice
	Lake F	76.16433	161.99595	Granitic	500	125	2100	No birds	No ice
Tarn Flat	Lago Gardo	74.96837	162.51650	Granitic	35,000	−40*	17,700*	No birds	No ice
	Lago Tonolli	74.96790	162.51640	Granitic	35,000	−70*	3100*	No birds	Partial
	Lake 1	74.99332	162.53232	Granitic	29,000	110	3800	No birds	Partial
	Lake 2	74.97862	162.55693	Granitic	27,000	100	4700	No birds	Partial
	Lake 3	74.97220	162.55772	Granitic	26,000	90	2900	No birds	Partial
	Lake 4	74.95342	162.52882	Granitic	24,000	130	6200	No birds	No ice
Inexpressible Is.	Lake A	74.89842	163.64420	Metamorphic complexes	6000	154	7200	No birds	No ice
	Lake C	74.91038	163.68525	Granitic	2100	21	4200	Several	Partial
	Penguin lagoon	74.89927	163.71910	Granitic	10	0*	26,700*	Many	Partial
	Lake F	74.90998	163.68042	Granitic	2000	154	1900	Few	Partial
	Lake G	74.89305	163.71738	Granitic	1500	110	2000	Few	Perennial
	Lake H	74.89605	163.73362	Granitic	100	115	1700	Few	Partial
	Lake I	74.90417	163.63627	Granitic	4000	161	1900	Many	Partial
	Lake L	74.89268	163.69843	Granitic	4000	100	4000	No birds	No ice
	Lagoon	74.89444	163.68333	Marine sediment	0	0	8000	Many	No ice
	Lake	74.77553	163.65377	Metamorphic complexes	13,000	190*	2500*	Few	Partial
Adelie Cove	Lake	74.73312	163.94987	Metamorphic complexes	50	1	1800	Few	No ice
Andersson Ridge	Lake 5	74.71378	162.66815	Granitic	55,000	1000	2200	No birds	No ice
	Lake 6	74.71340	162.67000	Granitic	55,000	1000	2400	No birds	No ice
	Lake 7	74.72317	162.69383	Granitic	55,000	1000	2300	No birds	Partial
Rhodes Head	Lake	74.70416	163.04371	Granitic	35,000	350	800	No birds	Partial
Northern Foothills	Lago degli Skua	74.70000	164.08333	Granitic	2000	117 ^S	2200*	Several	No ice
	Pozza Eneide	74.69722	164.09861	Granitic	800	55	400*	Several	No ice
	Lake Enigma	74.72498	164.03316	Granitic	4000	195*	28,630	No birds	Perennial
	Lago Carezza	74.71000	164.05833	Granitic	2000	167 ^S	7900*	Few	No ice
Snowy Point	Lake	74.61532	163.76125	Metamorphic complexes	30,000	200*	2400*	No birds	Partial
Gondwana	Lago Gondwana	74.61268	164.21378	Metamorphic complexes	8000	86 ^S	3000*	Many	Partial
	Lake B	74.63277	164.22692	Metamorphic complexes	900	60*	2000*	Several	Partial
Edmonson Point	Lake 13	74.33275	165.13817	Volcanic	100	0*	17,800*	Very many	Partial
	Lake 14	74.32942	165.13292	Volcanic	500	20*	4000*	Few	Partial
	Lake 15	74.32595	165.12563	Volcanic	100	3*	3600*	Few	Perennial
	Pond C	74.33020	165.10807	Volcanic	500	3	150	Few	No ice
	Pond D	74.32918	165.10892	Volcanic	700	3	80	Few	Partial
Edmonson Point	Pond E	74.33137	165.14013	Volcanic	0	18	30	Many	No ice
	Lake	74.33137	165.14013	Volcanic	0	18	30	Many	No ice
Edmonson Point North	Lago Pantano	74.31293	165.06942	Volcanic	500	3*	4600*	Several	No ice
	Lake	74.23333	164.75000	Volcanic	100	10	1000	Few	No ice
Crater Cirque	Lake	72.60000	169.35000	Granitic	30,000	150	15,300	Many	Perennial
Luther Peak	Lake	72.37057	169.88800	Granitic	8000	390	1500	No birds	Partial
	Lake North	72.34563	169.92427	Granitic	100	1	2500	Few	Partial
Helm Point	Lake	72.13712	170.15825	Granitic	50	200	400	No birds	Partial

* From Guilizzoni et al. (1989).

^S From <http://www.pnra.it/txtgazetteer.htm>.

ized in Table 1. The names of most lakes are not official and, whenever possible, we adopted the nomenclature previously used by other researchers (e.g., Guilizzoni et al., 1989). The general features of each ice-free area and its lakes were recorded during sampling; a scale was used to express ice cover conditions (no ice, partial cover, perennial cover) and the number of seabirds nesting nearby (no birds, few, several, many).

Superficial sediments (0–3 cm, at a fixed water depth of 20 cm) were collected from different points in the littoral zone. Samples were brought back to MZS station and stored at −20 °C for transport to Italy.

About 2 g of fresh sediments were extracted at low temperature using 5 mL acetone (90%, chromatography grade), a vortex, and a sonicator. They were then stored for 1 hour at −20

TABLE 2

High Performance Liquid Chromatography (HPLC) gradient method utilized for photosynthetic pigment analysis.

Time (min)	% Methanol	% Ammonium acetate (10 mM)	% Acetone	Flow (μL/min)
0	80	20	0	800
5	80	10	10	800
45	80	5	15	1250
50	80	0	20	1500
65	80	0	20	800
67	80	20	0	800
95	80	20	0	800

°C. These operations were repeated three times, and the extracts were centrifuged and filtered at 0.45 μm. Ammonium acetate 0.1 M (10% of the sample) was added to the sample just before analysis. Throughout the extraction procedure, samples were protected from light and high temperatures. The extracts were analyzed by Liquid Chromatography–Mass Spectrometry with an Atmospheric Pressure Chemical Ionization source coupled with a Photodiode Array Detector (APCI LC-MS/PDA). Reversed phase columns (two Spherisorb ODS2 Hypersil, 150 × 4.6 mm ID, 5 μm particle size equipped with ODS2 pre-column) were used along with a solvent system (Table 2) and slightly modified gradients (10 mM ammonium acetate, 50 μL of sample) according to the method described in Pinckney et al. (1996). LC-MS analysis was performed using a Thermo system comprising a Finnigan surveyor autosampler, a MS pump, and a Finnigan LTQ. APCI LC-MS analysis was performed in positive ion mode, and MS instrument settings were as follows: capillary temperature of 250 °C, APCI vaporizer temperature of 350 °C, discharge current of 5.5 μA, discharge voltage of 4 kV, sheath gas flow rate of 40 arbitrary units (a.u.), auxiliary gas flow rate of 14 a.u., and sweep gas flow rate of 0 a.u.

Pigments and more weakly acidic MAAs (palythenic acid, palythene, palythine) were detected at their maximum wavelength and characteristic *m/z*. Peaks were identified by their absorption

and characteristic MH⁺ and MS² spectra, and their elution order through comparison with literature data (e.g., Rozema et al., 2002; Whitehead and Hedges, 2002; Yuan et al., 2009). Their concentrations were determined by comparing HPLC peak areas with those of standard solutions prepared using commercially available purified pigments from the International Agency for ¹⁴C Determination, VKI in Hoersholm, Denmark (pheophytin *a*, pheophorbide *a*, chlorophyllide *a*, chl *c*2, chl *c*3, divinyl chlorophyll *a*, lutein, peridinin, fucoxanthin, violaxanthin, canthaxanthin, zeaxanthin, alloxanthin, neoxanthin, 19'-butanohydroxyfucoxanthin, echinenone, prasinoxanthin, antheraxanthin, diadinoxanthin, 19'-hexanoyloxyfucoxanthin, lycopene, myxoxanthophyll), and from Sigma Aldrich (chl *a* and *b*, β,β- and β,β-car). Concentrations of unavailable standard molecules were estimated from the peak area, assuming a specific extinction coefficient for each compound, as reported in the literature (Hurley and Watras, 1991; Villanueva et al., 1994; Jeffrey et al., 1997). When extinction coefficients were not reported or when no structurally or spectrally similar pigments existed, the coefficient of β,β-car was used (Jeffrey et al., 1997). Data quality was checked every six samples by analyzing blanks (acetone diluted with 10% acetic ammonium), which were always below the detection limit. Replicate and duplicate samples were analyzed, and the relative standard deviation (SD) was usually <20%. Results were expressed as μg g⁻¹ TOC, because comparison between long-term monitoring data on lake plankton and the resulting varved fossil record indicates that this unit of measurement most accurately captures variations in algal abundance and community composition (Leavitt et al., 1997).

TOC in sediments was determined through a modified Walkley-Black titration method. TC and TN were analyzed with an elemental analyzer (2400 Series II, Perkin-Elmer), whereas P and S were determined by atomic emission spectrometry (Optima 5000 DV, Perkin-Elmer). Data quality was checked for each batch of samples by analyzing blanks (MQ waters were used for dilutions) and by simultaneous analysis of Standard Reference Materials with certified values. Replicate and duplicate samples were run daily, and the relative standard deviation between duplicates was always ≤1%.

TABLE 3

Water content (W), TN, TC and TOC (in %), S, and TP (expressed in μg g⁻¹ dry weight) in superficial lake sediments; *N*, number of lakes sampled at each site.

Site	<i>N</i>	W	TN	TC	TOC	S	TP
Kar Plateau	1	21.1	0.13	0.50	0.30	389	659
Depot Is.	1	20.1	0.15	1.10	0.98	657	415
Mt. Murray	6	13.0–33.5	<0.10–0.20	0.30–1.26	0.10–0.56	111–478	122–518
Tarn Flat	6	11.8–23.8	<0.10–0.23	0.14–1.09	<0.10–0.64	69–614	168–610
Inexpressible Is.	9	11.2–25.2	0.13–0.30	0.14–1.30	<0.10–1.05	182–1232	226–4758
Vegetation Is.	1	8.8	0.15	0.30	<0.10	345	464
Adelie Cove	1	16.6	0.12	0.22	0.14	338	406
Andersson Ridge	3	10.2–13.9	0.10–0.12	0.4–0.7	<0.10	69–434	109–163
Rhodes Head	1	18.3	0.14	0.58	0.24	359	324
Northern Foothills	4	8.0–42.1	0.13–0.15	0.55–1.10	0.12–0.60	297–457	353–569
Snowy Point	1	21.0	0.16	0.66	0.44	1293	574
Gondwana	2	46.8–76.5	0.24–0.26	1.53–2.13	1.30–1.95	800–1659	900–1193
Edmonson Point	6	9.3–70.1	0.24–0.28	0.83–3.20	0.11–3.12	133–989	519–837
Edmonson Point North	1	25.3	0.15	0.20	0.12	218	560
Baker Rocks	1	16.0	0.24	1.22	0.16	372	648
Crater Cirque	1	17.3	0.25	0.15	<0.10	211	700
Luther Peak	2	21.8–24.6	0.20–0.30	0.45–1.40	0.20–1.06	310–392	499–601
Helm Point	1	19.8	0.30	0.35	0.23	335	342

TABLE 4

Summary of pigment and MAA features determined by High Performance Liquid Chromatography–Photodiode Array Detector–Mass Spectrometry (HPLC-PDA-MS) in sediments.

Compound	UV/Vis absorption bands (nm)	[M+H] (<i>m/z</i>)	Fragment ions (<i>m/z</i>)	Peak number
Palythenic acid*	338	329		01
Unidentified carotenoid 1	482			02
Unidentified UV photoprotective compound 1	308			03
Palythene*	354	285		04
Palythine*	322	245		05
Unidentified UV photoprotective compound 2	376			06
Unidentified carotenoid 2	430			07
Reduced scytonemin derivative	356,456,480			08
Unidentified UV photoprotective compound 3	354			09
Unidentified UV photoprotective compound 4	310			10
Reduced scytonemin	356,442,580	547		11
Chlorophyllide <i>a</i>	432,666	616		12
Reduced scytonemin derivative	384,460,570			13
Unidentified UV photoprotective compound 5	356			14
Unidentified chlorin 1	408,666			15
Scytonemin	388	545		16
Unidentified carotenoid 3	480,506			17
Unidentified UV photoprotective compound 6	356			18
Pheophorbide <i>a</i>	410,666	593	533	19
Unidentified UV photoprotective compound 7	322			20
Scytonemin	388	545		21
Unidentified carotenoid 4	450,464			22
Unidentified carotenoid 5	482,504			23
Unidentified carotenoid 6	460,496,530			24
Unidentified UV photoprotective compound 8	322			25
Unidentified chlorin 2	408,670			26
Fucoanthin	452	641	581/563/549	27
Hydroxyspirilloxanthin*	474,496,528			28
Unidentified UV photoprotective compound 9	384			29
Unidentified chlorin 3	408,666			30
Unidentified chlorin 4	406,654			31
Spirilloxanthin*	466,496,528			32
Unidentified carotenoid 7	466,496,528			33
Neoxanthin	414,436,464	583	547/491/375	34
Unidentified carotenoid 8	450,474,500			35
Violaxanthin	416,440,468	601	583/565/509	36
Unidentified carotenoid 9	468,496,530			37
Unidentified carotenoid 10	440,474,504			38
Unidentified carotenoid 11	482,504			39
Unidentified carotenoid 12	450,474,504			40
Unidentified UV photoprotective compound 10	328			41
Unidentified carotenoid 13	452,478,504			42
Unidentified UV photoprotective compound 11	386			43
Myxoxanthophyll	450,474,504	760	691/549/567	44
Unidentified carotenoid 14	420,450,476			45
Unidentified chlorin 5	406,672			46
Unidentified carotenoid 15	448,474,504			47
Unidentified carotenoid 16	420,456,476			48
Unidentified carotenoid 17	484			49
Unidentified carotenoid 18	448,472			50
Unidentified carotenoid 19	432,454			51
Unidentified carotenoid 20	400,428,456			52
Unidentified carotenoid 21	444,474,504			53
Unidentified carotenoid 22	420,446,474			54
Unidentified carotenoid 23	444,474,504			55
Unidentified carotenoid 24	420,450,476			56
Unidentified carotenoid 25	474			57
Unidentified UV photoprotective compound 12	326			58
Unidentified carotenoid 26	422,452,478			59
Unidentified carotenoid 27	370,444,476,498			60
Unidentified carotenoid 28	370,440,470,494			61
Unidentified carotenoid 29	368,426,446,474			62
Lutein	418,446,474	551	495/429/411	63

TABLE 4

Continued.

Compound	UV/Vis absorption bands (nm)	[M+H] (<i>m/z</i>)	Fragment ions (<i>m/z</i>)	Peak number
Zeaxanthin	422,452,476	569	511/549/533	64
Unidentified carotenoid 30	468			65
Unidentified carotenoid 31	482			66
Lactucaxanthin*	414,440,468			67
Unidentified carotenoid	456,480			68
Unidentified UV photoprotective compound 13	386			69
Unidentified carotenoid 32	420,452,476			70
Unidentified carotenoid 33	450,472,500			71
Unidentified carotenoid 34	364,444,468,500			72
Unidentified carotenoid 35	474			73
Unidentified carotenoid 36	420,446,472			74
Unidentified carotenoid 37	404,428,454			75
Unidentified carotenoid 38	366,440,470,498			76
Unidentified carotenoid 39	445,474,504			77
Bacteriopheophytin c*	400,660			78
Unidentified carotenoid 40	425,452,474			79
Unidentified carotenoid 41	418,446,474			80
Unidentified carotenoid 42	452,474,504			81
Unidentified carotenoid 43	422,448			82
<i>cis</i> -Caloxanthin*	418,446,474			83
Unidentified carotenoid 44	464			84
Unidentified carotenoid 45	364,440,468,498			85
Unidentified carotenoid 46	406,424,450			86
Bacteriochlorophyll a	366,606,770			87
Unidentified carotenoid 47	474			88
Unidentified carotenoid 48	452,468			89
Unidentified carotenoid 49	362,440,468,498			90
Unidentified carotenoid 50	418,440,468			91
Unidentified carotenoid 51	444,468,498			92
Unidentified carotenoid 52	444,472			93
Unidentified carotenoid 53	428,450,474			94
Unidentified carotenoid 54	478			95
<i>trans</i> -Canthaxanthin	474	565	473/459/427	96
Unidentified carotenoid 55	420,436,464			97
Unidentified carotenoid 56	420,444,470			98
Unidentified carotenoid 57	412,438,464			99
Unidentified carotenoid 58	434,460			100
<i>cis</i> -Canthaxanthin	370,466	565	473/459/427	101
Unidentified carotenoid 59	480,676			102
Unidentified carotenoid 60	420,446,474			103
Bacteriochlorophyll a	366,606,770			104
Bacteriochlorophyll b*	458,696			105
Chlorophyll b	468,650	907	569/567/629	106
Chlorophyll b epimer	468,650	907	569/567/629	107
Unidentified chlorin 6	420,654			108
Unidentified chlorin 7	424,664			109
Hydroxychlorophyll a	428,664	909		110
Hydroxychlorophyll a epimer	430,664	909		111
Unidentified chlorin 8	420,664			112
Unidentified chlorin 9	420,656			113
Chlorophyll a	430,664	893	555/583/615	114
Echinenone	466	551	495/427/413	115
Echinenone isomer	452	551	459/427/413	116
Chlorophyll a epimer	430,666	893	555/583/615	117
Pyrochlorophyll a	410,664	835		118
Bacteriopheophytin a (phytol)	358,526,750	889		119
Pheophytin b	438,654	885		120
Pheophytin b epimer	438,654	885		121
Hydroxypheophytin a	404,666	887		122
Hydroxypheophytin a epimer	406,666	887		123
Pyropheophytin b	436,654	827		124
Bacteriopheophytin d*	406,662			125
Bacteriopheophytin d*	406,662			126
Pheophytin a	408,664	871	593/533	127

TABLE 4
Continued.

Compound	UV/Vis absorption bands (nm)	[M+H] (<i>m/z</i>)	Fragment ions (<i>m/z</i>)	Peak number
<i>trans</i> -β,β-Carotene	422,452,476	537	481/413/399	128
<i>cis</i> -β,β-Carotene	420,446,470	537	481/413/399	129
Pheophytin <i>a</i> epimer	408,666	871	593/533	130
<i>cis</i> -β,β-Carotene	414,444,468	537	481/413/399	131
Pyropheophytin <i>a</i>	410,664	813		132

* Tentative assignment.

STATISTICS

Spearman rank correlation was used to assess correlations between variables. Non-metric multidimensional scaling (n-MDS) was used to produce two-dimensional ordinations of the rank order of similarities (Anderson and Underwood, 1997) in order to compare the lakes on the basis of pigment distribution alone: a matrix of similarities between each pair of these samples was calculated using the Bray-Curtis similarity coefficient (Bray and Curtis, 1957). As a measure of the goodness of fit of a two-dimensional nMDS ordination space, we used the STRESS index (Legendre and Legendre, 1998). We adopted a scaling for which a STRESS < 0.2 indicates good performance in terms of the ability of the two-dimensional ordination to account for the relative position of samples in the multivariate space. Prior to multivariate analysis, all data were log-transformed to reduce or remove skewness. Redundancy Analysis (RDA; ter Braak, 1986; Legendre and Legendre, 1998) was used to investigate environmental factors affecting the distribution of pigments (Colacevich et al., 2009). Binary, categorical variables were introduced as dummy variates (0,1). Categorical variable with multiple, ordered levels (e.g., Ice Cover: 0, 1, and 2), were entered as dummy factors coded by a uniform sequence of integers. All analyses were performed using R version 2.8.1 (R Development Core Team, 2006: <http://www.r-project.org>). In particular, multivariate analyses were performed using the vegan package (Oksanen et al., 2006).

Results

S, TP, TOC, and TC concentrations in superficial sediments from Victoria Land lakes (Table 3) varied by one order of magnitude.

A total of 132 compounds (including pigments, UV compounds, and MAAs; Table 4) were detected in superficial sediments. Chl *a*, pheophytin *a*, and native and reduced scytonemins were the most widespread pigments. Other very common compounds were chl *b*, β,β-car, and some UV photoprotective compounds such as palythenic acid (e.g., Bandaranayake, 1998; Carreto et al., 2005 and references therein). Among algal marker pigments, the most frequent were echinenone, fucoxanthin, lutein, canthaxanthin, and myxoxanthophyll. The highest pigment concentrations were measured in sediments from lakes at Edmonson Point, Edmonson Point North, Gondwana, and Luther Peak (Table 5). Concentrations of chl *a* were significantly correlated ($p < 0.01$) with those of chl *b* ($r = 0.77$), lutein ($r = 0.67$), neoxanthin ($r = 0.51$), echinenone ($r = 0.63$), myxoxanthophyll ($r = 0.53$), zeaxanthin ($r = 0.44$), fucoxanthin ($r = 0.55$), and bacteriochlorophyll *a* ($r = 0.40$).

No significant ($p < 0.01$) correlation was found between water nutrients and pigments, whereas cyanobacterial markers (myxoxanthophyll, cantaxanthin, and echinenone) and bacterio-

chlorophyll *a* were correlated with sedimentary nutrients. All marker pigments were negatively related to latitude (chl *a*, chl *b*, lutein significantly) whereas only scytonemins were significantly and positively correlated to latitude.

Non-metric multidimensional scaling ordination (n-MDS) based on the presence-absence matrix of pigments and UV photoprotective compounds was used to identify the most similar lakes. Figure 2 shows that Lake 14, Lake 15, and Pond E at Edmonson Point, Lago Pantano at Edmonson Point North, Lagoon at Inexpressible Is., Pozza Eneide in the Northern Foothills, the two lakes at Gondwana and Luther Peak, and the lakes at Helm Point, Crater Cirque, and Depot Is. are very similar and form a cluster (in the circle). *A posteriori* data inspection revealed that they have the highest number of pigments, many of which are present in these lakes alone. In general, these lakes were characterized by the absence of any ice cover, and/or were located near the seashore and/or penguin rookeries and nesting seabirds. Another approach for interpreting the n-MDS map is to take two very distant objects and attempt to interpret the dimensions. For example, Lake G and Lake F on Inexpressible Is. and Lake 5 at Andersson Ridge differed most from the other lakes. They were characterized by a very low number of pigments.

RDA was used to study the distribution of pigments as a function of environmental characteristics (distance from the sea, latitude, altitude, ice cover, bird presence and catchment lithology) and the water chemistry (conductivity, Cl^- , NO_3^- , SO_4^{2-} , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , total Nitrogen and Phosphorous, TN and TP, and Silicates) and sediments (TN, TP, TOC and S). Only the analysis conducted using the environmental variables as explanatory matrix was statistically significant. The permutation test performed on all eigenvalues indicated that the above explanatory variables accounted for a significant (999 permutations, $p < 0.05$) portion of pigment distribution variations. Overall, the first and second RDA axes accounted for 65% and 19% of the explained variance respectively (Fig. 3); in particular, altitude (along the first axis) and latitude (along the second axis) were the two most important variables accounting for the distribution of pigments.

Discussion

Data from two lakes (Lake 14 and Lake 15) at Edmonson Point revealed a sedimentation rate of 0.033 and 0.039 cm year^{-1} , respectively (unpublished data), indicating that the each cm of sediment has accumulated over a period of 30 and 25 years, respectively. These sedimentation rates are intermediate between those reported in Progress Lake (0.14 cm year^{-1}) and Lake Reid (0.23 cm year^{-1}) in Larsemann Hills Region (Hodgson et al., 2001, 2005; Squier et al., 2005) and the sedimentation rate of Lake Hoare in the Dry Valleys ($\sim 0.015 \text{ cm year}^{-1}$; Doran et al., 1999). The latter is a perennially ice-covered lake, whereas the

TABLE 5

Average concentrations (in $\mu\text{g g TOC}^{-1}$) of major pigments in Victoria Land lakes. The epimers (of chlorophylls, pyropheophytins, pheophytins, and carotenoids) were summed with the main isomers. The reduced scytonemin (Scyt red) includes the sum of reduced scytonemin and its derivatives. $\Sigma\text{-car}$, sum of all unidentified carotenoids; $\Sigma\text{-UV}$, sum of all unidentified UV photoprotective compounds, quantified using the Scytonemin extinction coefficient. Chlide *a*, chlorophyllide *a*; Phide *a*, Pheophorbide *a*; Fuco, fucoxanthin; Neo, neoxanthin; Viola, violaxanthin; Myxo, Myxoxanthophyll; Lut, lutein; Zea, zeaxanthin; Cantha, canthaxanthin; Bchl *a*, bacteriochlorophyll *a*; Chl *b*, chlorophyll *b*; Echi, echinenone; chl *a*, chlorophyll *a*; Phytin, pheophytin; $\beta\text{-car}$, $\beta\text{-carotene}$).

Site	Lake	Scyt red	Chlide <i>a</i>	Phide <i>a</i>	Scyt <i>a</i>	Fuco	Neo	Viola	Myxo	Lut	Zea	Cantha	Bchl <i>a</i>	Chl <i>b</i>	Echi	Chl <i>a</i>	Phytin <i>b</i>	$\beta\text{-car}$	Phytin <i>a</i>	Pyrophytin <i>a</i>	$\Sigma\text{-UV}$	$\Sigma\text{-car}$
Edmonson Point North	Lago Pantano	6.07	2.10	3.49	4.99	1.63	0.41	0.21	0.71	3.99			0.39	4.57		1.77	0.37	0.67	5.42	0.73		5.04
Backer Rocks	Lake	16.67			9.49	0.62			0.65	0.69		0.21		0.88	0.12	6.89		0.010	1.01	0.68	0.95	2.05
Mount Murray	Lake F	48.81			13.70	0.09			0.18			0.02	0.18	0.13		1.45			0.72	0.00	0.53	0.89
Luther Peak	Lake North	15.29		2.93	21.78	4.70	0.66	0.83	0.62	4.71		0.85		18.07	0.40	23.98	1.71	2.00	13.62	1.19	0.65	11.67
Luther Peak	Lake	115.71			118.89	1.06	0.16	0.20	2.44	0.47		0.43	0.82	0.29	0.76	8.73		0.62	1.39	0.28		4.10
Helm Point	Lake	12.27		2.13	0.78	0.65				0.32				1.88	0.07	11.05	0.36	0.12	10.19	1.39	0.56	1.17
Depot Island	Lake	4.41		0.27	0.26	0.46	0.06	0.09		0.54				2.32	0.02	3.06	0.14	0.03	2.61	0.26	0.14	0.13
Crater Cirque	Lake	2.40		1.62	0.42	0.77	0.10	0.07	0.35	0.65				2.14		4.35	0.29	0.17	4.68	0.34	0.33	0.20
Northern Foothills	Pozza Enside	34.45			13.04					0.36		0.73	0.77	0.17	0.45	12.72		0.65	2.16	0.18	0.71	5.08
Northern Foothills	Lago Carezza	16.54			8.79	0.06			0.17							2.02		0.01	1.22		0.44	
Northern Foothills	Lago degli Skua	5.30			1.97	3.23										13.80		0.38	4.07		2.22	9.10
Inexpressible Island	Lake F															1.35			0.03			
Mount Murray	Lake E	3.32			3.91											0.31			0.33		1.55	
Northern Foothills	Lake Enigma	1.65														0.18					2.09	11.03
Inexpressible Island	Lake G													0.73		4.61		0.02	1.46		4.64	13.38
Inexpressible Island	Lake H	2.05			4.41											2.24			3.09		6.34	
Inexpressible Island	Lake I	0.34			0.35											0.07			0.13		0.98	0.54
Tarn Flat	Lake 4	0.34			0.11											0.23			0.14	0.03	0.03	0.01
Mount Murray	Lake B	9.12			2.31											0.12	0.16		0.66	0.09	1.77	
Mount Murray	Lake A	20.47			8.98											0.62			0.57	0.09	0.11	0.09
Kar Plateau	Lake	37.23			6.49				0.17	0.46		0.26		0.89		1.26			1.70		4.73	5.19
Tarn Flat	Lake 1	3.61			11.50											3.17			0.45		3.41	2.40
Tarn Flat	Lake 3	0.36			0.64														0.07		1.95	0.32
Tarn Flat	Lake 2	2.94			2.73											0.62			0.25		1.69	0.74
Inexpressible Island	Penguin Lagoon				0.20	0.04											0.13		0.57		3.41	3.30
Mount Murray	Lake D	9.29			3.72														0.75		1.52	
Mount Murray	Lake C	14.87			1.40														0.43		3.19	6.14
Edmonson Point	Pond C	77.96			20.59				0.31		0.37			8.64		3.41		0.21	1.62	9.11	4.43	0.61
Edmonson Point	Pond D	101.28			17.18				0.63	0.20	0.03	0.24		1.19		3.43			2.26		6.06	0.99
Inexpressible Island	Lake C	0.29												0.00		0.00			3.28		1.48	1.09
Inexpressible Island	Lake A	0.33			0.08	0.01				0.07	0.01	0.03		0.14		0.41			1.26	0.17	2.06	2.14
Edmonson Point	Lake 13															0.89			1.88	0.15	16.93	3.09
Edmonson Point	Pond E									16.57			5.78	45.08		36.17	1.54	0.26	15.70	3.32	0.08	0.82
Edmonson Point	Lake 14	12.29		0.59	6.34				0.69	0.03	1.23	0.09	1.81	0.30	0.24	7.92		0.05	2.44	0.41		5.45
Edmonson Point	Lake 15	1.42		2.76	1.29	0.57	0.05		1.99	1.24	0.48	0.09	1.46	1.11	0.11	14.37		0.75	12.68	0.67		3.31
Gondwana	Lago Gondwana	28.99		0.31	9.46		0.05		0.33	0.11	0.02	0.06	0.38	0.63		2.91			0.39			1.37
Andersson Ridge	Lake 5	9.31			4.25	3.36			0.14	0.28			0.39	3.09	0.01	4.11			1.11	0.05	0.31	1.69
Gondwana	Lake B	0.86	0.39	0.14	0.18	0.11	0.05		0.14				0.39			7.17	0.04				21.35	0.55
Andersson Ridge	Lake 6				59.22				0.39	0.03				0.13		5.24					0.47	
Inexpressible Island	Lake L	1.84			0.90	0.02										2.03			1.17			

TABLE 5
Continued.

Site	Lake	Scyt red	Chlide <i>a</i>	Phide <i>a</i>	Scyt	Fuco	Neo	Viola	Myxo	Lut	Zea	Cantha	Bchl <i>a</i>	Chl <i>b</i>	Echl	Chl <i>a</i>	Phytin <i>b</i>	β,β -car	Phytin <i>a</i>	Pyrophytin <i>a</i>	Σ UV	Σ -car
Vegetation Island	Lake	45.42			35.31	9.58				0.35	1.81		0.36	0.42		2.18						
Inexpressible Island	Lagoon				0.69											16.32		0.31	0.26	0.28	0.70	6.04
Andersson Ridge	Lake 7					0.30										2.20						2.56
Tarn Flat	Lago Gardo	11.76			3.62											1.60						0.11
Tarn Flat	Lago Tonolli	8.19			1.72	0.83										1.16			5.26		2.56	1.42
Rhodes Head	Lake	54.56			0.16	0.03						0.33				1.48			2.54		0.68	2.36
Snowy Point	Lake	4.77		0.20	0.01											0.45	0.52		4.48	1.27	0.16	0.08
Adelie Cove	Lake	0.01							0.03	0.01						0.10						

Larsemann Hills lakes have a longer ice-free period than our studied lakes due to the milder climate conditions of the region. Thus, even if uncertainty about sedimentation rates at the other sampling sites made estimation of annual pigment fluxes impossible, it is plausible to hypothesize that generally the superficial 3 cm samples have accumulated over a period of some tens of years.

S, TP, TOC, and TC concentrations in superficial sediments, as well as average TOC/TN and TOC/S ratios (2.7 and 8.8, respectively), were in the same range as those determined in the previous investigation of Victoria Land lakes (Borghini et al., 2007), whereas a much higher number of compounds were detected in superficial sediments.

Assuming chl *a* as an indicator of overall photoautotrophic biomass; chl *b*, neoxanthin, violaxanthin, and lutein as indicators of Chlorophyta; echinenone, myxoxanthophyll, zeaxanthin, and canthaxanthin as indicators of cyanobacteria; and fucoxanthin as an indicator of Bacillariophyta and Crysophyta, results indicate that Cyanophyta is the most abundant algal group in Victoria Land lakes. The presence of lutein and fucoxanthin in most sediments indicates that Chlorophyta and diatoms are also widespread, along with anaerobic bacteria (as indicated by bacteriochlorophylls and their degradation products). The correlations of chl *a* with chl *b* and lutein and echinenone and myxoxanthophyll indicate that primary production is due especially to Chlorophyta and Cyanobacteria.

Chlorophylls are susceptible to a variety of transformation reactions. Many attempts have been made to identify specific sources of particular chl *a* products. While studying chl *a* oxidation products in Kirsjes Pond (Larsemann Hills), Walker et al. (2002) found a relationship between the extent of chl oxidation and the residence time of the pigment in an oxygenated water column. Chlorine steryl esters (CSEs) are usually regarded as specific markers of grazing activity, and Squier et al. (2005) suggested that their occurrence in a sediment core from Progress Lake (East Antarctica) could be used to infer a marked change in community composition during the last interglacial period when the pressure of grazing had a significant impact on the phytoplankton community. No CSEs were found in the superficial sediments of the studied lakes, indicating that this area is characterized by truncated food webs with low grazing rates.

In general, pigment profiles and concentrations in the surface sediments of Victoria Land lakes were in the same range as those reported for several lakes in the Larsemann Hills (Squier et al., 2002; 2005), and the detected taxa corresponded to those reported in previous microscopy studies on cyanobacterial and algal mats from some of the sampled lakes (e.g., Broady, 1987; Fumanti et al., 1997; Cavacini and Fumanti, 2005).

Multivariate (n-MDS) analysis suggests that there are large-scale gradients in the diversity of pigments, and altitude and latitude were the two most important variables accounting for the distribution of pigments. RDA analysis partly confirms the results of Discriminant Function and Canonical Correspondence analyses completed in a previous study (Borghini et al., 2008): these analyses indicate that the distribution of pigments in Victoria Land lakes depends mainly on distance from the sea and the nutrient status. Trend of decrease in species diversity with latitude has been attributed to the harshness of the environment such as the period of ice cover and light intensity and to geographical isolation (Jones, 1996). The positive relationship between scytomemins and latitude seems to indicate higher UV levels at higher latitude. Recently, light availability was found to be a major controlling factor of lake productivity in nutrient-poor lake ecosystems (Karlsson et al., 2009). The distance from the sea

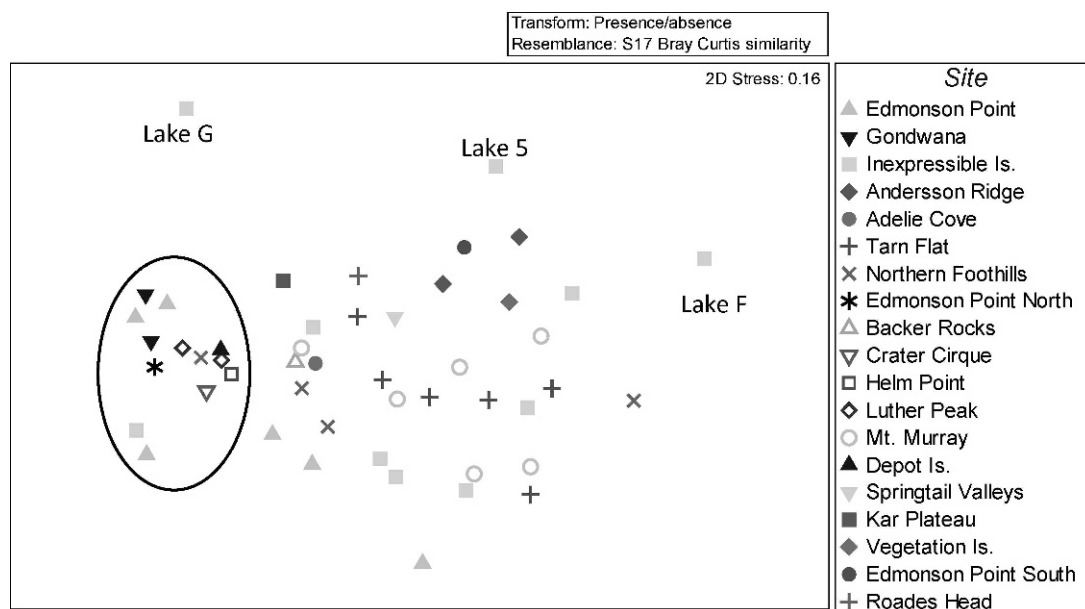


FIGURE 2. n-MDS bi-dimensional plot of pigments in Victoria Land sediments. The lake similarity matrix was based on the Bray-Curtis index and the presence/absence distribution of pigments. The STRESS index (a multivariate descriptive statistics) is less than 0.2, suggesting that the two-dimensional ordination plot adequately depicts the similarity matrix.

could influence microbial mat composition and structure because the lakes located close to the sea had a shorter ice cover period than the plateau lakes, likely due to their altitude and salt content causing a delay in freezing and earlier melt out; moreover, they are generally nutrient enriched from the sea. The Victoria Land (from Cape Adare, 71°S, to the southern end of Ross Island, 86°S) contains the most extensive coastal gradient in Antarctica and includes a variety of habitats. Important environmental factors including solar radiation, temperature, day length, and sea ice cover vary predictably along this gradient and are likely to exert a significant influence on ecological processes (Howard-Williams et al., 2006). In these conditions it may be expected that species

composition will change and species diversity may fall as latitude increases with the few remaining species adapted to extreme southern conditions. Differences in biomass and community composition within fast ice in two locations separated by five degrees of latitude, the same interval of the present study, along the Victoria Land coast were found by Ryan et al. (2006) and were attributed to significant differences in the physicochemical characteristics of each site.

However, RDA analysis explained about one-third of the total pigment variance, suggesting that pigment assemblages are affected by other variables. It is recognized that the latitudinal gradient approach is complicated by local variability at each

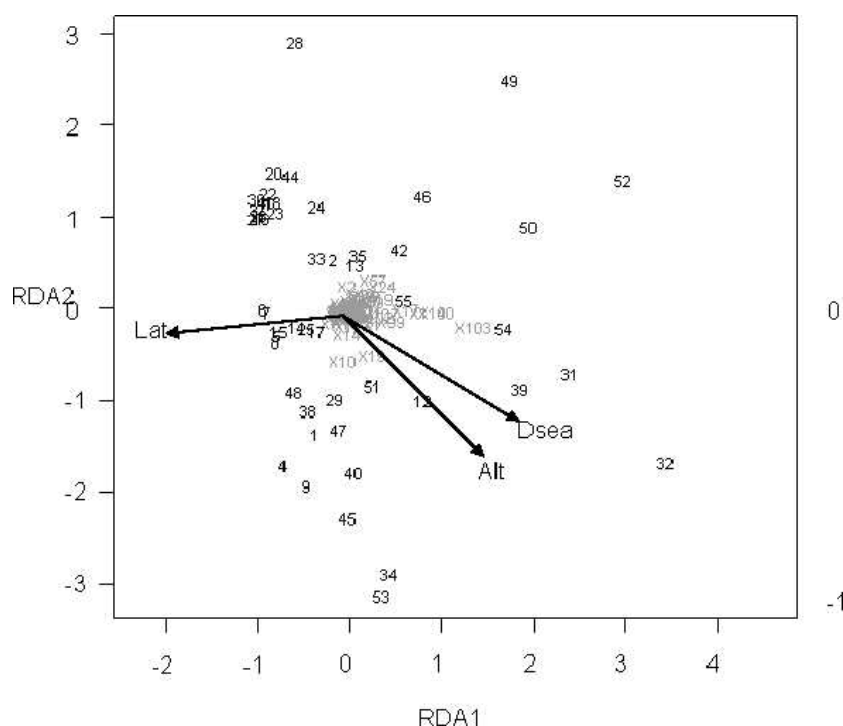


FIGURE 3. RDA multivariate model [Pigments = $f(\text{Latitude} + \text{Altitude} + \text{Ice} + \text{Seabirds} + \text{DSea})$] for the pigment concentration ($\mu\text{g g TOC}^{-1}$) matrix. Only explanatory variables (arrows) with detectable, large effects were depicted: Lat, latitude; Alt, altitude; DSea, distance from the sea. The numbers indicate the progressive numerical code for each lake, whereas the labels of the type “X followed by a number” are abbreviations for the pigments. These two sets of codes are here reported to give a rough indication of the dispersion of pigments (dependent and descriptive variables) and lakes (ordered objects) in the ordination space.

latitude due to altitudinal differences, topography, and microclimate (Howard-Williams et al., 2006). Hodgson et al. (2001, 2004b) found that geographical location, water depth, and conductivity can affect the composition of sedimentary pigments. The more saline lakes were characterized by higher abundances of chl *b* and carotenoids of green algae; diatom carotenoids occurred in all lakes but were relatively less abundant in freshwater ponds (Hodgson et al., 2004b). However, the environmental factors influencing mat composition and structure are complex and differ among the various taxa; also species of the same genus have different distribution. It was suggested that the oscillatorian species were likely distributed based on different conductivities of ponds in the McMurdo Ice Shelf, whereas Nostocales abundance seemed to be independent of conductivity (Jungblut et al., 2005). Likewise, in both continental and maritime Antarctica, *Prasiola crispa* (Chlorophyta) is found in the vicinity of bird colonies where there is considerable nutrient enrichment, whereas *P. calophylla* is found in habitats not experiencing nutrient enrichment and is absent from areas where there are high salt concentrations (Broady, 1989). In the Signy Is. lakes, Pearce (2005) found that the bacterioplankton community structure cannot be explained by physicochemical parameters alone, although nutrient concentrations and the timing and duration of ice cover can determine changes in these communities; biotic factors are probably also important. Latitude and altitude are surrogates of spatial environmental variations, and the distribution of compounds is probably a function of many environmental properties and factors (e.g., temperature, ice cover persistence, UV radiation, and nutrient availability) acting at different spatial scales.

Lacustrine water productivity varied by one order of magnitude in 15 subarctic lakes distributed within an altitude gradient reflecting a temperature gradient. This variation was mainly caused by variations in the duration of the ice cover and especially in the supply of organic carbon and nutrients (Karlsson et al., 2005). As for other terrestrial environments, Freckman and Virginia (1997) found that a variety of soil factors may together define suitable or inhospitable habitats for soil nematodes at local and regional scale; Powers et al. (1998) in the Taylor Valley soil did not identify a single soil property that fully explains the distribution, biodiversity, or community structure of these invertebrates.

Conclusions

In summary, the results from this study:

- (1) were used to compile a detailed database on sedimentary pigments that includes a larger number of compounds than found by previous studies and expands our knowledge of lakes in a relatively unknown but limnologically important region of Antarctica;
- (2) show that Cyanophyta is the most abundant and widespread algal group, followed by Chlorophyta and Bacillariophyta, in agreement with microscopy studies conducted by other researchers in a few lakes in the same area;
- (3) confirm that the density and diversity of autotrophic communities increase in naturally euthrophized water bodies without an ice cover located in coastal ice-free areas;
- (4) show that environmental variables, particularly latitude and altitude, are the main factors affecting pigment distribution;
- (5) support the usefulness of a pigment-based approach in the study of lacustrine biotic components.

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