Bacterial numbers in the freshwater bodies of a sub-Antarctic island

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Bacterial numbers in Marion Island (46°54'S, 37°45'E) lentic waterbodies (shallow lakes, lava lakelets and crater lakes) not influenced by animals varied between 2.4×10^5 and 24.5×10^5 cells ml^{-1}. A mountain tarn 1 100 m above sea level contained only 0.9×10^5 cells ml^{-1} and streams $(1.0-4.5) \times 10^5$ cells ml^{-1}. Waters influenced by seabird and seal manuring exhibited higher counts (up to 63.2×10^5 cells ml^{-1}) and NH_4-N and soluble reactive P concentrations accounted for 51% and 4% respectively of the total variation in bacterial numbers in 49 waterbodies.

Die aantal bakterieë in lentiese waters van Marion-eiland (46°54'S, 37°45'O), wat nie deur diere beïnvloed word nie, het van 2.4×10^5 en 24.5×10^5 selle ml^{-1} gewissel. In 'n bergmeertjie (1 100 m bo seevlak) is slegs 0.9×10^5 selle ml^{-1} bepaal terwyl die stroompies 1.0 tot 4.5×10^5 selle ml^{-1} bevat het. In waters wat deur voëls en robbe beïnvloed was, is seltellings van tot 63.2×10^5 ml^{-1} bepaal. NH_4-N en opgeloste reaktiewe P-konsentrasies het respektiewelik 51% en 4% van die totale variasie in bakteriegetalle in 49 waterliggame verklaar.

Introduction

Marion Island (46°54'S, 37°45'E) is a small island (290 km^2) situated in the southern Indian Ocean approx. 2 000 km SE of Cape Town. It experiences a typical sub-Antarctic oceanic climate, characterised by strong wind, high precipitation (± 2 500 mm per annum), general cloudiness and moderately cold weather throughout the year (mean temperature of coldest month, 3.2°C; of warmest month, 7.3°C). The island is volcanic in origin and consists of grey, preglacial and black, postglacial lava types. There is a striking contrast between the glaciated grey lava areas and the rugged black lava flows.

The lentic freshwater bodies of the island have been classified into four types (Grobbelaar 1975). *Lakes* (surface areas 1 000 — 100 000 m^2) occur mainly on the impervious grey lava and are generally less than 1.5 m deep. *Lava lakelets* occur predominantly on black lava and mostly have surface areas < 1 000 m^2. Lakes and lava lakelets may be further distinguished according to whether or not they are "biotically" influenced, i.e. enriched with nutrients through manuring by seabirds or seals. Owing to their shallowness and because of the prevailing strong winds, thermal stratification does not occur in the lakes and lakelets. *Crater lakes* are found in the craters of scoriaceous cones at mid to low altitudes (< 400 m above sea level). *Wallows*, caused by the activities of elephant seals (*Mirounga leonina*), are common on the shore zone. Coalescing wallows may form a fairly large depression which rapidly fills with water. In addition to wallows, small coastal pools occur which are heavily influenced by penguins, sub-antarctic skuas (*Catharacta antarctica*) and kelp gulls (*Larus dominicanus*).

The lotic waters of the island consist of streams, although the term "river" has been used in naming the more perennial of these. Due to the porosity of the black lava, most of the rain-water reaches the sea by subsurface drainage and streams occur predominantly on grey lava deposits.

Several bacteriological investigations have been carried out on saline and freshwater lakes in the Antarctic (Goldman *et al.* 1967, Koob & Leister 1972, Kriss *et al.* 1976) and maritime Antarctic (Ellis-Evans 1981a,b, Herbert & Bell 1973, Stanley & Rose 1967). However, very little freshwater bacteriological information is available for the sub-Antarctic region (Lindeboom 1979). In April and May 1982 direct counts of bacteria were carried out on a variety of waterbodies at Marion Island and these, along with some associated water chemistry parameters, are presented in this paper.

Materials and Methods

Fifty waterbodies were each sampled on one occasion over a two-week period in April/May 1982. Water samples were collected in sterile test tubes from a depth of *ca.* 20 cm near the edge of the waterbodies. Two samples from each waterbody were subjected to bacteriological enumeration as follows: Within 6 h of collection the tubes were shaken and 2 ml subsamples stained with 0.2 ml 0.1% acridine orange and filtered through 0.22 μm Nuclepore® membrane filters (Nuclepore Corporation, U.S.A.) prestained with Irgalan Black (Hobbie *et al.* 1977). For water containing especially low bacterial numbers a larger subsample was passed through the filter before adding the acridine orange. Bacteria on the filters were counted under oil immersion using a Zeiss Standard 14 microscope fitted with a IV FL epifluorescence condensor, HBO 50 mercury lamp, FT 510 dichromatic splitter, LP 520 barrier filter and BP 450-490 band-pass filter. At least 200 bacteria were counted per filter.

The remainder of the water sample was filtered and used for chemical analysis. NH_4-N was determined by the phenol-hypochlorite reaction (Solorzano 1969), NO_2-N by the Greiss-Ilosvay reaction (Mackereth *et al.* 1978) and NO_3-N as NO_2-N after reduction with spongy cadmium. "Soluble reactive P" was determined as the reduced phosphomolybdate-blue complex (Murphy & Riley 1962) and chloride estimated using an "Aquaquant" reagent kit (Cat. no. 14401, E. Merck, Darmstadt, West Germany) which depends on the formation

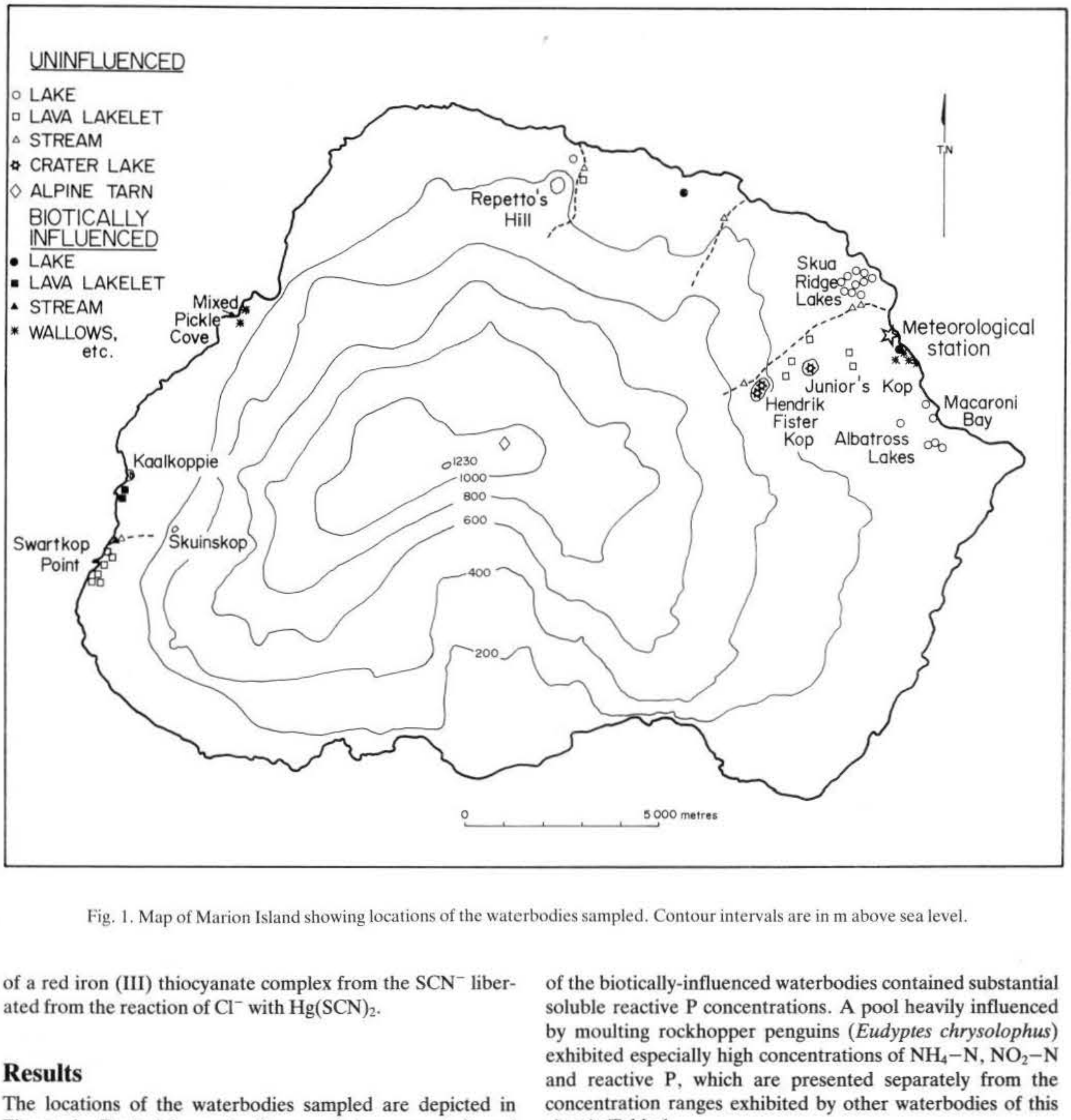

Fig. 1. Map of Marion Island showing locations of the waterbodies sampled. Contour intervals are in m above sea level.

of a red iron (III) thiocyanate complex from the SCN^- liberated from the reaction of Cl^- with $Hg(SCN)_2$.

Results

The locations of the waterbodies sampled are depicted in Figure 1. Bacterial counts (mean of two samples) and inorganic N, soluble reactive P, and Cl^- concentrations are summarised by waterbody type in Table 1. Differences in counts for duplicated samples generally increased with increasing bacterial numbers, but were mostly within 10% of the mean value. Also included in Table 1 are the water temperatures at the time of sampling, altitudinal ranges and distance from the nearest seashore.

Waterbodies not influenced by animals did not contain detectable levels of inorganic forms of N and only one (a small lava lakelet) contained a detectable concentration of reactive P. Inorganic N could also not be detected in biotically influenced lava lakelets. However, the two biotically-influenced lakes (Prinsloo Lake on the north coast and Gentoo Lake on the east coast) and all the smaller pools affected by seals and birds exhibited substantial levels of inorganic N. Only one stream influenced by animals (wandering albatrosses, *Diomedea exulans*) was sampled and contained an appreciable concentration of NH_4-N but no NO_2 or NO_3-N. Virtually all of the biotically-influenced waterbodies contained substantial soluble reactive P concentrations. A pool heavily influenced by moulting rockhopper penguins (*Eudyptes chrysolophus*) exhibited especially high concentrations of NH_4-N, NO_2-N and reactive P, which are presented separately from the concentration ranges exhibited by other waterbodies of this class in Table 1.

During the counting procedure no distinction was made between bacteria apparently free in the solution and those attached to particles. However, most of the bacteria in the plankton appeared to be free-living. Notes were made of the morphology of the cells counted. On average, rod-shaped bacteria made up approx. 75 per cent of the total count, cocci 22 per cent and the rest were spiral or crescent-shaped. Various proportions of the bacteria fluoresced orange or red, rather than yellow-green, especially in samples from heavily influenced waterbodies. Orange-red fluorescing bacteria have been interpreted as being metabolically active, due to a predominance of RNA over DNA (Hobbie *et al.* 1977). All bacteria in the sample from the rockhopper penguin-influenced pool fluoresced bright orange-red, as did a large proportion of those in wallow samples.

Biotically-influenced waterbodies contained greater numbers of bacteria than uninfluenced ones. West coast lakes and lava lakelets of the uninfluenced category also tended to

Table 1
Ranges of variable values for the various types of waterbodies.

Water body	Distance from shore (m)	Altitude (m above sea level)	Temperature at sampling (°C)	NH_4-N ($\mu g\ ml^{-1}$)	NO_2-N ($\mu g\ ml^{-1}$)	NO_3-N ($\mu g\ ml^{-1}$)	Sol. react. P ($\mu g\ ml^{-1}$)	Cl^{-1} ($\mu g\ ml^{-1}$)	Bacteria (10^5 cells ml^{-1})	N
Biotically uninfluenced										
Lakes	30–900	15–80	3–10	0	0	0	0	15–30	2.8–10.5	17
Lava lakelets	170–3000	5–250	6–10	0	0	0	0–0.6	10–50	2.4–24.5	12
Crater lakes	2450–4000	300–330	5–7	0	0	0	0	<5	3.8–5.8	3
Alpine tarn	7800	1100	0.5	0	0	0	0	<5	0.9	1
Streams	500–4200	10–300	6–10	0	0	0	0	10–20	1.0–4.5	6
Biotically influenced										
Lakes	10–300	5–10	9–10	0.29–0.30	0.01–0.05	0.09–0.45	0.16–0.22	15–100	45.0–63.2	2
Lava lakelets	100–140	5	6–7	0	0	0	0.19–0.31	15–20	21.5–40.0	2
Streams	500	10	6	0.20	0	0	0.11	15	12.0	1
Wallows, penguin puddles etc.	2–200	3–30	6–11	0.16–0.58 (2.93)*	0–0.08 (3.00)*	0–0.27	0–0.37 (5.12)*	20–500	12.0–50.0 (0,02)**	6

*Concentration in a rock pool highly contaminated with penguin guano and feathers.
**Count for a small pool heavily influenced by fur seals.

Table 2
Correlation coefficient matrix for data summarized in Table 1.

	Bacteria	NH_4-N	NO_3-N	Reactive P	NO_2-N	Chloride	Temp.	Distance from shore	Altitude
Bacteria	1								
NH_4-N	.717***	1							
NO_3-N	.558***	.592***	1						
Reactive P	.630***	.666***	.447**	1					
NO_2-N	.454**	.611***	.668***	.664***	1				
Chloride	.343*	.288*	.301*	.199	.399**	1			
Temperature	.345*	.345*	.245	.249	.359*	.212	1		
Distance from shore	−.354*	−.247	−.149	−.235	−.178	−.177	−.302*	1	
Altitude	−.303*	−.208	−.119	−.209	−.139	−.143	−.342*	.926**	1

exhibit higher bacterial counts than their counterparts on the east coast. Crater lakes contained bacterial numbers similar to those of the east coast lava lakelets. Streams not affected by animals exhibited bacteria counts corresponding to the lower part of the ranges found for lakes and lava lakelets. The mountain tarn (Table 1) was a small lava lakelet, the edge of which was covered by thin ice. This waterbody contained a low number of bacteria. One small pool very heavily influenced by fur seals (*Arctocephalus tropicalis*) possessed substantial concentrations of NH_4-N (0.58 $\mu g\ ml^{-1}$, NO_3-N (0.27 $\mu g\ ml^{-1}$) and P (0.31 $\mu g\ ml^{-1}$) but contained the lowest number of bacteria recorded for all the waterbodies (0.02×10^5 cells ml^{-1}; reported separately in Table 1). The reason for this cannot be explained.

The distinction between biotically-influenced and uninfluenced waterbodies is quantitative rather than qualitative and many of the latter category may receive some nutrients originating from vertebrate fauna activities (e.g. enhanced NH_4-N occurs in the precipitation near penguin rookeries). A small number of subantarctic skuas bathe in one of the Skua Ridge Lakes (designated as no. 4 in physico-chemical and primary production investigations; Grobbelaar 1974, 1975). This lake exhibited a significantly higher phytoplankton production than did the adjacent no. 3 lake although no difference in chemical composition was apparent (Grobbelaar 1975). Bacterial numbers in Skua Lake 4 (5.9 to 6.4×10^5 cells ml^{-1}) were higher than those ($3.4-3.6 \times 10^5$ cells ml^{-1}) in Skua Lake 3.

Table 2 presents the simple correlation matrix for all nine variables for the fifty waterbodies. Bacterial counts and the biogenic elements (P and inorganic forms of N) were highly correlated. The next obvious group of intercorrelated variables consisted of distance from the sea-shore and altitude, both of which were negatively associated with temperature but not significantly correlated with any of the chemical variables, even Cl^-. Bacterial numbers showed a significant positive association with temperature and were negatively correlated with altitude and distance from the shore.

The relative contributions of the temperature, chemical and positional variables to the variation in bacterial numbers for 49 of the waterbodies were evaluated by stepwise multiple regression analysis. The rockhopper penguin-influenced pool was excluded from this analysis. The regression results (Table 3) indicate that 51.4 per cent of the variation in bacterial counts was accounted for by NH_4-N levels. Including reactive P concentrations in the regression equation significantly increased the explained variation by 4.2 per cent. The contribution of all the other independent variables, collectively or individually, was insignificant at $P \leq 0.05$. Including the data for the penguin-influenced pool in the analysis decreased the importance of NH_4-N in the regression suite (explained variation 28 per cent, $P = 0.001$) but greatly enhanced that of NO_2-N (explained variation 30 per cent, $P = 0.001$). The status of the other independent variables remained unchanged, with reactive P contributing 4.6 per cent ($P = 0.05$) to the total variation and the rest being insignificant.

Table 3
Results of the multiple regression analysis of data summarised in Table 1.

Independent variable	% variation explained	P
NH_4-N	51.4	0.001
Reactive P	4.2	0.05
Distance from shore	2.7	N.S.
NO_2-N	2.9	N.S.
NO_3-N	2.2	N.S.
Cl^-	2.3	N.S.
Temperature	1.0	N.S.
Altitude	0.9	N.S.

Discussion

Bacterial numbers in waterbodies not influenced by animals at Marion Island varied from 0.9×10^5 cells ml^{-1} in a mountain tarn to 24.5×10^5 cells ml^{-1} in a lava lakelet on the west coast. These counts are within the range of those ($0.9 - 63 \times 10^5$ cells ml^{-1}) recorded for Arctic tundra ponds at Barrow, Alaska and for an Arctic lake (Ikroavik) near Barrow ($0.5 - 58 \times 10^5$ cells ml^{-1}; (Hobbie *et al.* 1980). Bacterial counts for lakes and lava lakelets on the east coast of Marion Island varied between 2.4 and 10.5×10^5 cells ml^{-1}, similar to those found at mesotrophic Heywood Lake at Signy Island, maritime Antarctic (Ellis-Evans 1981b) but slightly higher than for oligotrophic Moss Lake at the same site (Ellis-Evans 1981a). The mountain tarn at Marion Island contained similar numbers of bacteria to Moss Lake and also to Char Lake in the High Arctic, considered to be ultra-oligotrophic (Morgan & Kalff 1972). Lowest counts recorded for fresh waters in the southern subpolar region are from Lake Bonney on the Antarctic continent where 0.009×10^5 cells ml^{-1} were found at 5 m depth (Goldman *et al.* 1967).

Primary production rates in the island's nonbiotically-influenced waterbodies are amongst the lowest recorded in the world (Grobbelaar 1974), ascribed to the rigorous climate, poor water quality and low numbers of planktonic algae. Biotic enrichment stimulates C fixation rates, the magnitude of the enhancement depending on the extent of fertilisation. Wallows, which represent extreme degrees of enrichment, exhibited the highest aquatic primary production and the largest populations of zooplankton (Kok 1977). Laboratory experiments showed no significant increases in the growth response of algae when either N, P or micronutrients were added to freshwaters from the island but simultaneous addition of N and P increased algal growth potential up to 280 fold (Grobbelaar 1978). Simultaneous addition of N, P and trace elements had no effect on bacterial growth in freshwater samples from the island but addition of an energy source such as glucose, together with the inorganic nutrients, strongly stimulated growth (Lindeboom 1979). It therefore seems likely that, in addition to the direct influence of the increased nutrient status of biotically-influenced waterbodies, the enhanced bacterial numbers are also caused by a greater algal productivity in these bodies. In addition, they are also a response to the energy-rich proteins, lipids and uric acid in the animal excrement.

The correlation between freshwater bacterial counts and NH_4-N and reactive P concentrations is similar to a previous observation that much of the variation in soil bacterial numbers at Marion Island could be ascribed to soil NH_4-N and "available" P contents (Smith & Steyn 1982). Current studies are focusing on the activities of soil and freshwater bacterial populations at manured and non-manured sites.

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