

## A SEASONAL STUDY OF MARINE BACTERIA IN ADMIRALTY BAY (ANTARCTICA)

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**Abstract:** Bacterial numbers at standard depths (10/50, 100, 200, 300, and 400 m) in Admiralty Bay, King George Island, South Shetland Islands, were determined from April 1990 to January 1991. Neither CFU (Colony Forming Units on nutrient media, *ca.*  $10^3/l$ ), nor total bacteria (AODC—Acridine Orange Direct Count, *ca.*  $10^7/l$ ) numbers varied significantly over the 400 m water column; temporal variation was low, and means and ranges decreased with depth. Significantly more CFU were isolated from 100 m and 200 m after incubation at 15 °C than 1 °C ( $p < 0.05$ ). Close inshore in Half Moon Cove, bacterial numbers at 5 and 10 m depth were similar to those at the bay's centre; significantly more CFU were also isolated here after incubation at 15 °C. Mean cell volume and biomass in the upper 100 m was  $0.228 \mu\text{m}^3$  ( $n = 1636$ ), and  $50.63 \text{ fg C/cell}$  respectively. Bacterial carbon in the upper 100 m (mean  $3.415 \mu\text{g C/l}$ ) ranged widely (August,  $0.338 \mu\text{g/l}$ ; January,  $37.321 \mu\text{g/l}$ ). Rods dominated the total bacteria in most samples. Values of a number of these parameters to levels below those usually reported here during offshore summer cruises, underline the importance of long-term inshore bacteriological studies in the Southern Ocean, particularly over the austral winter.

### 1. Introduction

Land based year-round studies of marine bacterial communities in Antarctica (TANNER and HERBERT, 1981; ZDANOWSKI, 1981, 1985a, 1988a; DELILLE, 1993; *cf.* ZDANOWSKI and DONACHIE, 1993a) have by necessity considered only small inshore areas. Although the dynamics of such communities may differ to those in the open ocean (ZDANOWSKI, 1995) it must also be borne in mind that any two inshore sites may not be fully comparable in terms of bathymetry, hydrography, avifaunal influence (DELILLE, 1987, 1993; DELILLE and CAHET, 1991), or terrestrial run-off. Indeed, the effects of such features may be amplified by the application of methods that reflect disparate research aims; in this respect some procedures (*cf.* KARL, 1993) could well be open to improvement or standardisation.

Bacterial populations in the Southern Ocean can, however, vary considerably in terms of numbers and physiology over small distances and times scales (*cf.* ZDANOWSKI and DONACHIE, 1993b). Despite the fact that bacteria are important components of the Antarctic marine ecosystem (EL-SAYED, 1987; AZAM *et al.*, 1983; KARL *et al.*, 1991; KARL, 1993) most studies of their numbers, distribution, and biomass, have been conducted only for short periods during the austral summer. This approach has given rise to a paucity of data on these parameters for much of this region during the austral

winter. In order to add to the small database for this period, this paper describes the results of the first medium term study of marine bacteria in Admiralty Bay for several years, and indeed the first to consider bacteria at a number of depths at the centre of the bay over the austral winter and spring: CFU and AODC numbers at different depths at the centre of the bay were followed over an eight month period, and the total bacterial population additionally described in terms of cell size, carbon content, and morphology. Bacterial numbers and morphology were also followed close inshore to investigate the effects of a nearby penguin rookery on the bacterial population at this site.

## 2. Materials and Methods

Bacterial numbers were determined in water samples collected at a range of standard depths, at a point equidistant between Henryk Arctowski station (Point Thomas) and Hennequin Point, Admiralty Bay, King George Island (Fig. 1), over a *ca.* 450 m water column: 10/50 m (an integrated sample representing the upper 10 to 50 m of the water column, prepared by aseptically combining 100 ml from 10 m, 20 m, 30 m, 40 m, and 50 m) after ZDANOWSKI and DONACHIE (1993a, b), 100 m, 200 m, 300 m, and 400 m.

Water was taken with an ethanol rinsed (KRISS, 1962; ZDANOWSKI, 1982) one litre stainless steel Van Dorn bottle, from which the first *ca.* 100 ml from each depth was discarded by flushing through the tap (*cf.* KRISS, 1962, p. 75). Approximately 250 ml was then transferred to a sterile, opaque, screw-capped glass bottle, one bottle per depth. Sampling commenced at the greatest depth. All samples were maintained in a 'cool-box' to prevent freezing in winter, and warming in summer, and were processed in the

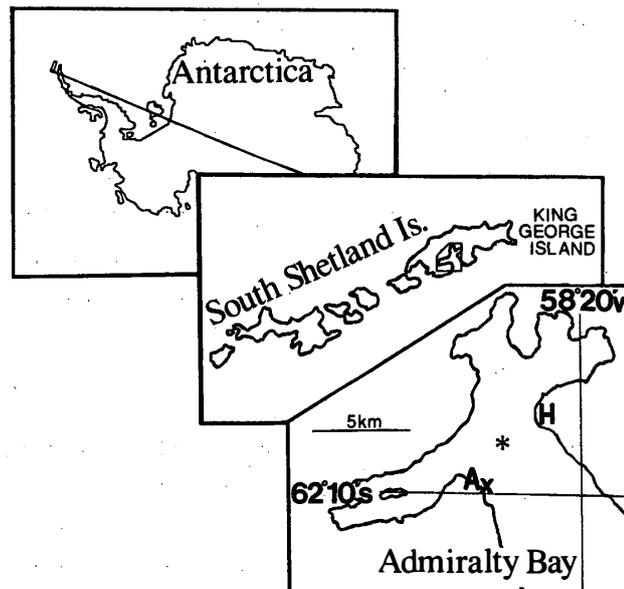


Fig. 1. Admiralty Bay, King George Island: location of sampling stations in this work (\*between Arctowski station (A) and Hennequin Point (H); ×—Half Moon Cove).

laboratory within three hours.

Sampling commenced on 14th April 1990, with one sample from 10/50 m; from 17th April this was extended to 100 and 200 m, and to 300 and 400 m from 2nd October. From 18th September water was also collected at 5 and 10 m, *ca.* 100 m offshore in Half Moon Cove (HMC) (Fig. 1), over a 12 to 15 m water column: this site is below an Adélie penguin (*Pygoscelis adeliae*) rookery, and sampling commenced before penguins returned to the site, and prior to any large scale terrestrial run-off of melting snow and ice in the area. Sampling at both sites ended on 15th January 1991.

Heterotrophic bacteria (Colony Forming Units, CFU) were enumerated in 5 to 25 ml of each seawater sample filtered through 47 mm diameter Millipore GS filters, pore size 0.22  $\mu\text{m}$ , settled on Natural Seawater Nutrient Agar (NASW) (ZDANOWSKI, 1982). Results are expressed as the mean number of colonies after 15 days incubation at 1 °C and 15 °C. To assess how incubation temperature affected the number of heterotrophic bacteria isolated at each depth, the respective counts at each temperature were log transformed and compared by t-tests of their log ratios.

Total bacteria (Acridine Orange Direct Counts, AODC) in seawater were counted in 5 to 20 ml samples fixed with 0.22  $\mu\text{m}$  filtered formalin to a final concentration of 1% in sterile glass ampoules. AODC were determined by epifluorescence microscopy according to ZIMMERMAN and MEYER-REIL (1974) under a Carl Zeiss Jena FLUOVAL 2 microscope fitted with an Apochromat HI 100/1.32; 160/0.17 objective with oil immersion. All bacteria in at least twenty fields were counted (CASSEL, 1965).

The percentage shares of rods, cocci, vibrio, and filaments in the total bacteria were determined in each AODC sample. Bacterial biovolumes and biomass were described in 24 samples from the upper 100 m (17th April to 1st January): calculations are based on the dimensions of 1636 randomly selected cells measured on a slide viewer. Biomass was derived from biovolume according to a conversion factor of 220 fg C  $\mu\text{m}^{-3}$  (BRATBAK and DUNDAS, 1984).

### 3. Results

#### 3.1. Bacterial numbers at the centre of the bay

From May to September CFU were uniformly distributed over the upper 200 m of the water column at up to  $3 \times 10^3/l$  (Fig. 2); a temporally short increase was noted to this depth in October, with CFU exceeding  $6 \times 10^3/l$  at 200 m. Mean CFU over the 400 m water column was *ca.*  $3.5 \times 10^3/l$ , with maximal levels in January; only in the upper 100 m did CFU attain  $10^4/l$ .

AODC showed similar patterns to CFU in terms of temporal and vertical distribution (Fig. 3). From May to mid-December counts rarely exceeded  $3 \times 10^7/l$ . Highest AODC throughout the 400 m water column were recorded in January, attaining  $10^8/l$  in the upper 100 m.

Although CFU and AODC data for the upper 200 m cover a wider temporal scale than 300 and 400 m, the vertical pattern is preserved when data covering the whole water column from October onwards is considered. Both CFU and AODC numbers showed the smallest range at 400 m (Fig. 4a, b).

Overall, 62 coincident CFU and AODC counts were recorded throughout the

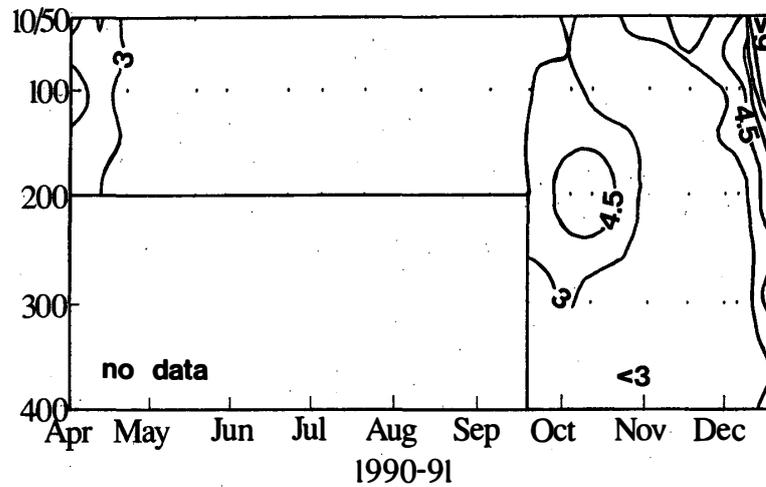


Fig. 2. Contour Plot of CFU ( $\times 10^3/l$ ) on NASW (mean of those determined at 1 °C and 15 °C) at standard depths at the centre of Admiralty Bay (April 1990 to January 1991). Fewer than  $3 \times 10^3$  CFU/l were noted to 200 m during much of the study. Numbers increased briefly in the upper 200 m in September to October, and tended to decrease with depth (x-axis: month; y-axis: depth, m).

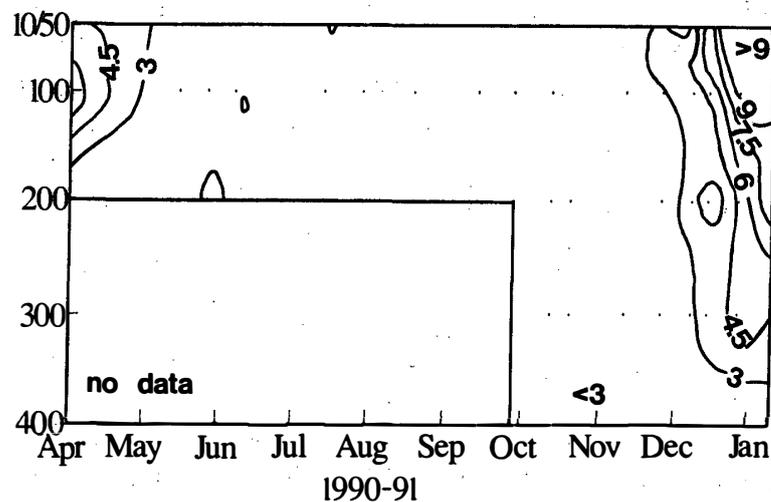


Fig. 3. Contour Plot of AODC ( $\times 10^7/l$ ) at standard depths at the centre of Admiralty Bay (April 1990 to January 1991). Fewer than  $3 \times 10^7/l$  were noted to 200 m through much of the study. (x-axis: month; y-axis: depth, m).

400 m water column: neither varied significantly with depth (one-way ANOVA; CFU- $F_{1,122}=0.72$ ,  $p=0.581$ ; AODC- $F_{1,122}=2.40$ ,  $p=0.127$ ). On average, CFU constituted up to 0.01% of AODC throughout the water column (Table 1), and at each depth showed non coincident maxima of 0.025 to 0.06%, most of which occurred during spring; lower values often coincided (Fig. 5). The size of this fraction varied little with depth. CFU and AODC were most highly correlated in the upper 10/50 m (Table 2).

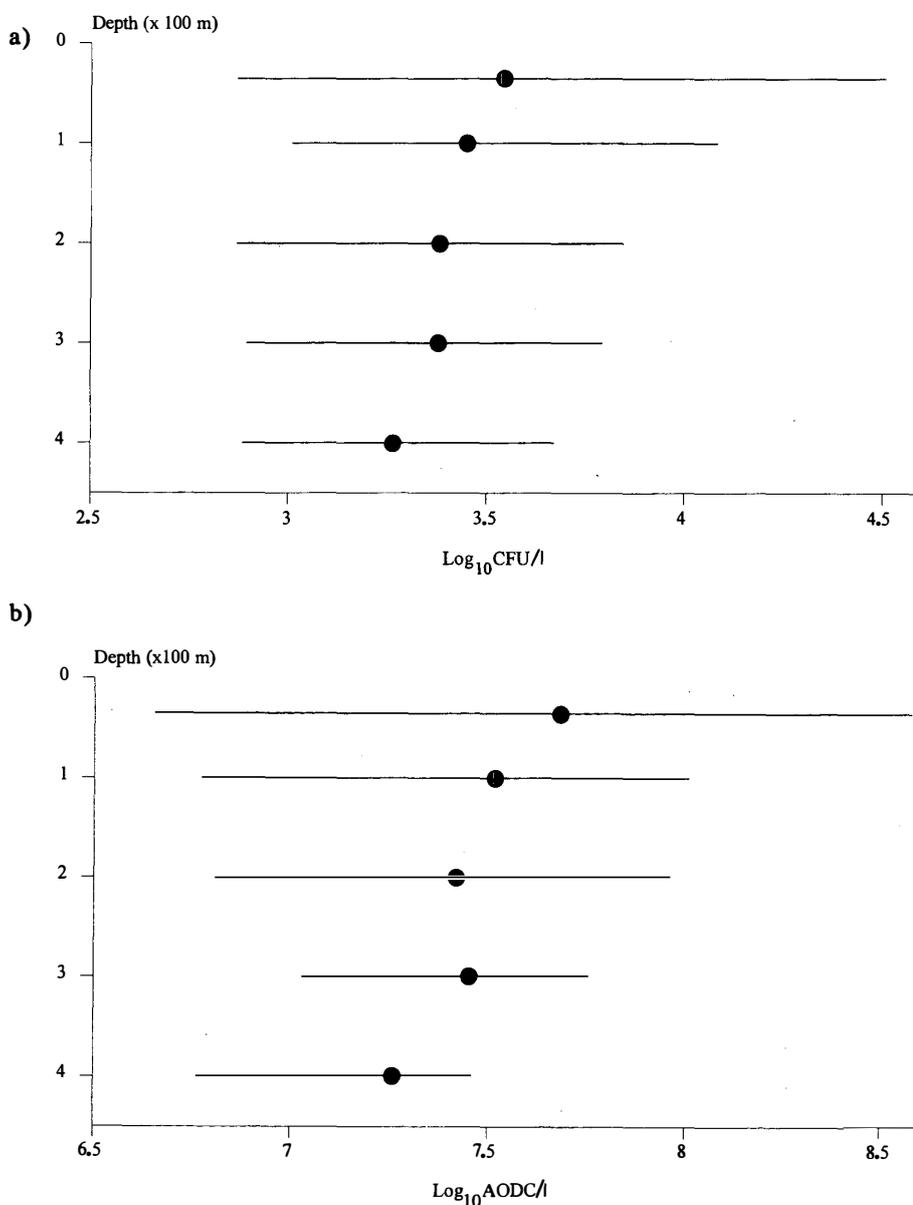


Fig. 4. Greatest range in (a) CFU and (b) AODC at the centre of the bay was noted at 10/50 m. Means (●) and ranges tended to decrease with depth.

Table 1. Mean CFU (1°C and 15°C) as a percentage of coincident AODC at the centre of the bay. Throughout the study CFU constituted a similar fraction of AODC at each depth.

Depth (m)	CFU × 10 <sup>3</sup> /l	AODC × 10 <sup>7</sup> /l	CFU : AODC (%)
10/50	3.51	4.80	0.007
100	2.81	3.29	0.009
200	2.41	2.63	0.009
300	2.39	2.85	0.008
400	1.83	1.82	0.01
5 HMC	11.1	4.83	*
10 HMC	12.5	14.1	*

\* see text.

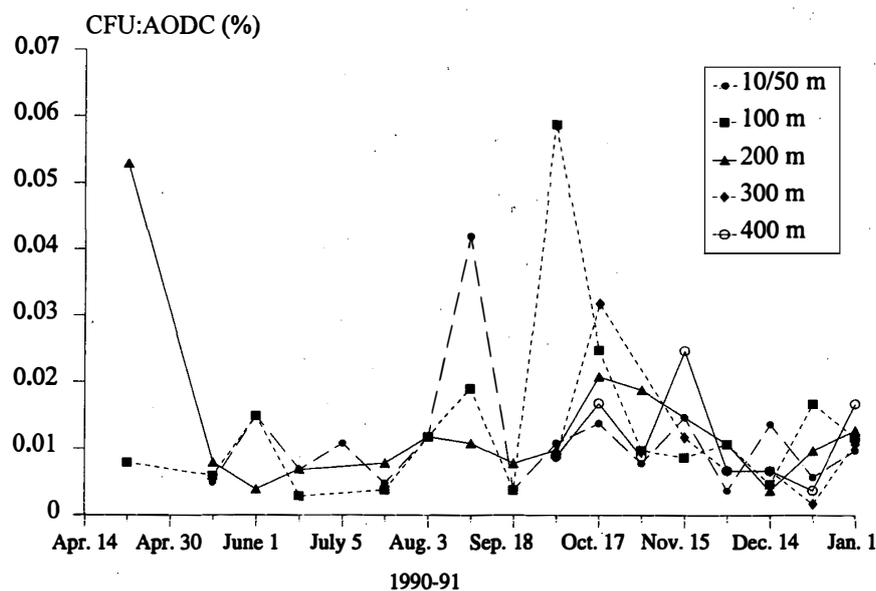


Fig. 5. CFU as a percentage of the coincident AODC at standard depths at the centre of Admiralty Bay. Throughout the study CFU constituted only a few hundredths of one percent of AODC.

Table 2. Comparison of coincident CFU and AODC determined at each depth (two sample t-test). Means varied significantly in all samples. (correlation, *r*: ns—not significant).

Depth (m)	<i>t</i>	<i>p</i>	<i>r</i>
10/50	$t_{29}=31.0$	<0.001	0.73
100	$t_{27}=33.2$	<0.001	ns
200	$t_{26}=37.5$	<0.001	0.51
300	$t_{13}=31.2$	<0.001	ns
400	$t_{13}=32.1$	<0.001	ns
5 HMC	$t_{15}=22.6$	<0.001	ns
10 HMC	$t_{12}=14.6$	<0.001	0.67

ns: not significant.

Table 3. Comparison of CFU numbers determined 1°C and 15°C in water samples from each depth (t-test of log ratios). Significant differences between each incubation temperature in terms of the number of CFU were noted at 100 and 200 m at the bay's centre, and at both sampled depths in Half Moon Cove.

Depth (m)	<i>t</i>	<i>p</i>
10/50	$t_{17}=1.27$	ns
100	$t_{16}=2.18$	<0.05
200	$t_{15}=2.82$	<0.02
300	$t_6=1.32$	ns
400	$t_6=1.83$	ns
5 HMC	$t_7=2.99$	0.02
10 HMC	$t_6=3.59$	<0.01

ns: not significant.

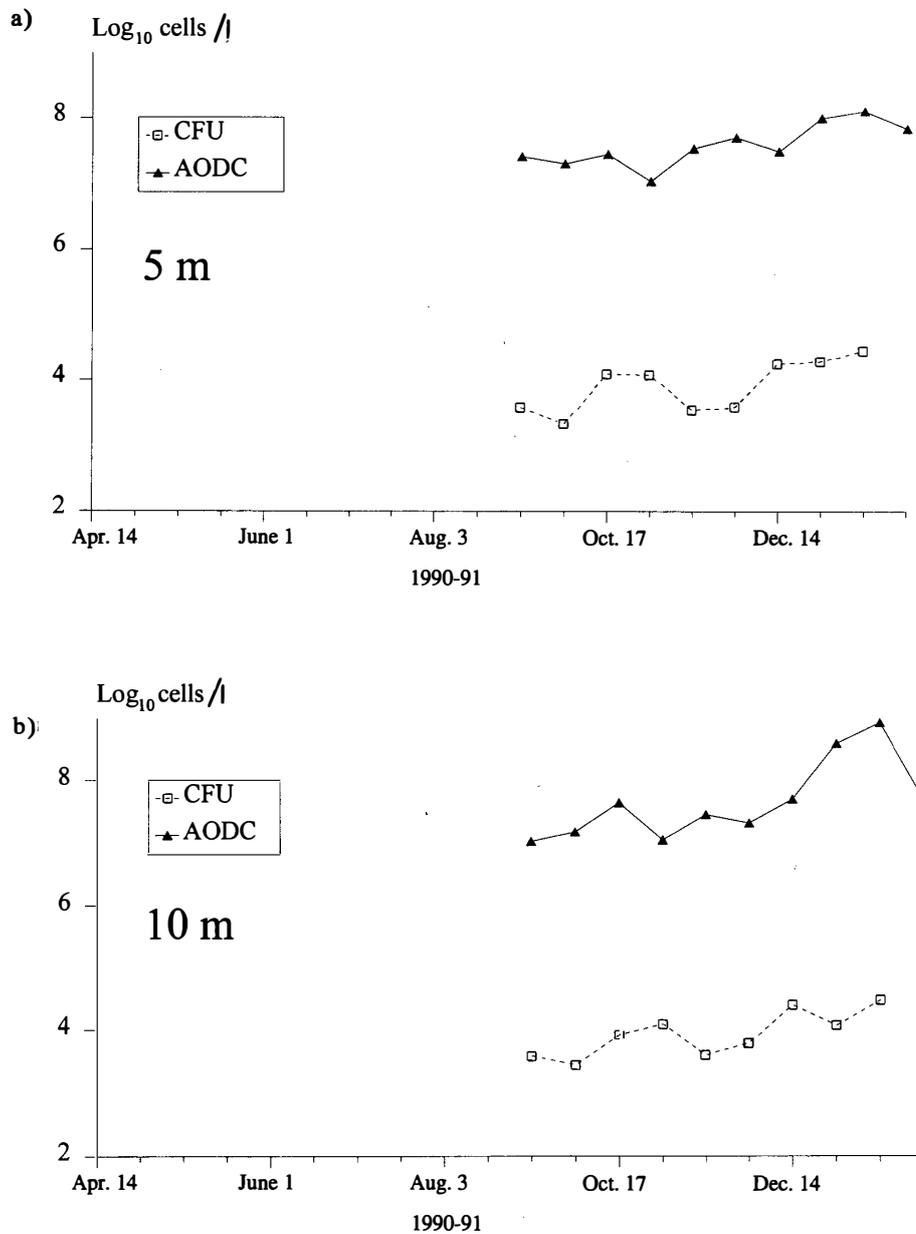


Fig. 6. Numbers of CFU (mean of those determined on NASW at 1°C and 15°C) and AODC isolated at (a) 5 m, and (b) 10 m depth in Half Moon Cove (1990-91).

Table 4. Cell morphology of the total bacterioplankton at each depth (%).

Depth (m)	Rods	Cocci	Vibrio/Filament
10/50	59.83	39.45	0.72
100	57.85	42.02	0.13
200	56.99	42.68	0.33
300	56.74	42.91	0.35
400	53.31	47.00	0.69
5 HMC	54.96	44.29	0.76
10 HMC	55.36	43.29	1.36

### 3.2. CFU and temperature

In samples from 100 m and 200 m the numbers of CFU determined on NASW after incubation at 1 °C and 15 °C varied significantly, with more being determined after incubation at 15 °C (Table 3). The number of CFU determined at each incubation temperature over the 400 m water column, however, did not vary with depth (one-way ANOVA of log ratios of CFU at each temperature:  $F_{1,128}=0.48$ ,  $p=0.754$ ).

### 3.3. CFU and AODC in Half Moon Cove

CFU numbers in Half Moon Cove showed an upward trend from the commencement of sampling (Fig. 6a, b), averaging  $11 \times 10^3$  and  $12.5 \times 10^3/l$  at 5 and 10 m respectively; maximum values at these depths were attained in January ( $35 \times 10^3$  and  $46 \times 10^3/l$  respectively, both at 15 °C). Significantly more CFU were isolated from each depth after incubation at 15 °C (Table 3). Lowest AODC counts were noted in September (5 m) and November (10 m) (Fig. 6a, b). At 5 m, CFU isolated at 1 and 15 °C constituted 0.018% and 0.022% of AODC respectively. Equivalent figures for 10 m were 0.005% and 0.002%. The structure of the total bacterial population in the cove was similar to that at the centre of the bay (Table 4).

### 3.4. Cell morphology, biovolumes, and bacterial biomass

Differences between depths and sites in terms of the percentage of each cell type were negligible (Table. 4). Rods tended to dominate the bacterioplankton, although in the upper 10/50 m this fraction's size varied from 21 to 96% with highest levels coinciding with highest CFU counts. *Vibrio* and filamentous forms usually comprised less than 1% of the population at each depth.

Rods were divided into 8 length classes of 0.5  $\mu\text{m}$  increments; over 60% of rods

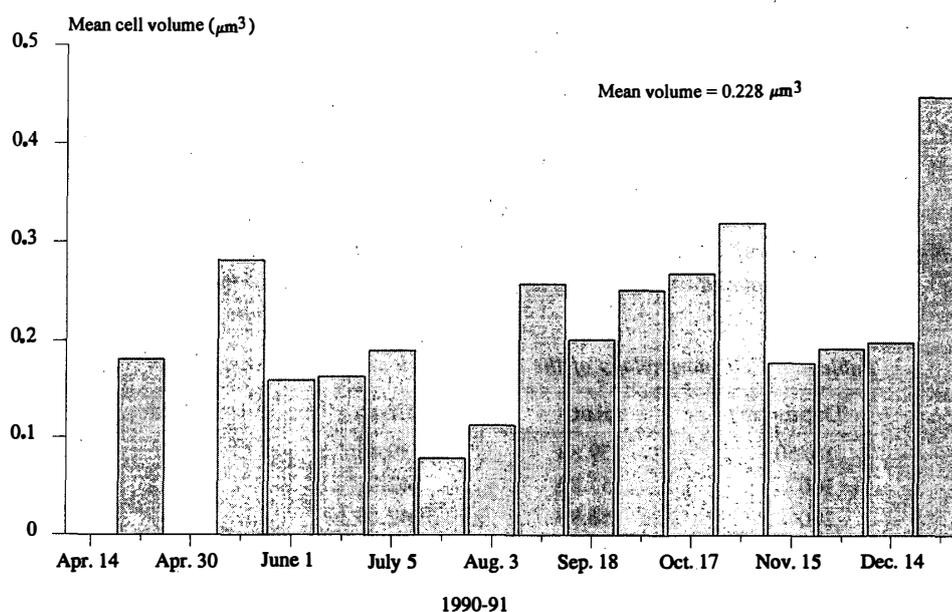


Fig. 7. Mean bacterial cell volume in the upper 100 m of Admiralty Bay (April 1990 to January 1991). Mean volume in each sample generally fell between 0.15 and 0.3  $\mu\text{m}^3$ .

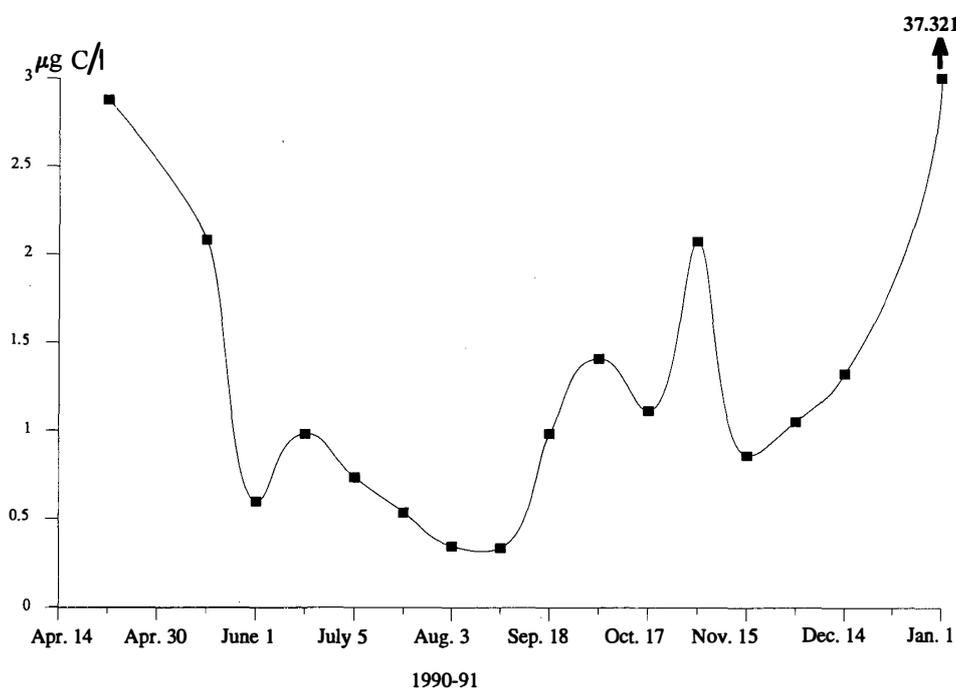


Fig. 8. Lowest amounts of bacterial carbon ( $\mu\text{g}/\text{l}$ ) in the upper 100 m of the bay were noted in winter. Bacterial carbon on 1st January exceeded that on all other dates by 1 to 2 orders.

measured 0.5 to 1  $\mu\text{m}$  in length, and only 0.04% exceeded 2  $\mu\text{m}$ . Cocci were divided into 6 diameter classes of 0.2  $\mu\text{m}$  increments; the largest class contained cells of 0.2 to 0.4  $\mu\text{m}$ , but overall 80% measured 0.2 to 0.8  $\mu\text{m}$ . Between 17th April and 3rd August cells to 0.5  $\mu\text{m}$  length/diameter dominated the bacterioplankton; from 24th August those over 0.5  $\mu\text{m}$  increased in prominence. As one would expect, small numbers of large cells constituted large fractions of the total biovolume, and where present comprised considerable fractions of the total carbon. With this in mind, the mean cell volume in the upper 100 m was 0.228  $\mu\text{m}^3$  ( $n=1636$ ,  $\text{SD}=0.356 \mu\text{m}^3$ ), ranging from 0.088  $\mu\text{m}^3$  (18th July) to 0.45  $\mu\text{m}^3$  (1st January) (Fig. 7), with a mean cell biomass of 50.63 fg C/cell. Bacterial biomass ranged from 0.338 to 37.321  $\mu\text{g C}/\text{l}$  (24th August and 1st January respectively), mean 3.415  $\mu\text{g C}/\text{l}$  ( $n=16$ ) (Fig. 8).

#### 4. Discussion

Marine microbiological studies conducted in Admiralty Bay during this study from April 1990 to January 1991, showed that neither numbers of heterotrophic (CFU) nor total (AODC) bacteria at the bay's centre varied significantly with depth. The strong tidal influences (MADEJSKI and RAKUSA-SUSZCZEWSKI, 1990) in this area, together with upwellings resulting from the bay's topography, promote the development of a largely homogenous water column; this was reflected in the similar CFU fractions of AODC at each depth. The low stability of the water column in the bay has been well documented, with LIPSKI (1987) reporting the absence of both a thermocline and halocline throughout the year, and terrestrial run-off in summer giving rise to only local variations. Pronounced increases in bacterial numbers during spring (*cf.* Figs. 2 and 3) in the upper

100 m followed blooms in both the phytoplankton (KOPCZYŃSKA, pers. commun.) and tintinnid populations (WASIK and MIKOŁAJCZYK, pers. commun.). The scale of the increase of *ca.* 1 order over two weeks exceeded all others at this station and confirm the strong seasonal patterns described for the Antarctic marine environment by CLARKE (1988).

The temporal stability and small range noted in CFU numbers at the centre of the bay contrasted with the findings of ZDANOWSKI (1985a), who reported CFU at the same station ranging over three orders between mid-May and August of 1979. This author also noted that CFU numbers peaked after a dramatic decrease in the numbers of krill (*Euphausia superba*) that had been present until mid-May (ZDANOWSKI, 1988a) of that year. ZDANOWSKI and DONACHIE (1993a) compared these findings with the simultaneous low krill and bacterial abundances noted in this study, during which no krill were caught from early May to October, despite regular trawls; salps in fact dominated the zooplankton in the bay during 1990 (MENSHENINA and RAKUSA-SUSZCZEWSKI, 1992; pers. obs.). CFU numbers in the bay were, however, comparable to those reported by ZDANOWSKI (1982, 1985a, 1995) for the Bransfield Strait, and by KIM (1991) for the Bransfield Strait immediately prior to this study. With respect to other Antarctic inshore sites, counts were up to four orders lower than for Adélie Land (DELILLE and MALLARD, 1991), with similar differences existing between the work of DELILLE and CAHET (1985), DELILLE (1987), and DELILLE *et al.* (1988) and CFU in Half Moon Cove. As both areas host seasonally large avifauna populations, such contrasting counts may be attributable to differing levels of terrestrially derived bacteria (PIETR, 1993) through the proximity of sampling points to the shore and terrestrial run-off.

With respect to the Total bacteria, their numbers in Antarctic seawater tend to vary between  $10^7$  and  $10^8/l$  (HODSON *et al.*, 1981; ZDANOWSKI, 1985b; BAILIFF *et al.*, 1987) though most published data concerns only the austral summer. AODC counts in this work tended towards the lower end of this range, with minima of  $10^6/l$  at most depths during winter. Comparable numbers have been reported for the Bransfield Strait (ZDANOWSKI, 1985b, 1988b), the Gerlache Strait (KARL *et al.*, 1991), and surface waters between Elephant Island and the South Orkneys (ZDANOWSKI and DONACHIE, 1993b).

#### 4.1. CFU as a fraction of AODC

The CFU (*i.e.* heterotrophic) fraction of AODC reflects, in part at least, the trophic status of the habitat in question. For example, DONACHIE (1995) noted that CFU constituted up to *ca.* 21% of AODC in krill, *E. superba*, stomach, whilst in the surrounding water column CFU comprised much less than 1% of AODC. The low CFU:AODC values seen throughout the water column of Admiralty Bay during winter again reflect the water column's homogeneity at this time; peaks at most depths during spring reflect both enhanced nutrient levels and increased stability (KOPCZYŃSKA, 1980, 1981; LIGOWSKI and KOPCZYŃSKA, 1993). Values recorded during this study were, however, lower than previously reported for the bay (ZDANOWSKI, 1985b). In terms of the number of dividing cells, only 0.8% of the total bacteria in the upper 100 m between 2nd October and 1st January were in a dividing state, further evidence of low nutrient availability during winter (*cf.* DELILLE *et al.*, 1988; DONACHIE, 1995).

#### 4.2. CFU numbers and temperature

The isolation from a number of depths of significantly more CFU after incubation at 15°C than 1°C confirms that these bacterial populations growing at low, stable temperatures, show optima for growth and activity at temperatures up to 15°C higher (MORITA, 1975). DELILLE and PERRET (1989), however, reported no such difference in numbers of CFU determined at 4°C and 18°C (Kerguelen Archipelago and Adélie Land). With respect to marine bacterial populations in Admiralty Bay, ZDANOWSKI (1995) described increases in their specific growth rates ( $Q_{10}$  2.14 to 2.25) as temperature rose from 0 to 15°C; such a response is reflected in this work in the highest numbers of bacteria in the surface waters, and the overall highest correlation between CFU and AODC in the upper 10/50 m.

#### 4.3. Cell morphology, biovolume, and bacterial biomass

The bacterioplankton is rarely described in terms of cell morphology. During this study rods comprised on average the largest overall fraction, although their contribution actually varied in any one sample from 21 to 96%. Highest numbers actually coincided with the highest counts of heterotrophic bacteria, after the phytoplankton bloom collapsed. In this respect, ZDANOWSKI (1995) described how both the total and saprophytic bacterial populations in the bay during spring were dominated by rods. Furthermore, DAWSON *et al.* (1985) related large proportions of rod-shaped bacteria at stations close inshore to high levels of nutrients derived from the phytobenthos and terrestrial input, and the evidence clearly supports their suggestion that this group reflects the nutrient status of this environment.

The mean volume of a bacterial cell in the upper 100 m of the water column varied considerably but, as with bacterial numbers, lowest values were recorded in winter: the minimum of 0.088  $\mu\text{m}^3$  (18th July), however, actually compares with values reported for a south eastern Portuguese lagoon (0.107  $\mu\text{m}^3$ , *cf.* BARBOSA, 1991), and for late summer in the Central and Western Baltic Proper (0.091  $\mu\text{m}^3$ , *cf.* GÖCKE and RHEINHEIMER, 1991). The latter authors did consider as high a maximum biovolume of 0.145  $\mu\text{m}^3$  determined in the Bay of Bothnia, but this is only one third of the maximum, albeit rather extreme value of 0.45  $\mu\text{m}^3$  noted in midsummer during this study, some time after the phytoplankton bloom had subsided (KOPCZYŃSKA, pers. commun.).

Bacterial carbon levels over much of the study were below values reported for this region, with only the maximum (in January) exceeding 4  $\mu\text{g C/l}$ . The low rates of primary production and an unstable water column during winter contribute to the low bacterial biovolumes and biomass at this time (minima in July and August, respectively). For example, KARL *et al.* (1991) reported bacterial carbon in the Gerlache Strait during the austral summer ranging from 4 to 28  $\mu\text{g C/l}$ , and a similar range was reported by ACOSTA POMAR *et al.* (1993, Table 3) for total summer picoplankton in the Ross Sea. Besides this study only one author (ZDANOWSKI, 1995) has described bacterial biomass in Admiralty Bay: values of 8.4  $\mu\text{g C/l}$  in April/June 1983, and 1.46  $\mu\text{g C/l}$  in September/October of the same year fall within the range determined in this study.

To conclude, the seasonal changes in bacterial numbers, cell volumes, and bacterial biomass determined in Admiralty Bay, testify to the pronounced seasonal patterns in biological activity in the Antarctic marine environment. In addition to these marked

seasonal changes, however, bacterial populations in this region do vary numerically, physiologically, and morphologically over longer temporal scales (*cf.* ZDANOWSKI and DONACHIE, 1993b; ZDANOWSKI, 1995); this is particularly true with respect to the numbers of heterotrophic bacteria and the structure of the bacterioplankton population. Although the difficulties encountered in comparing different areas within Antarctica are clear, this study has underlined the importance of longer term inshore bacteriological studies, particularly those covering the austral winter.

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