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# Heterotrophic Activity, Bacterial Types and Abundance in Different Ecosystems of the Queen Maud Land

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**Abstract**

Microbiological studies from the marine, limnetic, terrestrial and glacial ecosystems were carried out during the Ninth Indian Expedition (1989-90) to estimate the bacteria! numbers, to characterise the generic types and also to estimate the uptake of $^{14}C$ glucose and glutamic acid by these heterotrophs. Bacterial communities in different habitats appeared to be having distinct ecosystem specialisation both in terms of number and activity. In the maritime Antarctic, their biomass was found to be substantially high. Vertical profiles of bacterial counts biomass decreased gradually and uniformly with increasing depth in the polynyas. A higher recovery of viable population from 100m was seen in the pelagic waters within the Antarctic Convergence. Distribution of bacterial populations in freshwater, terrestrial and glacial habitats in the Schirmacher Oasis showed a remarkable ecological adaptation. They were more abundant in the habitats where there was accumulation of organic matter. Interestingly, marine bacterial populations were persistent and highly active in the materials of marine origin (eg. flesh and bone remains of petrels) that were found far away in glacial and/or terrestrial regions. Production of extracellular enzymes in the freshwater bacterial strains was more pronounced than those of the marine origin and measurements of heterotrophic uptake of labelled substrates showed a distinctly different uptake pattern in the freshwater, polynyal and offshore natural microbial assemblages. Results of these studies are discussed with an emphasis on the role of microbial population in the oxidation and biotransformation of organic matter and in the trophodynamics of the Antarctic regimes.

## Introduction

Much of the microbiological research in the Antarctica is still in the "what is there?" phase of discovery (Franzmann *et al.,* 1990; Huntley *et al.,* 1991 and Labourn-Parry *et al.,* 1991). Studies on micro-organisms and microbial processes began only very lately and our understanding of these aspects has radically changed over the last 2 to 3 decades. Earlier investigators thought that bacteria.would strongly be inhibited by the perpetual low temperatures and the rate of oxidation of dissolved organic matter would be extremely low (see Sorokin, 1971). However, recent studies carried out with new enumeration and assay techniques have disproved such contentions (Hanson, 1982; Hempel, 1985; Holm-Hansen, 1985; Tanoue and Hara, 1986; Verlencar era/., 1988 and Hand, 1980).

The Indian efforts on understanding the Antarctic microbiology were initiated by Matondkar (Matondkar and Gomes, 1983) followed by Shivaji (1987) and Matondkar and Parulekar (1987) leaving much of the microbial community characteristics unelucidated.

This paper deals with the bacteriological studies carried out on their types, abundance and activity, during the Ninth Indian Expedition to Antarctica.

## Materials and Methods

Sampling from different ecosystems carried out in the Indian sector of the Southern Ocean and in the Schumacher Oasis of the Queen Maud Land included water, sea-ice, phytoplankton, zooplankton, krill gut contents of seabirds (marine); lake water and ice, sediments, lichens, mosses, cyanobacterial mats (limnetic); dry soils, dead remains of snow petrels (terrestrial); glacial ice and faeces and feather droppings of Adelie and Emperor penguins (glacial). Oceanographic stations in the maritime Antarctic and freshwater lakes in the Schumacher Oasis, from where the samples were collected, are shown in Figs 1 and 2. Quantitative enumeration of viable populations of bacteria from all the above samples was carried out following standard microbiological methods. Membrane (0.22 μm; Millipore) filtration technique for ice and water samples and serial dilution and spread plate techniques for all other samples were employed for enumeration purposes. Water and ice samples were suitably preserved and brought to NIO for the determination of Total Direct Counts. Acridine Orange was employed for staining bacterial cells and total bacterial cells were counted by epifluorescence microscopy as per the procedure given by Parsons *et al.,* (1984). Seawater nutrient agar and ZoBell's marine agar 2216e were used for enumerating viable marine bacterial populations. Nutrient agar prepared with the lake water was used for enumerating viable bacterial populations from the terrestrial, glacial and limnetic samples. All the plates were incubated at ambient temperatures of 0 to 8°C for 12 to 15 days and the bacterial colonies counted. After enumeration, over 1100 colonies from all these samples were picked up randomly and purified for further investigations. Biochemical characterisations of most of these strains was carried out by following Oliver (1982). Many marine and freshwater strains were also screened for the production of extracellular enzymes following the procedures of Baumann and Baumann (1981) and Espeche *et al.,* (1987).

In one set of experiments, production of extracellular enzymes such as amylase, gelatinase, lipase, citrate kinase and urease by 150 marine strains and 100 freshwater strains of bacteria was examined. In another set of experiments, about 450 strains of marine bacteria isolated from seawater samples were screened for 8 different extracellular enzymes. Production of these enzymes was examined to understand the extent of the bacterial role in the breakdown of organic matter.

The rate of uptake of C labelled glucose and glutamic acid by the natural microbial assemblages in the lake, glacial ice, sea ice and seawater was measured following the method of Gocke (1977). The percentage of $^{14}C$ substrates assimilated by the heterotrophic bacteria was calculated for each of 5 different concentrations provided for all the samples.

Particulate organic carbon (POC) was also determined following Parsons *et al.,* (1984) from the water samples brought to NIO under deep frozen conditions.

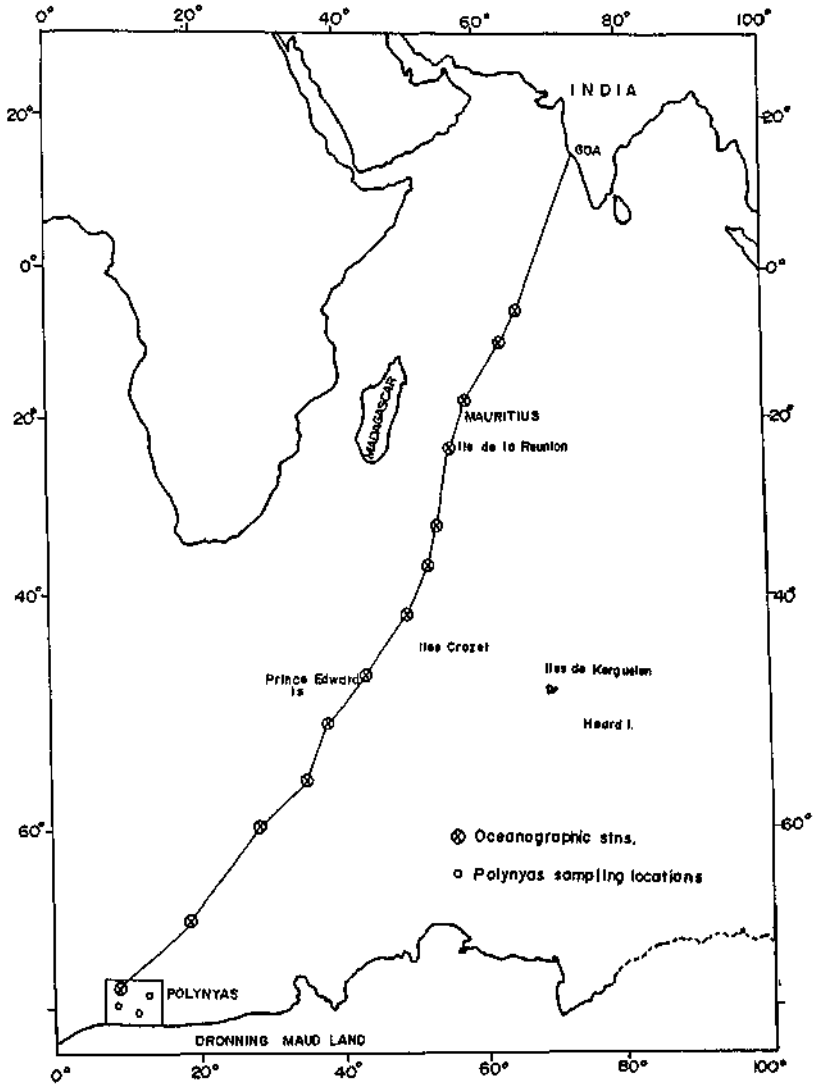



*Fig. 1. Sampling locations.*

## Results

Vertical distribution pattern of bacterial densities in the polynal stations in the India Bay is shown in Fig.3. Although the total direct counts are of 4 order of magnitude higher (4.58 X $10^4$ to 13.71 X $10^4$ $ml^{-1}$) than those of the viable (5.0 to 205.0 cells $ml^{-1}$) bacterial counts, the bacterial populations as a whole, decreased with increasing depth (Fig.3). In the open waters, the vertical distribution pattern of viable bacterial populations consistently showed a peak'at 100m (Fig.4) unlike that of the total populations which was found to be generally

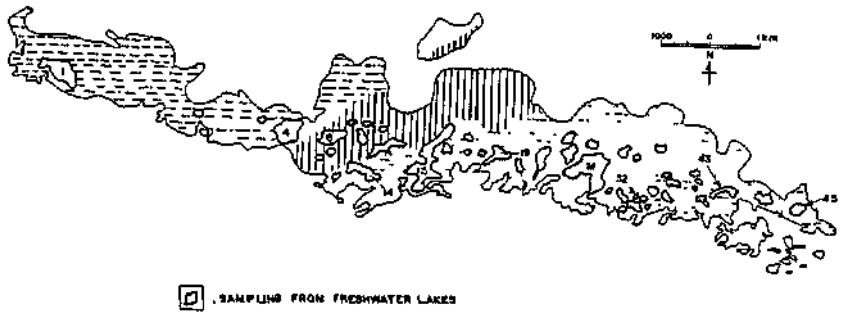



*Fig. 2. Map of Schirmacher Oasis, showing freshwater lakes sampled.*

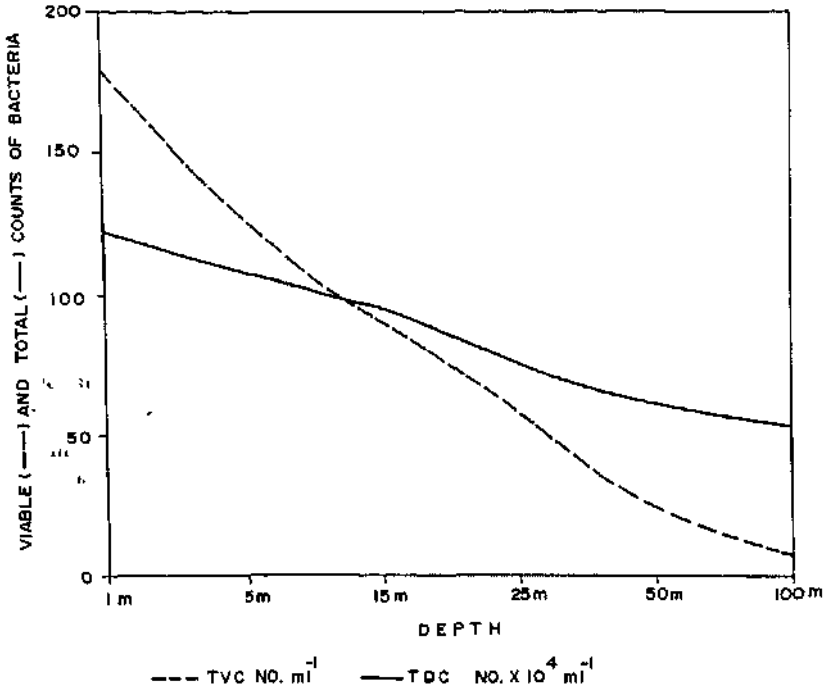



*Fig. 3. Vertical distribution of bacteria in polynyas.*

high around 15 or 30 metres coinciding with the active photosynthetic depth. The relative abundance of viable bacterial population in other types of marine samples is furnished in Fig.5. When compared to the mean viable cell numbers in the sea water, the gut contents of krill harboured the highest densities as against the lowest numbers found in the hard ice samples.

Distribution of bacterial populations in the freshwater, terrestrial and glacial ecosystems of the Schirmacher Oasis showed a remarkable ecological adaptation, being more abundant

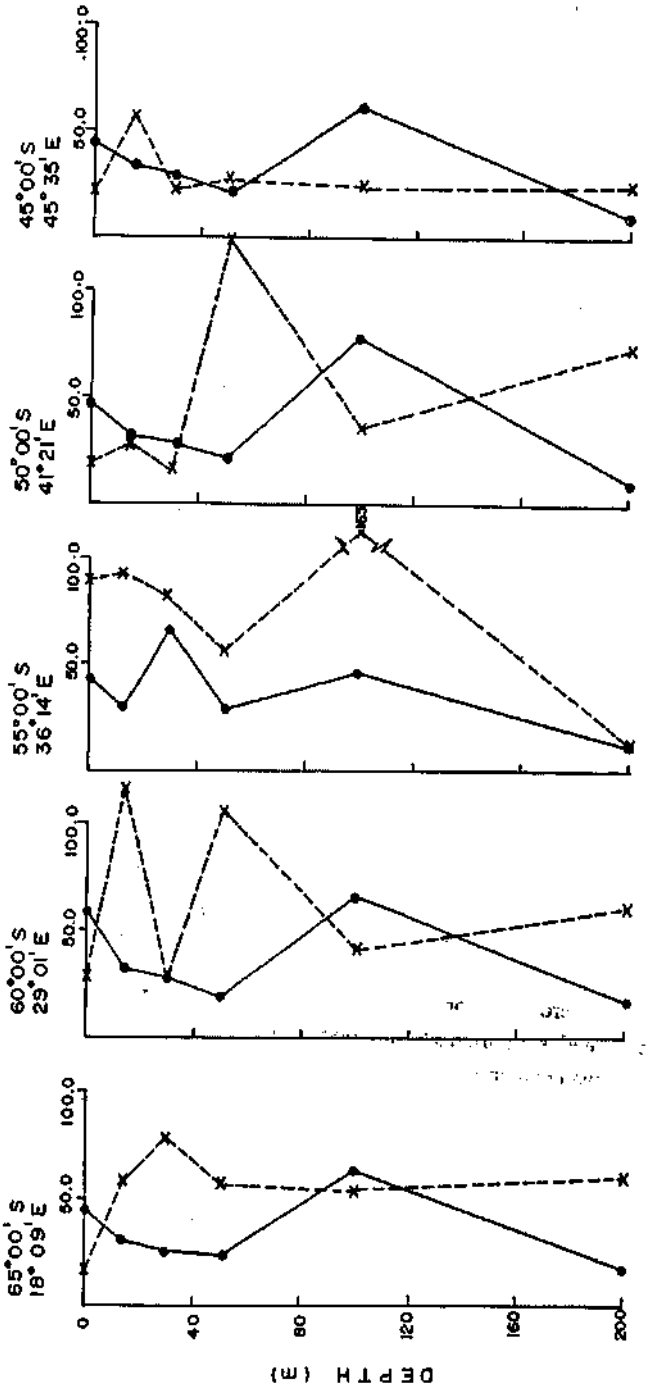



*Fig.4. Vertical distribution of bacteria in Antarctic waters.*

**Table 1: Viable Bacterial Populations in the Limnetic, Terrestrial and Glacial Environs of the Queen Maud Land, Antarctica**

| Type of sample | n | TVC | |
|---|---|---|---|
| | | range | mean |
| Lake water (no. ml ) | 45 | 0.20 - 99.40 | 16.72 |
| Sediment (no. x$10^3$ $g^{-1}$ dry wt.) | | | |
| Sandy silt | 11 | 0.74 - 0.86 | 0.78 |
| Silty sand | 23 | 170.00 - 699.89 | 112.93 |
| Silty clay | 15 | 6.36 - 163.61 | 46.96 |
| Sandy clay | 12 | 86.76 - 106.66 | 96.16 |
| Clayey sand | 13 | 29.09 - 78.62 | 62.66 |
| Clay | 12 | 187.30 - 316.79 | 247.45 |
| Sand | 16 | 0 - 66.36 | 16.00 |
| Mosses (no. x $10^3$ $g^{-1}$ dry wt.) | | | |
| Green, live | 5 | 373.80 - 696.77 | 527.29 |
| Green, dried | 5 | 394.67 - 607.71 | 469.67 |
| Black, live | 5 | 27.00 - 43.46 | 36.23 |
| Black, dried | 5 | 2032.00 -3319.00 | 2604.33 |
| Lichens (no. x $10^3$ $g^{-1}$ dry wt.) | 10 | 70.00 - 149.00 | 106.33 |
| Red alga live | 5 | 469.71 -2366.37 | 1684.77 |
| dried | 5 | 6360.00 -6433.00 | 6391.00 |
| Bl. gr. algal mat(no. >:$10^5$) | 10 | 616.00 -1963.29 | 1264.34 |
| Dry soils (no. x $10^2$ $g^{-1}$ dry wt.) | 5 | 4.84 - 9.63 | 5.83 |
| Peng, rookery ice(no. x $10^2$ $ml^{-1}$) | 10 | 2.82 - 36.69 | 6.91 |
| Peng, guano (no. x $10^3$ $g^{-1}$ wet wt.) | 10 | 63.00 - 196.56 | 106.38 |
| Peng, feathers (no. x $10^2$ $g^{-1}$ dry wt.) | 5 | 100.00 - 180.55 | 124.82 |
| Snow petrel flesh & bones (no. X $10^3$ $g^{-1}$ dry wt.) | | | |
| Salt tolerant | 8 | 661.94 -3498.82 | 2408.99 |
| General | 8 | 232.48 - 394.00 | 366.76 |
| Glacial ice(no. $ml^{-1}$ ) | 15 | 2.00 - 2.80 | 2.40 |

in the habitats where more organic matter (Table 1) accumulation was seen. Mosses, whether live or dead, supported a higher population of bacterial flora than that of lichens. In the crystal clear waters in most of the freshwater lakes of Schirmacher, bacterial populations were very scanty unlike in the benthic bluegreen algal carpets (Table 1). The average values of viable bacterial counts from various habitats in the limnetic, terrestrial, glacial and marine ecosystems are depicted in Fig.5.

A

Table 2: Percentage of Limnetic and Maritime Bacterial Strains Positive for One, Two and Three Enzymes

a: one and two enzymes

*Freshwater strains* (100)

| | Amy | Cit | Gel | Lip | Ure |
|---|---|---|---|---|---|
| Amylase (amy) | 38 | | | | |
| Citrate kinase (cit) | 36 | 30 | | | |
| Gelatinase (gel) | 72 | 52 | 24 | | |
| Lipase (lip) | 28 | 20 | 40 | 16 | |
| Urease (ure) | 14 | 24 | 28 | 28 | 20 |

*Marine bacterial strains* (150)

| | Amy | Cit | Gel | Lip | Ure |
|---|---|---|---|---|---|
| Amylase (amy) | 38 | | | | |
| Citrate kinase (cit) | 22 | 10 | | | |
| Gelatinase (gel) | 24 | 22 | 82 | | |
| Lipase (lip) | 32 | 16 | 64 | 54 | |
| Urease (ure) | 12 | 18 | 12 | 24 | 12 |

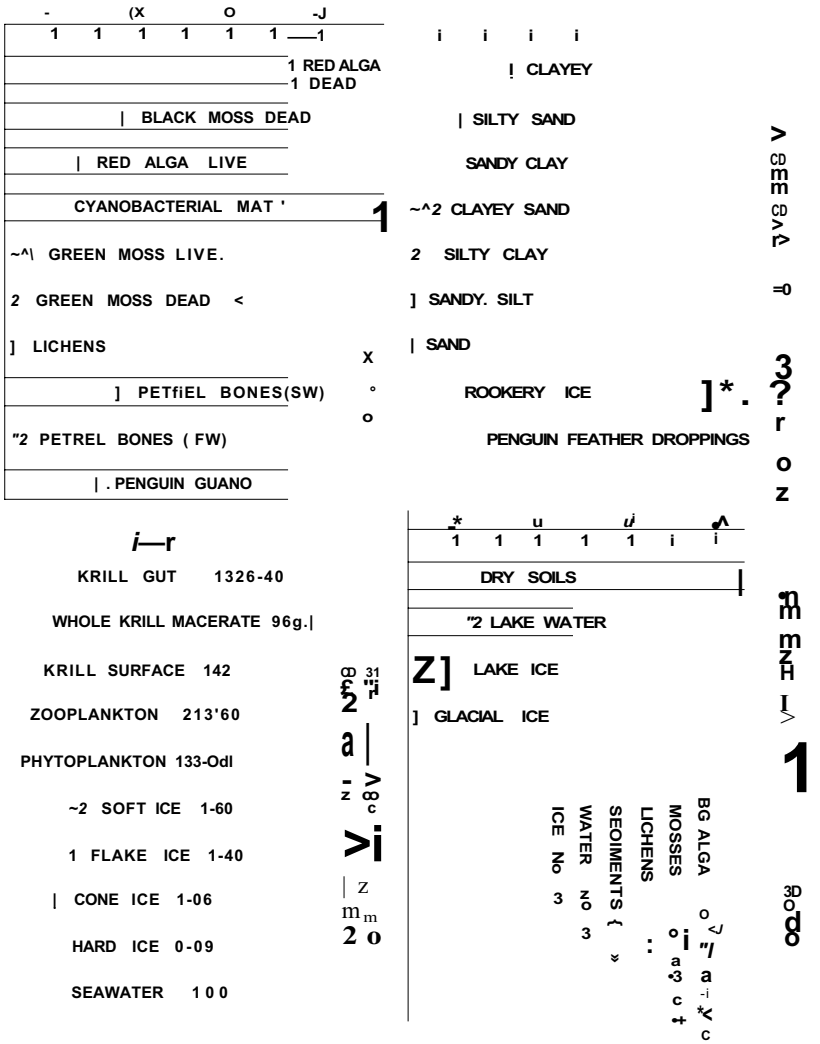
b: three enzymes

| | ACG | ACL | ACU | AGL | AGU | ALU | CGL | CGU | GLU |
|---|---|---|---|---|---|---|---|---|---|
| Freshwater strains (100) | 32 | 36 | 24 | 38 | 18 | 16 | 20 | 24 | 16 |
| Marine strains (150) | 16 | 12 | 14 | 22 | 14 | 8 | 8 | 4 | 12 |

A - amylase; C - citrate kinase; G - gelatinase; L - lipase; U - urease

Figures in parentheses are the number of bacterial strains examined.

Large numbers of freshwater strains were found to produce many of the examined enzymes and on a comparative basis, more number of freshwater bacterial strains elaborated two or more enzymes (Table 2). From the marine samples, the percentage of bacterial strains positive for these enzymes and the vertical distribution pattern of these enzyme activities are illustrated in Fig 6. Generally, many of these strains elaborated proteases (gelatinases), lipases and chitinases. Production of alginate lyase, cellulase and citrate kinase was limited to lesser numbers of strains. Urease was elaborated by a minor percentage at most sampling depths. Majority of these isolates were found to possess multienzyme system. Clustering of the biochemical characteristics of all the bacterial strains indicated 20 different types. Of these, strains assignable to Gram negative genera *Pseudomonas, Vibrio, Chromobacterium, Aeromonas, Acinetobacter* and *Moraxella* constituted the bulk of the examined isolates. Nearly 18% of the isolates representing 4 different types were Gram positive and 15% were unidentifiable. Strains belonging to *Pseudomonas, Aeromonas* and *Vibrio* showed versatile enzyme activities irrespective of their source of origin.


1 RED ALGA
1 DEAD
BLACK MOSS DEAD
RED ALGA LIVE
CYANOBACTERIAL MAT
GREEN MOSS LIVE.
GREEN MOSS DEAD
LICHENS
PETfiEL BONES(SW)
PETREL BONES ( FW)
PENGUIN GUANO
CLAYEY
SILTY SAND
SANDY CLAY
CLAYEY SAND
SILTY CLAY
SANDY. SILT
SAND
ROOKERY ICE
PENGUIN FEATHER DROPPINGS
KRILL GUT 1326-40
WHOLE KRILL MACERATE 96g.
KRILL SURFACE 142
ZOOPLANKTON 213'60
PHYTOPLANKTON 133-Odl
SOFT ICE 1-60
FLAKE ICE 1-40
CONE ICE 1-06
HARD ICE 0-09
SEAWATER 100
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BG ALGA
MOSSES
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WATER
ICE No 3

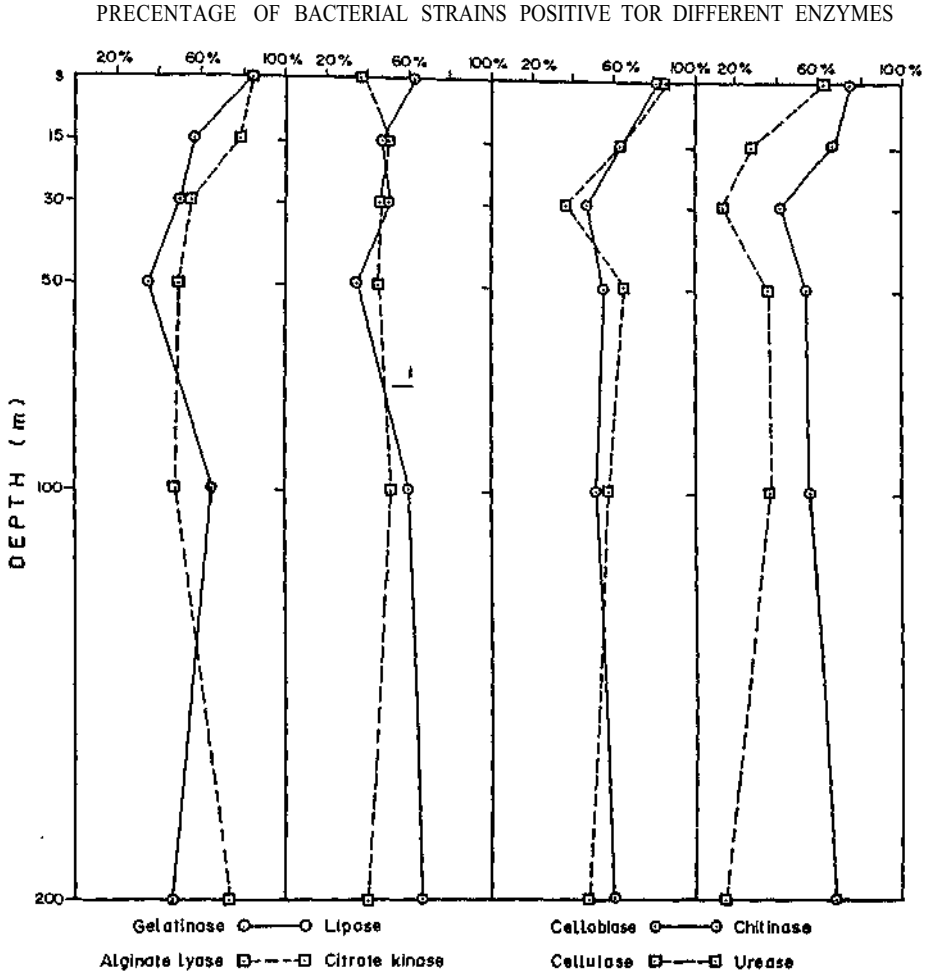


*Fig.6. Vertical distribution pattern of enzymatic activities of the marine bacterial strains.*

Heterotrophic uptake of C glucose showed a distinctly different uptake pattern in the freshwater, polynyal and offshore natural microbial assemblages (Table 3). The fraction of C glucose incorporated is far lower in the lake environs. Whereas in the polynya this incorporation rate was moderate and it was most pronounced in the pelagic waters within the Antarctic Convergence. Although the apparent values of the fraction of uptake of labelled glucose appeared to be uniform, the amount taken up actually showed an increase almost proportionately with the increasing substrate concentration. This trend of the increased uptake might signify both rapid turnover of natural organic material and multiphasic incorporation by the native aquatic microflora. Large differences in the rates of uptake of $^{14}$C glucose and glutamic acid were seen in all the habitats examined (Fig. 7). The percentage

Table 3: Fraction of $^{14}C$ Glucose Assimilated in One Hour by the Natural Heterotrophic Microbial Assemblages in Different Water Bodies

| | Substrate concentrations (|ig C L"') | | | | |
|---|---|---|---|---|---|
| | 6.80 | 13.60 | 20.40 | 27.20 | 34.00 |
| | *a. Freshwater lakes* | | | | |
| LakeM | 0.0016 | 0.0011 | 0.0012 | 0.00.17 | 0.0019 |
| Lake 8 | 0.0015 | 0.0011 | 0.0015 | 0.0017 | 0.0017 |
| Lake 32 | 0.0017 | 0.0011 | 0.0017 | 0.0018 | 0.0020 |
| Lake 43 | 0.0021 | 0.0010 | 0.0015 | 0.0020 | 0.0021 |
| | *b. Polynyal stations* | | | | |
| Stn 1 lm | 0.0031 | 0.0021 | 0.0023 | 0.0034 | 0.0O33 |
| 25 m | 0.0023 | 0.0024 | 0.0021 | 0.0026 | 0.0030 |
| 50 m | 0.0027 | 0.0021 | 0.0023 | 0.0024 | 0.0029 |
| 100 m | 0.0035 | 0.0021 | 0.0023 | 0.0024 | 0.0032 |
| Stn 2 lm | 0.0039 | 0.0034 | 0.0036 | 0.0037 | 0,0030 |
| 25 m | 0.0045 | 0.0036 | 0.0039 | 0.0034 | 0.0036 |
| 50 m | 0.0042 | 0.0035 | 0.0032 | 0.0038 | 0.0O35 |
| 100 m | 0.0047 | 0.0031 | 0.0038 | 0.0036 | 0.0037 |
| | *c. Open waters within the Antarctic Convergence* | | | | |
| Stn 1 lm | 0.0028 | 0.0018 | 0.0015 | 0.0015 | 0.0012 |
| 50 m | 0.0016 | 0.0018 | 0.0015 | 0.0018 | 0.0012 |
| 100 m | 0.0026 | 0.0019 | 0.0019 | 0.0018 | 0.0013 |
| 200 m | 0.0034 | 0.0022 | 0.0015 | 0.0018 | 0.0019 |
| Stn 2 lm | 0.0027 | 0.0027 | 0.0025 | 0.0O26 | 0.0028 |
| 50 m | 0.0019 | 0.0022 | 0.0023 | 0.0020 | 0.0021 |
| 100 m | 0.0046 | 0.0040 | 0.0022 | 0.0024 | 0.0020 |
| 200 m | 0.0032 | 0.0045 | 0.0024 | 0.0023 | 0.0025 |
| Stn 3 lm | 0.0410 | 0.0416 | 0.0316 | 0.0219 | 0.0232 |
| 50 m | 0.0398 | 0.0431 | 0.0396 | 0.0175 | 0.0201 |
| 100 m | 0.0491 | 0.0483 | 0.0445 | 0.0204 | 0.0237 |
| 200 m | 0.0439 | 0.0433 | 0.0453 | 0.0203 | 0.0250 |
| Stn 4 lm | 0.0460 | 0.0512 | 0.0281 | 0.0199 | 0.0171 |
| 50 m | 0.0421 | 0.0310 | 0.0267 | 0.0144 | 0.0161 |
| 100 m | 0.0324 | 0.0384 | 0.0246 | 0.0189 | 0.0175 |
| 200 m | 0.0271 | 0.0283 | 0.0223 | 0.0164 | 0.0173 |
| Stn 5 lm | 0.0621 | 0.0579 | 0.0598 | 0.0582 | 0.0448 |
| 50 m | 0.0515 | 0.0566 | 0.0501 | 0.0581 | 0.0600 |
| 100 m | 0.0823 | 0.0537 | 0.0591 | 0.0513 | 0.0582 |
| 200 m | 0.0553 | 0.0662 | 0.0586 | 0.0577 | 0.0529 |

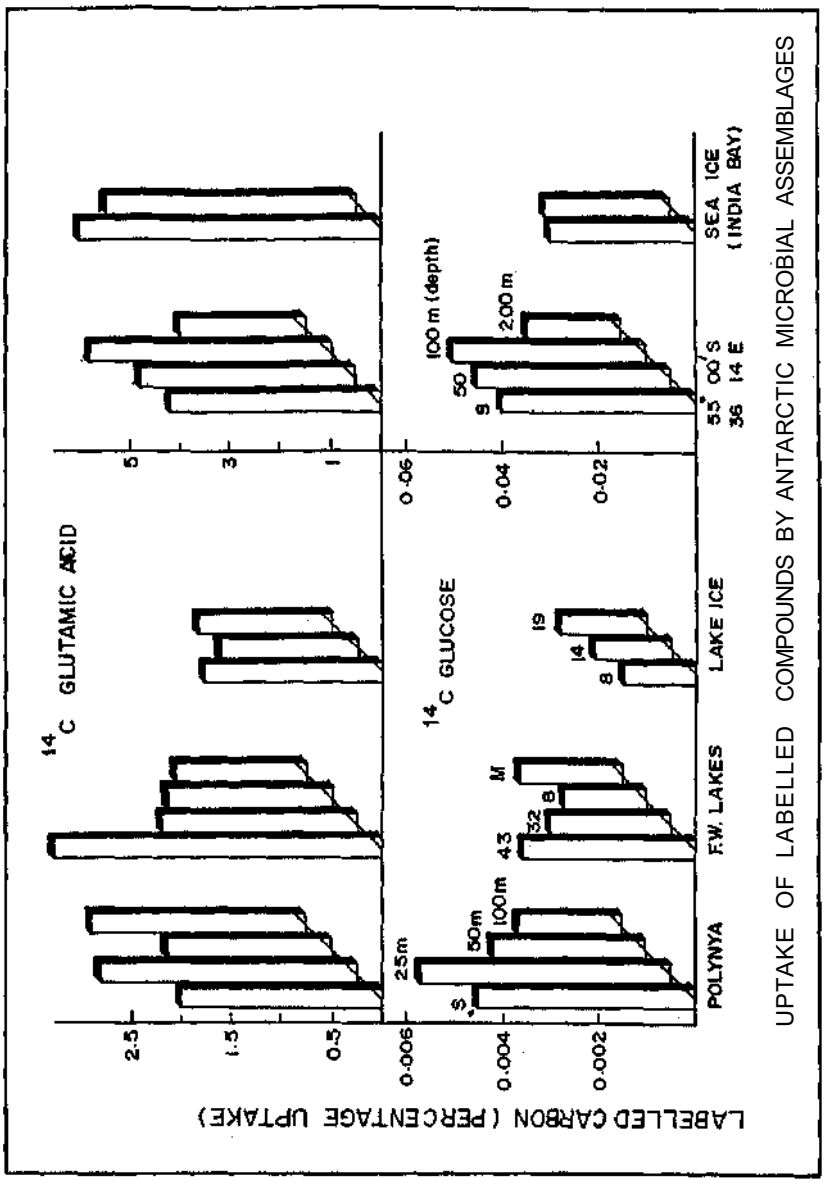


*Fig. 7. Comparison of uptake pattern of labelled glucose and glumatic acid by the microbial heterotrophs from different ecosystems. Please refer to figures 1 and 2 for more details.*

uptake of labelled glutamic acid was 2 to 3 orders of magnitude higher than that of labelled glucose suggesting that the natural microbial assemblages do show a preferential utilisation of the substrates from the surrounding water.

## Discussion

Microbial heterotrophy and diversity, once considered negligible in the Antarctica, are now emerging as important links in the secondary production. Detrital material, particularly derived from shoaling krills and large rookeries of penguins, appears to impact the Antarctic trophodynamics at many points. Clarke (1985) rightly suggested that importance of microbial communities both in terms of their quantity as well as variety, is still to be understood realistically.

In the limnetic ecosystems, viable bacterial populations observed during the present study compare well with those of Matondkar and Gomes (1983), Shivaji (1987), Franzmann *et al.,* (1990) and Karl *et al,* (1991). But, there appeared to be a large variation in their numbers from one lake to the other. Even the density of bacteria in the sediments varied widely. This must imply the local influence of the growth of cyanobacterial mats and mosses. It was seen that the quantitative abundance of bacteria was far greater in the lakes that are bordered with dense growth of mosses, lichens as well as those with cyanobacterial carpets. Besides highlighting the exceedingly high abundance of viable bacteria in the cyanobacterial mat, live, dying algae and mosses, it can be inferred that the quantitative abundance of heterotrophic microbes is a direct indication of the rich availability of organic nutrients in such substrata. One would thereby derive an important ecological clue with regard to their habitat associations. This might be due to the rich availability of organic nutrients that can sustain a relatively higher bacterial population. Texture of the sediment appeared to play an important regulatory role in the quantitative abundance. Wynn-Williams (1985) has indicated the occurrence of specialised microbial groups under different habitats and is of the opinion that the edaphic and biogeographical parameters bear a strong influence on the distribution of bacteria both in terms of the type as well as the quantity. Another point of interest was that many samples of marine origin (e.g., flesh and bone remains of sea birds and penguin rookery wastes) were found far away in the terrestrial and glacial environs. When these samples were plated on the seawater nutrient agar, more numbers of salt tolerant bacteria than those of terrestrial origin still persisted in these samples indicating a long survival and activity outside marine environment.

Bacterial biomass in the polynyal and pelagic waters was far greater. Although well within the ranges reported from other maritime zones, these values in fact, far exceeded the viable populations observed in the open ocean or those found in the slope regions of the tropical seas (Ramaiah, 1989) and in other Antarctic regions (Dawson *et al.,* 1985; Dawson and Liebezeit, 1983).

Higher the bacterial biomass, higher would be their contribution to the biotransformation of organic material. Such high bacterial abundance is also suggestive of the bacterioplankton as a quantitatively important component in the marine Antarctic food webs (Fuhrman and Azam, 1980; Matondkar and Qasim, 1983 and Mitchell and Holm-Hansen, 1991). As also evidenced in the present study, occurrence of uniformly higher bacterial numbers in the Antarctic marine waters is supported by large quantities of organic carbon (Table 4)

Table 4 : Distribution of Particulate Organic Carbon (POC) (µg. L⁻1) at Different Depths in the Antarctic Waters

| Depth (m) | Range of POC |
|---|---|
| 1 | 45.21-242.49 |
| 15 | 444.24 - 542.52 |
| 30 | 116.26-590.39 |
| 50 | 166.77-361.88 |
| 100 | 165.56-351.84 |
| 200 | 151.50-242.84 |

available for their growth and multiplication (Bolter and Dawson, 1982). Marine Antarctic bacteria which have the capacity to decompose the detrital biopolymers of both plankton and animal origin play a very important role in the transformation and availability of food to the macro heterotrophs (Holm-Hansen, 1985). A crucial role is played by the microbes in the mineralisation and natural fertilisation of penguin guano (Tartur and Myrcha, 1984). These transformations are brought about by their ability to produce various types of enzymes. During the present study, high and varied extracellular enzyme production by most of the examined marine bacterial strains was observed which must be responsible for a faster turnover rate of organic matter. The fact that cellulose production was more pronounced in these psychrophiles when compared with many species of bacteria isolated from the tropical seas (Ramaiah, 1989), can be utilised in future investigations to exploit these psychrophiles in the production of cellulases. Although the decomposition may be slow at the prevailing temperatures, microbial enzymes appear to remain quite active and therefore, must be helpful in the oxidation of organic matter.

Pivotal role of heterotrophic communities in the primary productivity of the polar seas is already being realised (Horner, 1985; Grossi *et al*., 1987 and Smith and Clement, 1990). However, Kottmeier and Sullivan (1987) suggest that detailed investigations are necessary on the heterotrophic processes in the Antarctic ecosystems. Smith and Clement (1990) reported an increased assimilation rate for glucose and thymidine incorporation with the increasing salinity. In the present study, it was observed that the fraction of C assimilated increased northwards within the Antarctic Convergence with the increasing temperature suggesting a temperature dependent heterotrophic process. Seawater temperature rose from ~2°C at stn 1 to 8-10°C at Stn 5. Our study forms the first of its kind on the measurement of heterotrophic activity in the Indian Ocean Sector of the Southern Ocean. From this study, it is clear that there is a distinct ecosystem specialisation in the uptake kinetics and that, temperature is the key factor in regulating the uptake kinetics of the *in situ* microbial assemblages.

From this investigation it is substantiated that the microbial component plays the most crucial role of biotransformation even under extremely cold conditions. Their enzymatic and heterotrophic processes are of basic importance be it in the terrestrial and Southern Ocean trophodynamics or in the biogeochemical cycling of organic matter. In this study we have made an extensive sampling and compared the quantitative abundances of bacterial populations and some of their characteristics from different ecosystems of Queen Maud Land and

the Indian Sector of the Southern Ocean, However, a lot still needs to be done particularly in the spheres of taxonomy, biotechnological prospecting and biochemical characteristics of these little known, microscopic and fascinating creatures.

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