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SEASONAL FLUCTUATION OF BACTERIAL NUMBERS NEAR THE ANTARCTIC CONTINENT

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Abstract: Bacterial numbers were monitored on a regular basis near Australia's Davis Base from May 1987 until January 1988. Samples were collected from depths between the surface and 80 m. Numbers were low (mean: *ca.* 1.1×10^5 cells/m*l*) during the winter period but rose to between 2.5×10^5 and 8.0×10^5 cells/m*l*, depending on depth, in October, when considerable amounts of detrital material were present in water column and available for heterotrophic activity. A significant decrease in bacterial numbers occurred in early December to $<0.5\times10^5$ cells/m*l*, but recovered later in December to $>5\times10^5$ cells/m*l*; the increase in numbers occurred at the same time as a bloom of the alga *Phaeocystis pouchetii*. The greatest population of bacteria ($>1\times10^6$ cells/m*l*) was recorded in January, by which time the algal bloom was decreasing.

1. Introduction

Bacteria are ubiquitous in the Antarctic marine ecosystem, much as they are in other marine environments. It has been reported that bacterial activity in the Antarctic Ocean is similar in magnitude to that in more temperate regions (HANSON *et al.*, 1983), even though a lowering of activity due to the extreme nature of the environment (temperatures being nearly always below 1°C) might be expected. MCMEEKIN (1988) has isolated a large numbers of bacterial strains from the Antarctic region and has shown that most are capable of growth at these temperatures, and that some are able to survive at temperatures as low as −5°C.

Little is known about seasonal fluctuations in the populations of bacteria in the Antarctic Ocean, especially under the sea-ice during winter. This paper reports results obtained from a study of bacterial numbers at two inshore sites close to the Antarctic Continent over the period from May 1987 to January 1988. No attempt was made to identify the bacteria present. Studies of the annual cycles of uni-cellular algae and zooplankton populations have been made previously at nearby sites (PERRIN *et al.*, 1987; TUCKER and BURTON, 1988, 1990); this paper complements these previous studies.

2. Materials and Methods

Water samples were obtained from May 1987 until November 1987 at Site 1 (Fig.

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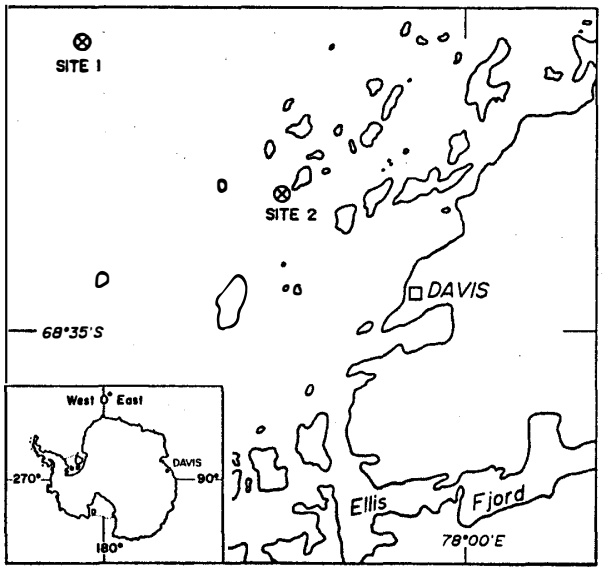

Fig. 1. Map of the Davis region showing the location of the sampling sites.

1), which was approximately 10 km offshore from Davis Base (68°35'S, 78°00'E). The water depth at this site was 120 m. Samples obtained during December 1987 and January 1988 were collected from a second site (Fig. 1, Site 2) closer to the mainland due to the inaccessibility of the original site as a result of deterioration of the ice cover used for access. The water depth at this site was 30 m.

Samples were collected in 2*l* Kemmerer Bottles constructed entirely of polycarbonate and Teflon deployed through a hole drilled in the sea-ice with a Jiffy Ice Drill, or from a small boat after the sea-ice had dispersed. The samples were placed in polycarbonate containers and fixed with formalin to a final concentration of 2% (v/v). Samples were stored at room temperature until processed. Bacteria and *Phaeocystis pouchetii* cell counts were obtained, after staining with acridine orange, using fluorescent microscopic techniques (FRANZMANN *et al.*, 1986). Cell counts of at least 40 microscope fields were undertaken in order to obtain accurate cell numbers. *P. pouchetii* forms large colonies containing many individual cells; preservation of samples with formalin broke down the mucilage that held the colonies together (EBERLEIN *et al.*, 1985), allowing accurate determination of cell numbers.

3. Results

Profiles of bacterial populations were obtained on 12 occasions from May 1987 to January 1988. The results obtained are presented in Fig. 2. No data were obtained beneath a depth of 30 m after early December because sampling was moved to a shallower site, as explained above. Even though we recognize that the environments at the two sites were different, we have chosen not to separate the data. We feel this is justifiable because the coastal region in which this study was undertaken is continually swept by the Prydz Bay cyclonic gyre (SMITH *et al.*, 1984) and previous biological and biochemical studies (GIBSON *et al.*, 1990; TUCKER and BURTON, 1990) have found little difference between sites inside and outside the coastal islands (Fig. 1).

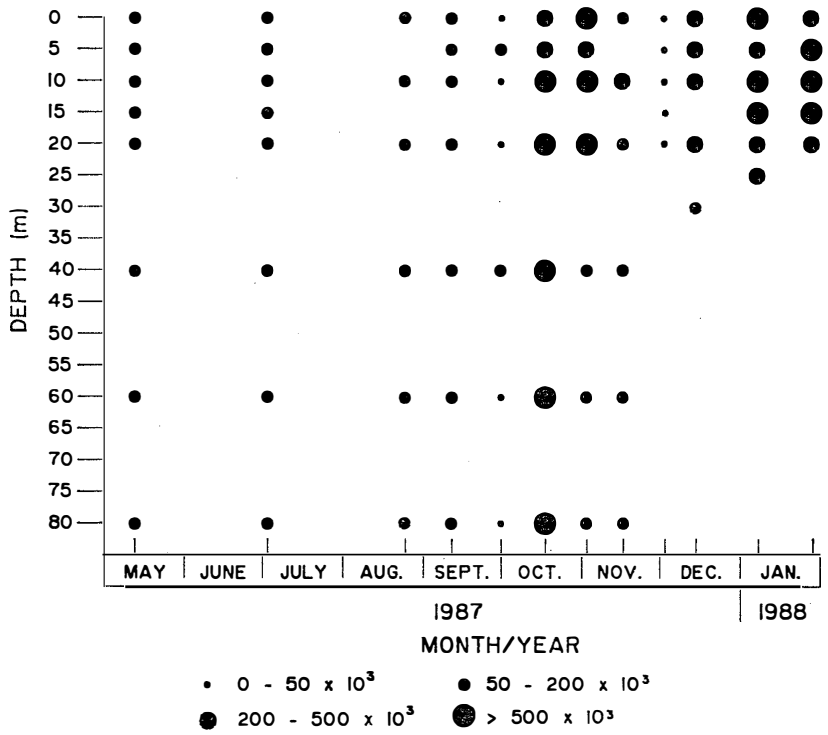

Fig. 2. Bacteria cell counts (cells/ml), May 1987–January 1988. Samples were not collected from beneath 30 m after November 1987 due to a change in sampling site (see Sections 2 and 3).

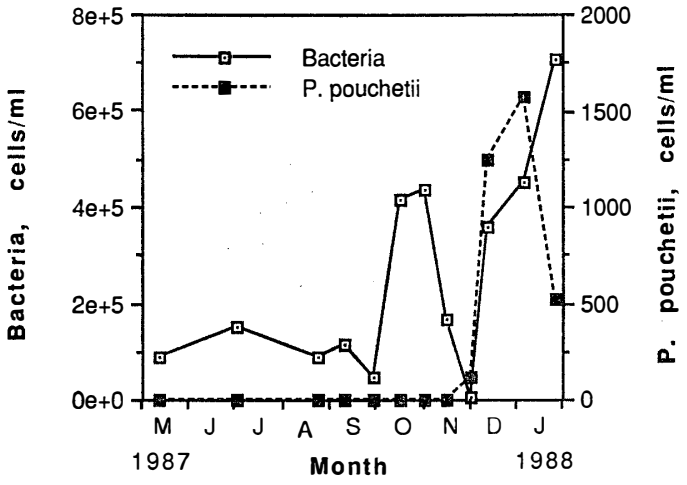

Fig. 3. Average bacteria and Phaeocystis pouchetii *cell counts (cells/ml) from 0 to 20 m.*

During the winter period (May–early September), bacterial numbers averaged 1.1×10^5 cells/m*l*. As shown in Fig. 3, which shows average cell counts between 0 and 20 m, no significant variation was observed either between different sampling dates or depths during the period May–September 1987. Bacterial numbers, however, had fallen considerably by 30 September 1987 to an average of 4.5×10^4 cells/m*l*.

A sudden and significant increase in bacterial numbers had occurred by the date the next profile was obtained, 17 October 1987 (Figs. 2 and 3). The increase was most apparent at depths below 10 m. A large amount of mucilaginous organic material was also observed to be present in these samples. The next profile (2 November 1987) again showed high bacterial numbers in the top 20 m of the water column, with counts dropping off in samples taken from deeper water. The decrease had spread to the whole water column by 16 November 1987, and continued into early December, when, in samples collected 2 December 1987, very low numbers of bacteria ($<1\times10^4$ cells/m*l*) were found. As Fig. 3 shows, the population size at this time was well below that recorded during the winter period.

By the middle of December, the bacterial populations had recovered to densities of approximately 4×10^5 cells/m*l* at all depths. During January, the population continued to increase at most depths, especially at 10 m, where the cell counts increased to a maximum of over 1×10^6 cells/m*l*. The average population (Fig. 3) was still increasing when the final set of samples was collected in late January, 1988, due mainly to high cell counts in the 10 m region.

4. Discussion

Bacterial numbers during the period from May to September were essentially constant, suggesting that little growth of the bacteria beneath the sea-ice was occurring. The density of bacteria, *ca.* 1.1×10^5 cells/m*l*, was of the same order of magnitude reported for the Prydz Bay region during March and April (PAINTING *et al.*, 1985) and the Antarctic Peninsula region during late winter (HANSON *et al.*, 1983). The combination of low light levels, low temperature, and the little material available for heterotrophic activity (no extracellular organic material was noted in the water column during this period, *v.i.*) probably resulted in the maintenance of the low population of bacteria without any significant growth in total numbers. No attempt was made to measure the rate of primary production or of cell division in order to estimate cell activity. The drop in cell counts in late September is difficult to explain; considerable currents were observed under the ice at the sampling site during winter and it is possible that the body of water sampled on this occasion had a history sufficiently different to the previous water masses sampled to result in different bacterial numbers.

The increase in bacterial numbers in October coincided with the appearance in the water column of a considerable amount of mucilaginous material. The majority of bacteria observed ($>80\%$) during this period was closely associated with this organic material. PERRIN *et al.* (1987) reported that increased numbers of the sea-ice diatom *Nitzschia cylindrus* and other diatom species occurred in the sea-ice during this period in an earlier year at a nearby site. It is probable that the mucilaginous material was produced by sea-ice algae. Bacteria have been found to be efficient utilizers of organic substances released by phytoplankton (WOLTER, 1982; AZAM *et al.*, 1983). Thus, when organic material is available for utilization, an increase of activity, and thus population, would be expected.

The equally dramatic drop in bacterial numbers which followed soon after this population increase probably resulted from grazing by bacteriophagous organisms. The

sudden increase in the bacterial population could be expected to have led to a concomitant increase in the numbers of organisms, such as choanoflagellates and protozoans, that predate bacteria (MARCHANT, 1985). Even though the populations of these organisms were not monitored, it is reasonable to assume that numbers increased to take advantage of the ready food supply. The drop in numbers of bacteria to a level well below the winter 'steady-state' suggests that predation of the bacteria was sufficient to reduce total population numbers once bacterial growth itself slackened.

After the bacterial population reached its minimum (2 December 1987), another sharp increase in bacterial numbers occurred, a trend which continued until the end of the study in January 1988. This increase coincided with a similar increase in the numbers of the alga *P. pouchetii*, which was by far the dominant algal phytoplankton species throughout December 1987 and early January 1988, as has been observed immediately after sea-ice break-up at other coastal Antarctic sites (SAKSHAUG and SKJOLDAL, 1989). *P. pouchetii* is a uni-cellular alga that forms large colonies consisting of thousands of individual cells embedded in mucilage. In this colonial stage, *P. pouchetii* excretes 46–64% of its photoassimilated carbon, mostly as polysaccharides (GUILLARD and HELLEBUST, 1971; LANCELOT and MATHOT, 1987). This material would be available for utilization by bacteria (and other organisms), especially when the colonies of the alga become senescent and are disrupted.

Figure 3 shows average cell numbers (0–20 m) for the alga as well as bacteria. Before the beginning of December, algal numbers were insignificant. A correlation occurred during December 1987 and early January between cell counts of *P. pouchetii* and bacteria, suggesting that organic material produced by the alga was an important nutrient source for the bacteria.

The population of *P. pouchetii* had decreased by the end of January 1988, but bacterial numbers remained at similar, if not higher, levels to earlier in the month. This result suggests that bacterial utilization of dead cells and the organic mucilage of senescent colonies was occurring; bacteria were now observed to be associated with dead colonies.

There appeared to be little or no delay (on the time scale of our sampling program) in the response of bacterial numbers to the increase in the population of *P. pouchetii*. A similar observation has been made during a bloom of the same alga in the North Sea (BILLEN and FONTIGNAY, 1987). *P. pouchetii* is known to produce large amounts of acrylic acid (up to 2% of the total organic material excreted) (GUILLARD and HELLEBUST, 1971; EBERLEIN *et al.*, 1985). This compound, which is a broad spectrum bacteriocide (SIEBURTH, 1961), is a breakdown product of the osmoregulator β-dimethylsulfoniopropionate (ANDREAE *et al.*, 1983). The strain of *P. pouchetii* found close to the Antarctic Continent has been found to produce large quantities of dimethylsulfide (the other decomposition product of β-dimethylsulfoniopropionate) and thus acrylic acid (GIBSON *et al.*, 1990). If acrylic acid were to act as a significant bacteriocide in the ocean near Davis, the contemporaneous sharp increase in bacterial numbers with the algal bloom would not have been expected.

Laboratory experiments reported by DAVIDSON and MARCHANT (1987) suggested that the acrylic acid produced by the algal colonies has an effect only in the immediate neighbourhood of and inside actively growing colonies. However, senescence of the

colonies, with consequent lowered or non-existent acrylic acid production, allowed bacteria to attack the colonies, resulting in dissolution of the supporting mucilage and an increase in bacterial numbers. The results of DAVIDSON and MARCHANT (1987) are in agreement with those found in this study: the bacterial populations increased in size at the same time as the bloom of *P. pouchetii* as a response to the organic material excreted from the algal colonies, and they continued to increase after algal numbers dropped, as bacteria utilized the organic material in senescent and ruptured algal colonies.

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