*Polarforschung 72 (1), 31 - 40, 2002 (erschienen 2004)*

# Free-Living and Associated Bacteria in the Coastal Waters of Ardley Cove (King George Island, Antarctica): Quantitative Changes from February to October

by Vladimir V. Ilinskiy[1] and Alexander N. Gorshkov[2]

**Abstract:** We determined the numbers of free-living and associated (aggregated or bonded with particles) bacteria in the coastal water of King George Island at an offshore (St. 1) and a nearshore station (St. 2) as a function of physico-chemical parameters. Water samples were collected between March and October at St. 1 and between April and October at St. 2. Direct counts of total bacteria varied from $0.53 \cdot 10^8$ to $5.02 \cdot 10^8$ cells $l^{-1}$. Associated microorganisms accounted for 5 to 20 % of the total number of bacteria. Strong Spearman and Pearson correlations were observed ($R = 0.82$; $P = 0.001$) between the numbers of free-living and associated bacteria at St. 1. These two groups of bacteria were nearly evenly distributed in the horizontal transects from inshore to offshore waters at depths of 1-10 m in Ardley Cove. There were no substantial differences in the numbers of either free-living or associated bacteria in vertical transects too. Their number at St. 1, but not at St. 2, correlated significantly with all tested environmental parameters (salinity, temperature, solar radiation, nitrate, phosphate and chlorophyll a concentrations), except nitrite concentrations in water. The most probable reason for these correlations is that a common seasonal trend is characteristic of most tested parameters during the March to October period.

**Zusammenfassung:** Es wurden Zahlen von freilebenden und angehefteten (aggregierten, oder an Partikel gebundenen) Bakterien im Meeresgebiet vor King George Island an einer küstenfernen (St. 1) und einer küstennahen Station (St. 2) in Bezug auf die physikalischen und chemischen Eigenschaften der Wasserkörper bestimmt. Die Wasserproben wurden zwischen März und Oktober an St. 1 und zwischen April und Oktober an St. 2 genommen. Direktzählungen der Bakterien lagen zwischen 0,53 und 5,02 $10^8$ Zellen $l^{-1}$. Angeheftete Mikroorganismen machen 5-20 % der Gesamtzahl aus. Signifikante Korrelationen (Spearman und Pearson) konnten an St. 1 zwischen den angehefteten und freien Zellen beobachtet werden ($R = 0,82$; $P = 0,001$). Diese beiden Bakteriengruppen waren in den Tiefenzonierungen der küstennahen und küstenfernen Stationen der Ardley Cove zwischen 1 m und 10 m gleichartig verteilt. Die Zellzahlen von St. 1, aber nicht von St. 2, korrelierten signifikant mit den Umweltparametern (Salinität, Temperatur, Einstrahlung, den Konzentrationen von Nitrat, Phosphat und Chlorophyll a), nicht aber mit der Konzentration von Nitrit. Die naheliegendste Erklärung für diese Beziehungen ist der gleichartige saisonale Trend, dem diese Parameter in der Periode von März bis Oktober unterliegen.

## INTRODUCTION

Bacteria with sizes less than 1 µm play the main role in the microbial loop. Their direct consumption by zooplankton is restricted or even impossible because of the particularities of the constitution of the zooplankter oscular apparatus. Thus, aggregated or particle-bonded (associated) bacteria have to be regarded as an important additional food source for large planktonic organisms, and detritus provides the main food source for Antarctic krill (PAINTING et al. 1985). In this case, the food chain, usually involving bacteria, has special paths.

There is another important ecological aspect also involving associated bacteria. In spite of the fact that their percentage of the total number of the bacteria in the World Ocean is rather insignificant and seldom exceeds 20 % (AZAM et al. 1983), their specific heterotrophic activity (i.e. activity per cell) considerably exceeds that of free-living bacteria, and for the Southern Ocean per cell activity for the first group may be higher than for the last group by a factor of 50-100 (HODSON et al. 1981, FUKAMI 1995). Some recent data indicate that attached bacterial activity may play a key role in the breakdown and dissolution of particulate detritus and organic micro- and macro-aggregates at all in aquatic ecosystems (SIMON et al. 2002, ANESIO et al. 2003). Though relatively much scientific information has accumulated concerning the total bacteria number (TBN) in the Southern Ocean (KARL 1993), little is known about the amount of associated micro-organisms and the relationships between free-living and associated bacteria during Antarctic year. Some data of full year observations are available only for coastal waters of Adelie Land, which is located in the Indian sector of Southern Ocean (DELILLE & MALLARD 1991), and for an offshore station, located 110 km southwest of the Kerguelen Islands ((DELILLE 2003). There are some results of austral summer observations on spatial changes of total cell number and the fraction of bacteria, attached to particles already present for Admiralty Bay, King George Island (DAWSON et al. 1985).

The goal of this study was to investigate quantitative seasonal changes in associated and free-living bacteria direct counts in conjunction with important environmental parameters in the coastal waters of King George Island, at shallow and deep-water stations, located in Ardley Cove. Nearly all observations were conducted during the austral autumn and the austral winter periods, which have been poorly investigated in comparison with the austral summer time (KARL 1993). Here, we report the results of the first microbiological and hydrochemical studies ever conducted in the Ardley Cove area.

## MATERIAL AND METHODS

### *Study area*

The study was carried out between March and October 1989 in Ardley Cove, King George Island, one of the South Shetland Islands. This cove is one of the several side-arms of Maxwell Bay. The latter is approximately 14 km long and 6 to 14 km wide with a maximum depth of 520 m, separated from the Bransfield Strait by a 430 m deep sill. Ardley Cove is located

---

[1] Department of Hydrobiology, Moscow State Lomonosov University, Biology Faculty, Vorob'evi gori, 119899 Moscow, Russia; <ilinskiivladimir@mtu-net.ru>.
[2] Arctic and Antarctic Research Institute, ul. Beringa 38, 199226 St. Petersburg, Russia.

Manuscript received 4 April 2002; accepted 26 September 2003

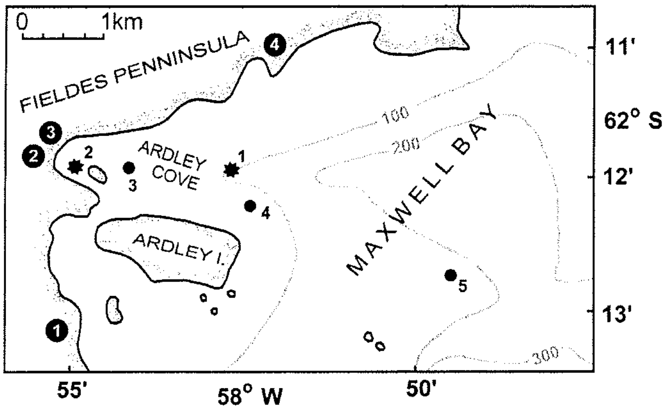


**Fig. 1:** Location of sampling stations in Ardley Cove and Maxwell Bay. Long-time sampling station: *1 deep-water offshore station St. 1; *2 shallow water station St. 2; (•3, •4, •5: stations along the transect shore - sea. National research stations: (1) China; (2) Chile; (3) Russia; (4) Uruguay.

**Abb. 1:** Probenlokationen in Ardley Cove und Maxwell Bay. Langzeit-Stationen: *1 Tiefwasser-Station St. 1; *2 Flachwasser-Station St. 2; •3, •4, •5 Stationen entlang des Profils Strand – Meer. Nationale Forschungsstationen: (1) China, (2) Chile, (3) Russland, (4) Uruguay.

in the northwest part of Maxwell Bay and flanked by Ardley Island and the Fieldes Peninsula (Fig. 1). St. 1 (the maximum depth was 100 m) is located at a distance of 1 km from the shore and St. 2 (the maximum depth was 20 m) at a distance 20 m from it (Fig. 1). A transect from shore to open water was sampled (stations 3, 4, 5), located inside and outside the Ardley Cove (Fig. 1) in February and May. The Russian wintering Antarctic station Bellingshausen was used as the base for our work. It is located on the shore of Ardley Cove, near the Chilean wintering Antarctic station Teniente Marsh. Some stations of other countries are located in adjacent areas (Fig. 1). A small creek disgorges into the Ardley Cove near Bellingshausen Station. The coast of Ardley Cove is glacier-free, a small snow-bound area remains in summer in the mountainous part only.

### *Sampling*

Subsurface water samples were collected at St. 1 during March-October and St. 2 during April-October periodically on the same days in main with a 5 l water sampler at depths of 1, 10 and 20 m. At St. 1 more detailed vertical sampling at depths 1, 5, 10, 15, 20 and 75 m was performed in March and April. During the short (c. two weeks) period with ice cover in September, water samples at St. 2 were taken from a drill hole. No observations at St. 1 were conducted at this time. Some samples were collected in February and May at depths of 1, 5 or 10 m in the horizontal transects from the shore to the open waters of Maxwell Bay. All samples were kept cold after collection and transported to the laboratory within two hours.

### *Physico-chemical parameters and chlorophyll a*

Salinity was measured with a GM-65 salinometer and $O_2$ concentrations were determined by the modified Winkler method (GARRITT & CARPENTER 1966). Standard seawater procedures (ORADOVSKIY 1977) were used to determine nutrients concentrations. Water transparency was measured with Secci disk.

Chlorophyll *a* content was determined using acetone extracts according to the recommendations of the SCOR-UNESCO working group (Determination of photosynthetic pigments etc., 1966).

### *Determination of bacterial parameters*

The TBN were determined using the acridine orange direct count (AODC) method with a ML-2 (LOMO) epifluorescence microscope (IL'INSKII 1995). The aqueous solution of acridine orange was added to formalin-fixed water samples. Free-living and associated bacteria were counted separately. Standard deviations varied from 5 to 15 % and from 5 to 40 % for the means of numbers of the free-living bacteria (FLBN) and of associated bacteria (ABN), respectively.

Statistica 5.1 for Windows programs package was used for all statistic's procedures.

## RESULTS

### *Abiotic parameters*

The air temperature varied significantly (from +4 to –15 °C) during the sampling period (Fig. 2), water temperature in the surface layers (1-20 m) at the two stations was low and almost constant between –1.2 and +1.4 °C at St. 1 and between –0.42 and –1.9 °C at St. 2 (Fig. 2). The most significant temperature changes occurred at the initial phase of our observations. From the last days of March to the middle of April air and water temperature declined markedly. There were no drastic changes in salinity and oxygen concentration: they slowly increased during the austral autumn and were stable during the austral winter (Fig. 2).

| Station no | Date | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 26-03 | 30-03 | 10-04 | 23-04 | 03-07 | 16-07 | 31-07 | 16-08 | 19-09 | 17-1 |
| | Transparency (m) | | | | | | | | | |
| 1 | 6.0 | - | 7.0 | 6.5 | 9.0 | 8.0 | 11.0 | 10.5 | 12.0 | 10.5 |
| 2 | - | 8.0 | - | - | 10.0 | - | 10.0 | - | 12.0 | 10.5 |

**Tab. 1:** Water transparency (Secci depth) at offshore (1) and nearshore (2) stations in Ardley Cove during March to October.

**Tab. 1:** Wassertrübung (Secci-Tiefe) an strandfernen (1) und strandnahen Stationen (2) in Ardley Cove während der Monate März bis Oktober.

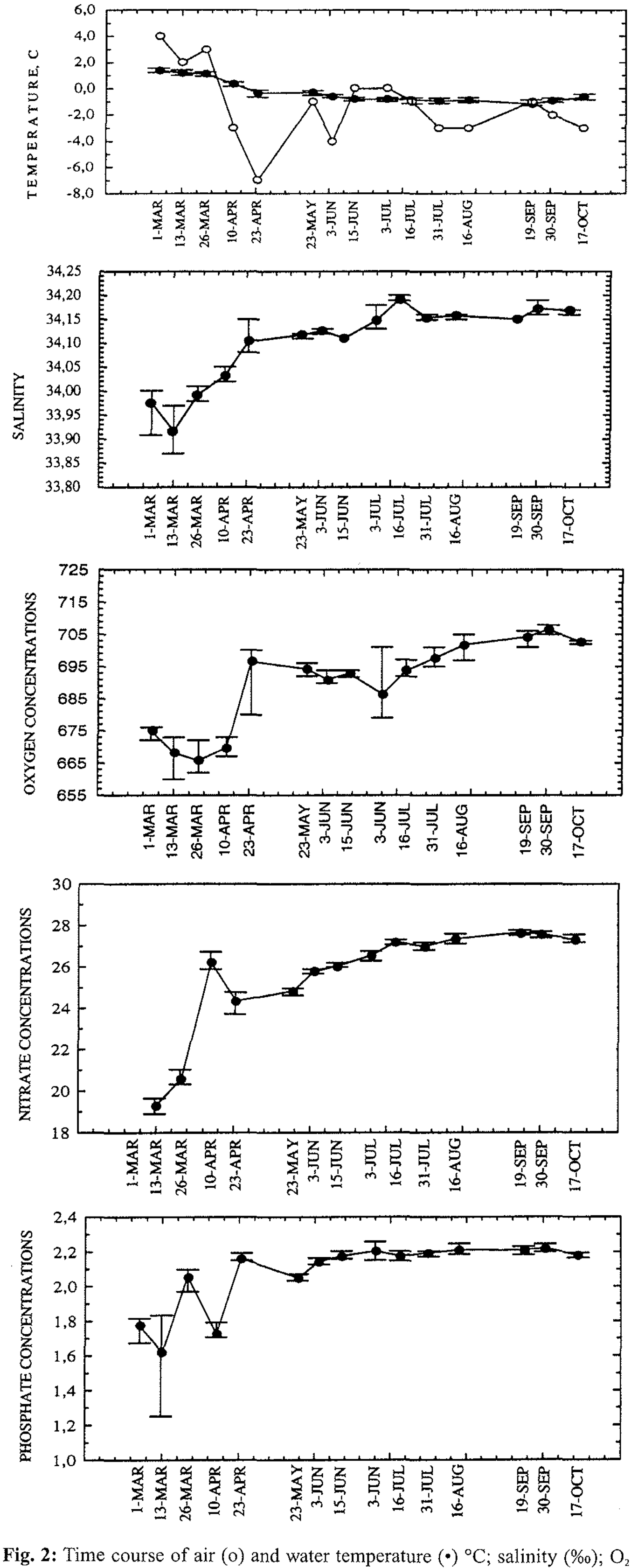


**Fig. 2:** Time course of air (o) and water temperature (•) °C; salinity (‰); $O_2$; $NO_3^-$ and $PO_4^{3-}$ concentrations (µg-at $l^{-1}$) in surface layers at offshore station St. 1 in Ardley Cove (average values for 1-20 m depth). Horizontal lines = max. and min. values.

**Abb. 2:** Zeitlicher Verlauf der Luft- (o) und Wassertemperatur (•) °C, Salinität (‰), $O_2$, $NO_3^-$ und $PO_4^{3-}$ -Konzentrationen (µg-at $l^{-1}$) in Oberflächenschichten der küstenfernen Station St. 1 in Ardley Cove (Mittelwerte für Tiefen von 1–20 m); horizontale Linien = Max- und Min-Werte.

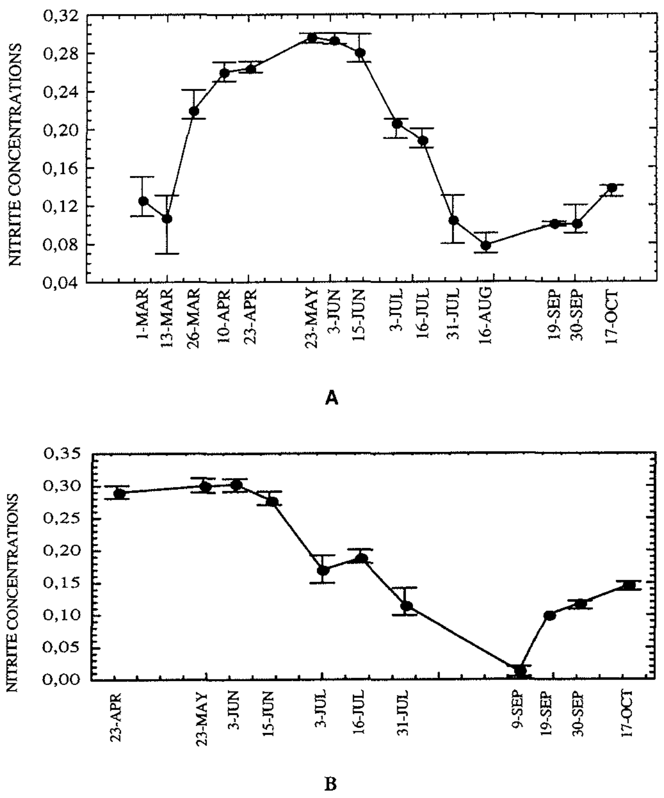


**Fig. 3:** Time course of $NO_2^-$ concentrations (µg-at $l^{-1}$) at St. 1 (A) and St. 2 (B) in Ardley Cove (average values for 1-20-m depth). For further explanations see Fig. 2.

**Abb. 3:** Zeitlicher Verlauf der $NO_2^-$-Konzentrationen (µg-at $l^{-1}$) an St. 1 (A) und St. 2 (B) in Ardley Cove (Mittelwerte für Tiefen von 1–20 m). Weitere Erläuterungen vgl. Abb. 2.

Water transparency in Ardley Cove varied between 6.0 and 12.0 m (Tab. 1) during the March-October period. Transparency values were low between March and April and high in September.

A sharp decline of solar radiation occurred in the austral autumn and the minimum fell on the austral winter months June and July. These values rapidly increased subsequently.

Marked oscillations in nitrate and phosphate concentrations occurred in March and April; a weak increase in these parameters was observed later in the year (Fig. 2). The time course of nitrite concentrations at St. 1 was quite different from that of other hydrochemical parameters (Fig. 3). Concentrations increased markedly in March and April, were almost stable for some time, decreased during the austral winter months June and July and finally stabilized during the August-October period. The total level of the vital biogenic compounds nitrate and phosphate remained high during the whole observation period.

The spatial distribution of abiotic parameters in the surface water layers (1 m and 5 m) of Ardley Cove at stations 3, 4 and 5, located between the coast and open waters (Tab. 2), demonstrated the following trend: the water temperature was lower near the shore and higher at the offshore stations. As for the nutrient concentrations, they were slightly higher near the shore than at the offshore stations.

| Station No | Sampling depth (m) | Water temp. (°C) | Salinity (‰) | $O_2$ ($\mu$g-at $l^{-1}$) | $PO_4^{3-}$ ($\mu$g-at $l^{-1}$) | $NO_3^-$ ($\mu$g-at $l^{-1}$) | $SiO_3^{2-}$ ($\mu$g-at $l^{-1}$) |
|---|---|---|---|---|---|---|---|
| 3 | 1 | -0.68 | 34.13 | 691 | 2.11 | 25.1 | 71.9 |
| | 5 | -0.52 | 34.14 | 691 | 2.15 | 25.0 | 73.8 |
| 4 | 1 | -0.40 | 34.13 | 689 | 2.10 | 24.7 | 70.7 |
| | 5 | -0.40 | 34.13 | 681 | 2.11 | 24.6 | 71.0 |
| 5 | 1 | -0.20 | 34.14 | 679 | 2.06 | 24.6 | 69.1 |
| | 5 | -0.19 | 34.13 | 680 | 2.03 | 24.7 | 69.1 |

**Tab. 2:** Abiotic parameters along the transect shore - sea during May

**Tab. 2:** Abiotische Parameter entlang des Profils Strand - Meer im Mai

| Parameters | Stat. No | Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | S | $PO_4^{3-}$ | $SiO_3^{2-}$ | $NO_2^-$ | $NO_3^-$ | $SR_{10}$ | Chl *a* | FLBN | ABN |
| Water temper. (T) | 1 | $-0.73^1$ | $-0.73^1$ | $-0.56^1$ | - | $-0.59^1$ | $0.50^1$ | $0.76^1$ | $0.55^1$ | $0.48^2$ |
| | 2 | - | $-0.43^2$ | $-0.60^1$ | $0.59^1$ | $-0.44^2$ | $-0{,}55^1$ | - | - | - |
| Salinity. (S) | 1 | X | $0.75^1$ | $0.77^1$ | $-0{,}28^3$ | $0.81^1$ | $-0.41^1$ | $-0.64^1$ | $-0.63^1$ | -0.53 |
| | 2 | X | $0.31^3$ | $0.63^1$ | $-0.60^1$ | $0.57^1$ | - | - | $-0.50^3$ | - |
| $PO_4^{3-}$ | 1 | | X | $0.80^1$ | $0.37^1$ | $0.77^1$ | $-0.30^3$ | $-0.57^1$ | $-0.50^2$ | $-0.50^2$ |
| | 2 | | X | $0.46^2$ | $-0.48^2$ | $0.42^2$ | - | - | - | - |
| $SiO_3^-$ | 1 | | | X | $-0.42^2$ | $0.80^1$ | - | $-0.34^3$ | $-0.53^2$ | $-0.53^2$ |
| | 2 | | | X | $-0.76^2$ | $0.73^1$ | $0.42^2$ | - | - | - |
| $NO_2^-$ | 1 | | | | X | $-0.55^1$ | $-0.47^1$ | - | - | - |
| | 2 | | | | X | $-0.81^1$ | $-0.50^2$ | - | - | - |
| $NO_3^-$ | 1 | | | | | X | - | $-0.56^2$ | $-0.42^3$ | $-0.47^3$ |
| | 2 | | | | | X | $0.35^3$ | - | - | - |
| $SR_{10}$ | 1 | | | | | | X | $0.60^1$ | $0.71^1$ | $0.42^3$ |
| | 2 | | | | | | X | $0.81^1$ | - | - |
| Chl *a* | 1 | | | | | | | X | $0.35^3$ | - |
| | 2 | | | | | | | X | - | - |
| FLBN | 1 | | | | | | | | X | $0.82^1$ |
| | 2 | | | | | | | | X | - |

**Tab. 3:** Spearman correlation matrix (only significant correlation coefficients with $P \leq 0.05$) of biological and abiotic parameters in the offshore (St. 1) and nearshore (St. 2) waters of Ardley Cove from March to October ($SR_{10}$ sum of solar radiation at sea level for ten days before sampling). - no significant correlation; 1 $p \leq 0.001$; 2 $p \leq 0.01$; 3 $p \leq 0.05$.

**Tab. 3:** Spearman Korrelations-Matrix (nur signifikante Koeffizienten $P \leq 0{,}05$) der biologischen und abiotischen Parameter der strandfernen St. 1 und strandnahen St. 2 der Ardley Cove für den Zeitraum März bis Oktober ($SR_{10}$ = Summe der solaren Strahlung auf Meereshöhe für 10 Tage vor der Probenahme. - keine signifikante Korrelation; 1 $p \leq 0{,}001$; 2 $p \leq 0{,}01$; 3 $p \leq 0{,}05$.

| Parameters | T | S | $PO_4^{3-}$ | $SiO_2^{2-}$ | $NO_2^-$ | $NO_3^-$ | $O_2$ | Chl *a* |
|---|---|---|---|---|---|---|---|---|
| Correlation coefficients | 0.59 | 0.74 | 0.66 | 0.57 | 0.93 | 0.83 | 0.73 | 0.73 |

**Tab. 4:** Correlation coefficients ($P \leq 0.001$) between parameter changes at St. 1 and at St. 2 for samples collected at both stations at the same days only during April to October.

**Tab. 4:** Korrelationskoeffizienten ($P \leq 0{,}001$) einiger Parameter für an gleichen Tagen im Zeitraum April bis Oktober an St. 1 und St. 2 gesammelte Proben

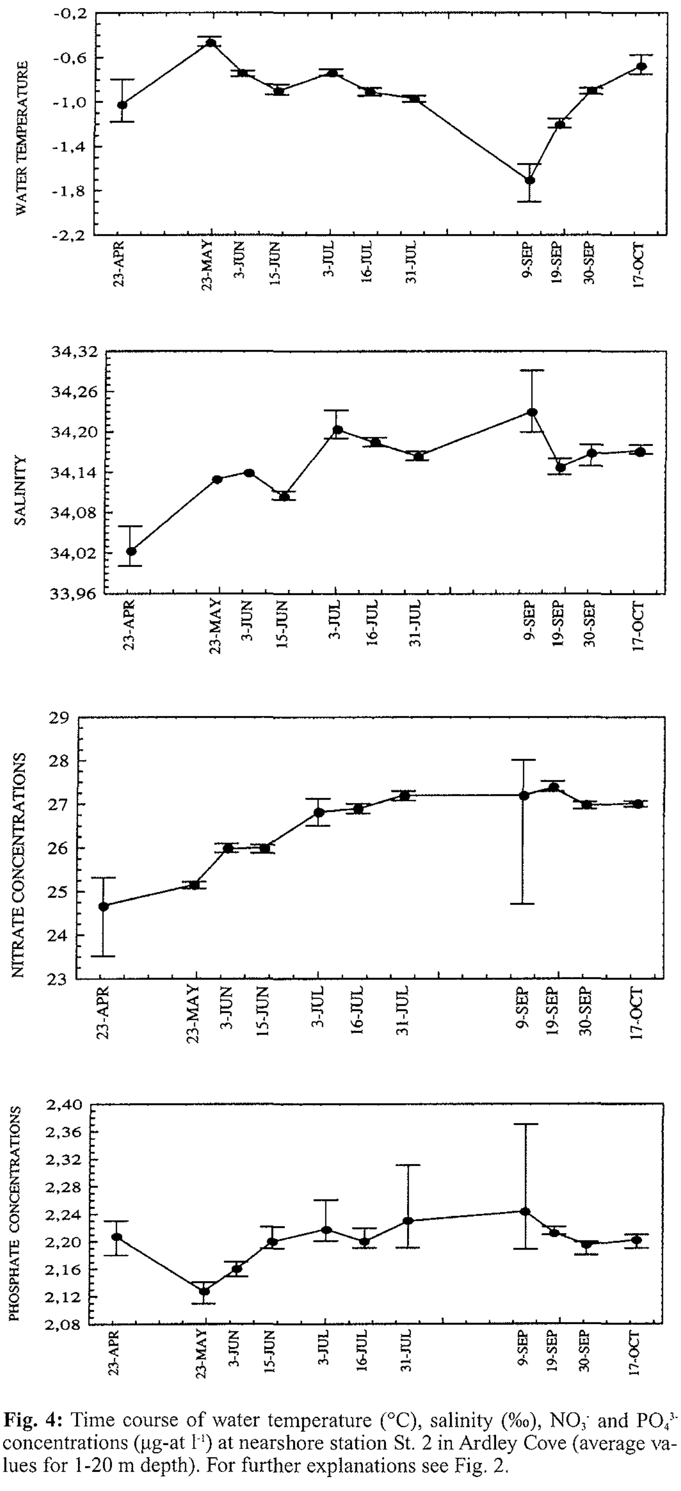


**Fig. 4:** Time course of water temperature (°C), salinity (‰), $NO_3^-$ and $PO_4^{3-}$ concentrations (µg-at $l^{-1}$) at nearshore station St. 2 in Ardley Cove (average values for 1-20 m depth). For further explanations see Fig. 2.

**Abb. 4:** Zeitlicher Verlauf von Wassertemperatur (°C), Salinität (‰), $NO_3^-$ and $PO_4^{3-}$-Konzentrationen (µg-at $l^{-1}$) an der küstennahen Station St. 2 in Ardley Cove (Mittelwerte für Tiefen von 1–20 m). Weitere Erläuterungen vgl. Abb. 2.

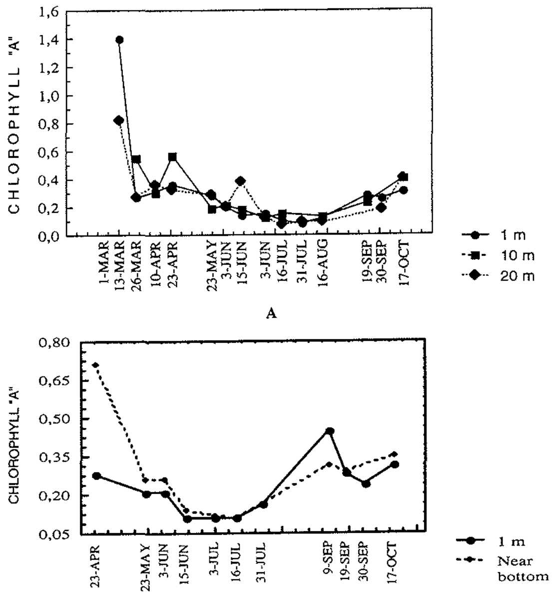


**Fig. 5:** Time course of chlorophyll *a* concentrations (µg $l^{-1}$) in the surface layers at St. 1 (A) and St. 2 (B) in Ardley Cove.

**Abb. 5:** Zeitlicher Verlauf der Chlorophyll *a*-Konzentration (µg $l^{-1}$) in den Oberflächenschichten an St. 1 (A) und St. 2 (B) in Ardley Cove.

The time course for abiotic parameters was nearly the same at St. 2 in Ardley Cove as at St. 1 in the late April-October period (Fig. 3B, Fig. 4). The most marked difference occurred in the water temperature, which was usually lower at St. 2 than at St. 1.

Significant pair correlations occurred between the values of most abiotic parameters at the deep-water station (St. 1), the nitrite concentrations being the only exception (Tab. 3). The number of significant correlations between the same parameters at the nearshore station was relatively low (Tab. 3). Strong correlations were observed between abiotic parameters at St. 1 and those at St. 2. (Tab. 4).

### *Chlorophyll a*

The chlorophyll *a* concentration at St. 1 was at maximum at the beginning of our observations in March (near 1.5 µg/l) and drastically declined later (Fig 5A). The chlorophyll *a* concentration in winter was at a low level until August, followed by a slow increase in the value of this parameter.

Chlorophyll *a* concentrations at St. 2 declined considerably only at a depth of 20 m and during the April-May period (Fig 5B). Only one peak of chlorophyll *a* concentrations occurred at both depths during the short ice cover period in Ardley Cove (in September).

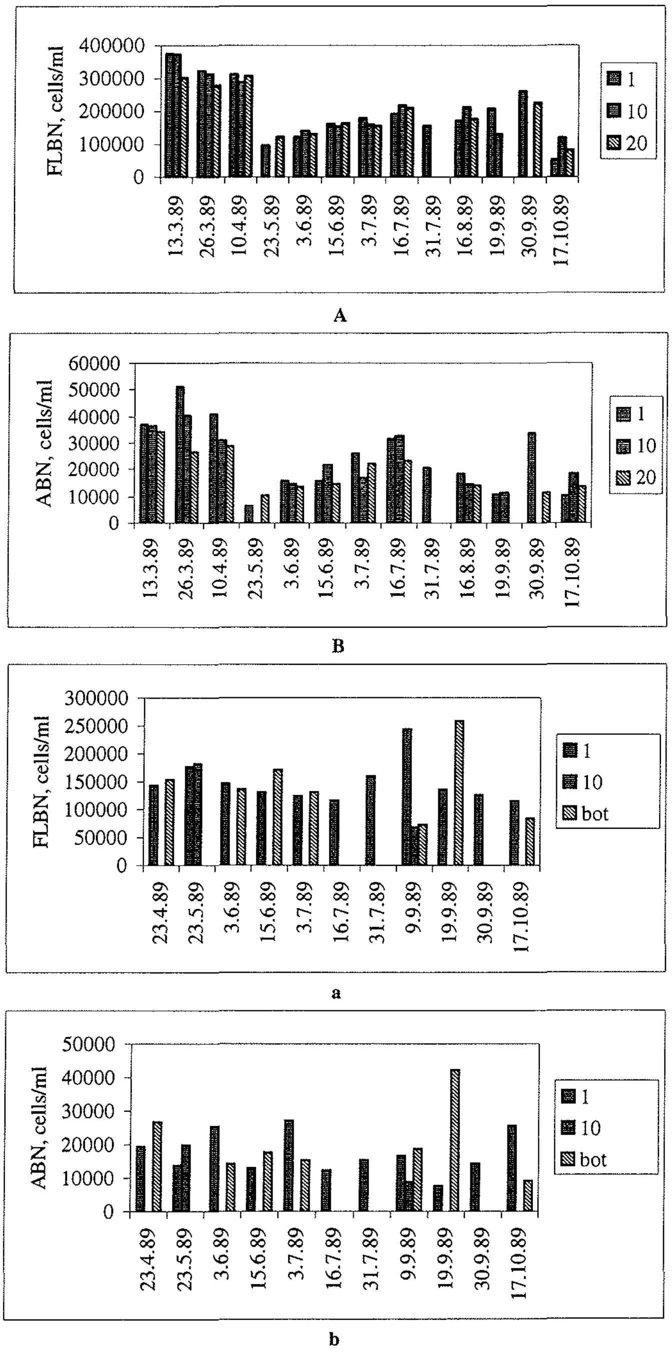
### *Microbiological parameters*

TBN varied at St. 1 in Ardley Cove from March to October within the range of (1.02 ±0.18) x $10^8$ cell $ml^{-1}$ to (5.59 ±0.09) x $10^8$ cells $l^{-1}$. Free-living bacteria dominated strongly the total bacterial population (Fig. 6A, B). The percentage of associated bacteria varied from 5 to 17 % of TBN (Tab. 5). The maximum value FLBN occurred at the beginning of the period under study (March) and declined in April and May. No marked changes occurred subsequently (Fig. 6A). Only one noticeable peak of FLBN was observed at a depth of 1 m in September, shortly after the disappearance of the sea ice cover. The time course of ABN at St. 1 had nearly the same pattern as FLBN. Only the ABN values were nearly one order of magnitude lower (Fig. 6B).

| Stat. No. | sampl depth | Data | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 13 | 26 | 10 | 23 | 23 | 3 | 15 | 3 | 16 | 31 | 16 | 9 | 19 | 30 | 17.1 |
| | | 3 | 3 | 4 | 4 | 5 | 6 | 6 | 7 | 7 | 7 | 8 | 9 | 9 | 9 | 0 |
| St. 1 | 1 | 9 | 14 | 12 | - | 6 | 12 | 9 | 13 | 14 | 12 | 10 | - | 6 | 11 | 17 |
| | 10 | 9 | 10 | 11 | - | - | 10 | 12 | 10 | 13 | - | 6 | - | 5 | - | 14 |
| | 20 | 10 | 9 | 9 | - | 8 | 9 | 8 | 13 | 9 | - | 8 | - | 8 | 5 | 14 |
| St. 2 | 1 | - | - | - | 12 | 7 | 15 | 9 | 18 | 10 | 9 | - | 6 | 5 | 10 | 18 |
| | 10 | - | - | - | - | 10 | - | - | - | - | - | - | 11 | - | - | - |
| | bottom | - | - | - | 15 | - | 9 | 9 | 10 | - | - | - | 20 | 14 | - | 10 |

**Tab. 5:** ABN percentage from TBN in the offshore St. 1 and nearshore St. 2 waters at Ardley Cove for the period of March to October.

**Tab. 5:** Anteil der angehefteten Bakterien an der Gesamtzahl der Bakterien an der strandfernen St. 1 und der strandnahen St. 2 in Ardley Cove von März bis Oktober.



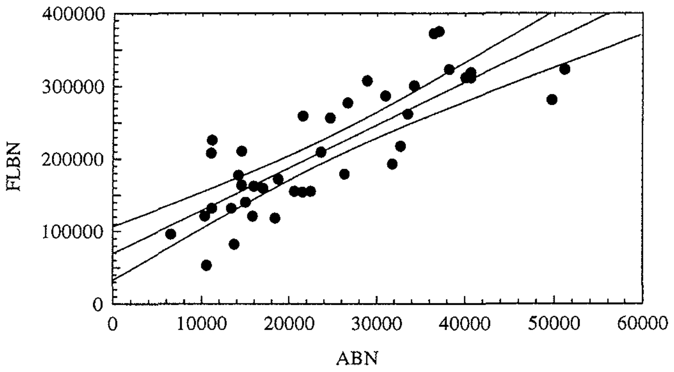


**Fig. 7:** Correlation between free-living bacteria number (FLBN) and associated bacteria number (ABN) at St. 1 (according to data for whole observation period of March to October).

**Abb. 7:** Beziehung zwischen Zahlen freilebender (FLNB) und angehefteter (ABN) Bakterien an St. 1 für den Gesamtzeitraum März bis Oktober.

There is a strong positive Spearman rank correlation between FLBN and ABN ($R = 0.82$; $P = 0.001$). We use some ordinary tests to check normality of FLBN and ABN data. As for FLBN, the distribution is near normal. D-statistic in Kolmogorov-Smirnov test is non-significant ($p > 0.20$) and W-statistic in Schapiro-Wilks-W test is non-significant either ($p < 0.21$). Test for ABN data normality give not that clear results: D statistic was unsignificant too ($p > 0.20$), but W statistic was significant ($p < 0.04$). With the assumption about the normal distributions both FLBN and ABN data we performed Pearson pair correlation for these two parameters. R-value is the same as for Spearman rank correlation ($R = 0.82$) and the regression equation for this relationships is $ABN = 69403 + 5.9 \cdot ABN$ (Fig. 7).

We can observe significant correlations both for FBLN and ABN with most of the tested abiotic parameters at St. 1, the nitrite concentrations being the only exception (Tab. 3). The R-values vary from 0.42 to 0.71 with a significance levels (P)

---

**Fig. 6:** Time course of free-living bacteria number (FLBN) and associated bacteria number (ABN) at 1 m, 10 m and 20 m depth at St. 1 (A, B) and at 1 m, 10 m and near bottom (bot) at St. 2 (a, b) in Ardley Cove.

**Abb. 6:** Zeitlicher Verlauf der Bakterien-Zahlen freilebender (FLBN) und angehefteter (ABN) Bakterien in 1 m, 10 m und 20 m Tiefe an St. 1 (A, B) und in 1 m, 10 m Tiefe und am Grund (bot) an St. 2 (a, b) in Ardley Cove.

from 0.05 to 0.001. Less pronounced but still significant correlation between FLBN values and chlorophyll *a* concentrations (R = 0.35; P = 0.05) is revealed (Tab. 3).

The TBN values at St. 2 varied within the narrow range of (0.77 ±0.04) x $10^8$ cell $l^{-1}$ to (3.01 ±0.09) x $10^8$ cells $l^{-1}$ and free-living bacteria clearly dominated the population in an analogy to St. 1. No drastic FLBN changes occurred from April to October, except for a small peak value in the middle of September at a depth of 1 m (Fig. 6A). As for ABN long-time changes, no clear-cut trend was observed, and only one relatively high peak value occurred after the ice cover disappearance in the middle of September (Fig. 6B).

No significant Spearman rank correlation could be established between ABN and FLBN values at St. 2. No signifycant correlation is established between FLBN changes at St. 1 and FLBN changes at St. 2. This holds also true for changes in ABN. This correlation, however, is revealed between chlorophyll *a* concentrations at these stations (R = 0.73; P <0.001) (Tab. 4).

Overall, the FLBN and ABN in the surface (1-20 m) layers at St. 1 during more detailed samplings at interstitial depths in March and April were near evenly distributed. They were only slightly higher in most cases at a depth of 1 m (Fig. 8). No marked changes in FLBN and ABN occurred near the bottom, at a depth of 20 m at St. 2 (Fig. 6A, B) and at a depth of 75 m at St. 1 (Fig. 8).

The FLBN and ABN in the surface water layers (1 m and 10 m) of Ardley Cove at the stations, located between coast and open waters were evenly horizontally distributed in February, but TBN was markedly higher at the offshore stations, especially at St. 5 in May (Tab. 6). At this last station the ABN values were over four times higher than that at St. 3 and St. 4. The ABN contribution to TBN at St. 5 reached 41 % (Tab. 6). This percentage also varied from 6 to 19 % at all other stations in February and in May. The chlorophyll *a* concentration was measured only in May and it was nearly the same at all transect stations (Tab. 6).

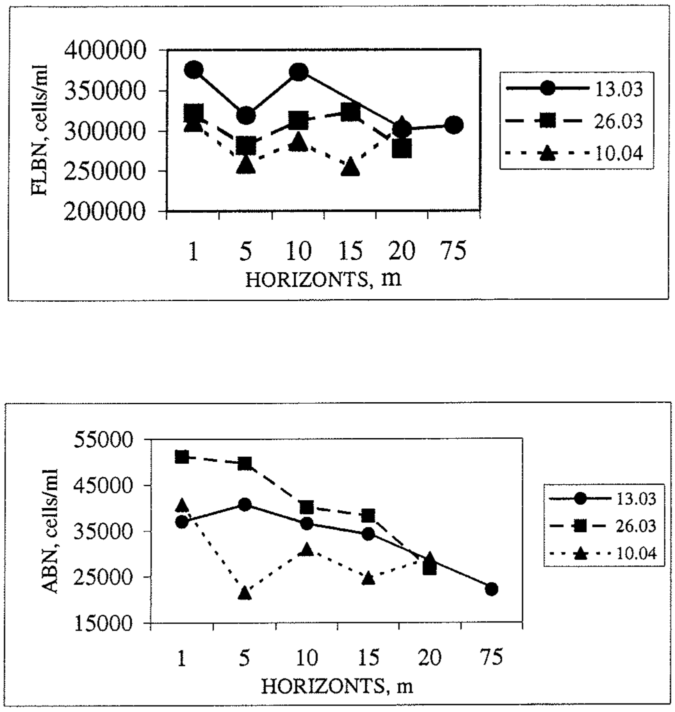


**Fig. 8:** Vertical distribution of free-living bacteria number (FLBN) and associated bacteria number (ABN) at St. 1 for different sampling periods.

**Abb. 8:** Vertikale Verteilung freilebender (FLBN) und angehefteter (ABN) Bakterien an St. 1 für verschiedene Probennahmen.

## DISCUSSION

The TBN values observed in Ardley Cove from February to October were close to the minimal and maximal values (1.4 x $10^8$ to 5.1 x $10^8$ cells $l^{-1}$) of this parameter in the Bransfield Strait and southern Drake Passage in summer time (MULLING & PRIDDLE 1987) and noticeably exceeded total cell numbers, observed in Admiralty Bay during austral summer time (DAWSON et al. 1985). These microbiological data probably indicate that Ardley Cove ecosystem functions at higher levels of both organic and inorganic nutrients. We have no data for

| Datum | Station No | Sampling depth | FLBN (cells $l^{-1}$)$10^6$ | ABN (cells $l^{-1}$)$10^5$ | TBN (cells $l^{-1}$)$10^6$ | % ABN from TBN | Chl *a* ($\mu g\ l^{-1}$) |
|---|---|---|---|---|---|---|---|
| 28 Febr. | 3 | 1 | 209 ±9 | 255 ±66 | 235 ±16 | 11 | - |
| | | 10 | 235 ±7 | 150 ±1 | 250 ±7 | 6 | - |
| | 4 | 1 | 220 ±15 | 290 ±21 | 249 ±13 | 12 | - |
| | | 10 | 271 ±2 | 352 ±27 | 306 ±17 | 12 | - |
| | 3 | 1 | 272 ±11 | 248 ±39 | 297 ±17 | 11 | - |
| | | 10 | 222 ±10 | 261 ±4 | 248 ±1 | 6 | - |
| 04 May | 3 | 1 | 103 ±10 | 199 ±10 | 123 ±11 | 19 | 0.39 |
| | 4 | 1 | 143 ±12 | 208 | 164 ±12 | 13 | 0.28 |
| | 5 | 1 | 118 ±16 | 825 ±160 | 201 ±18 | 41 | 0.35 |

**Tab. 6:** Distinct groups and total bacteria number, percentage, and chlorophyll *a* concentration at stations along the shore - sea transect across Ardley Cove and Maxwell Bay.

**Tab. 6:** Bakteriengruppen und gesamte Bakterienzahl und Chlorophyll *a* entlang des Schnitts über Ardley Cove und Maxwell Bay.

organic nutrient load, but nitrate and phosphate concentrations in Ardley Cove area according to our present observations data exceed the levels of the same nutrients in Admiralty Bay, presented by other researchers (DAWSON et al. 1985).

The spatial distribution of microbiological parameters in the surface water layers at stations, located between the coast and open waters, demonstrated that the vicinity of land in the Ardley Cove area has no significant influence on the TBN values in water. It is in contrast to the previous numerous observations if the surface effluents were rich in bio-available organic matter (RHEINHEIMER 1992). This may be due to the relatively short distance between our sampling stations and the shore. Even vertical distribution of bacteria number without elevated values near the bottom at St. 1 and St. 2 allows to expect the same even distribution of nutrients.

Associated bacteria were an important component of the microbial community in Ardley Cove. ABN here varied from 5 to 41 % of TBN, and did not depend on the season (Tab. 5, 6). These values were significantly higher than in the Southern Ocean open waters, where the ABN contribution to TBN was only 1 to 6 % (SAJIN & KOPILOV 1989) or 2 to 4 % (FUKAMI 1995). But they are closer to the values of 1-40 %, observed in Admiralty Bay (DAWSON et al. 1985) and to the values of 1-23 %, observed in coastal marine waters in temperate environments (ALBRIGHT et al. 1986, IRIBERRI et al. 1987). This may be due to the relatively small station depths in Ardley Cove and the land vicinity. As a result, the coastal waters in Ardley Cove were enriched in suspended particles, and this factor usually led to an increase in the ABN contribution to TBN (ALBRIGHT et al. 1986). The relatively low water transparency in Ardley Cove (Tab. 1) confirms this conclusion.

The vertical distribution of suspended particles may be sufficiently even too, because we did not observe higher ABN values in the near-bottom layer at St. 2 (Fig. 6). A higher percentage of associated bacteria (up to 50 % of TBN) was reported early for the Adelie Land coastal waters (DELILLE 1987) due to the enrichment of coastal waters by organic matter from the numerous penguin populations. Taking into account the higher activity of associated bacteria, we suggest their important role in the coastal ecosystems of Antarctic islands.

Significant correlation between ABN and FLBN values at St. 1 suggest a dynamic equilibrium between these two groups in deep water station. We did not observe the same correlation for St. 2, and the main reasons may be the smaller sampling size for this station in couple with the later start time of our observations at St. 2 as compared with St. 1. Different effects of environmental factors characterize St. 1 and St. 2, and, as a result, we found no significant correlations between FLBN values at St. 1 and at St. 2. The same pattern occurred with the ABN values at these two stations. A strong correlation between FLBN and ABN has been observed in marine temperate environments, in particular in the coastal waters of France (JACQ et al. 1985).

Temporal changes in both FLBN and ABN were more noticeable at the marine station (St. 1), where observations started in March (Fig. 6A, 6B), more than one month earlier than at the coastal station (St. 2). During this month, we observed most drastic changes in almost all environmental parameters in Ardley Cove. Of special importance for primary producers was the sharp decrease in solar radiation during March and April. As a result, the chlorophyll *a* concentrations drastically decreased in Ardley Cove (Fig. 6). As for micro-organisms, their number markedly decreased one month after the decrease in the phytoplankton number, in April-May (Fig. 6A, 6B). We found only a weak but significant correlation between chlorophyll *a* concentrations and FLBN. As for other Antarctic ecosystems, correlations between bacterio- and phytoplankton indexes varied from "closely related" to "full absence" (KARL 1993). Some recent results for offshore Southern Ocean waters suggested a strong coupling between phytoplankton and bacterio-plankton through DOC release and uptake under non-bloom conditions during austral summer (MORAN et al. 2001).

Water temperature is an additional abiotic parameter influencing microbial activity and growth. It slowly decreases during March-April from about +1.3 °C to c. -0.3 °C (Fig. 2). Nevertheless, this small decrease may be of importance for changes in microbial activity in combination with other environmental effects. The literature contains no reliable data on the temperature effects on TBN changes during the transitional period from autumn to winter for Antarctic waters. This period is only poorly investigated in Antarctic early in comparison with the austral summer time. The change from autumn to winter is not only a matter of temperature but also a matter of available organic nutrients decreasing (KARL 1993), and the latter affects microbial growth and activity. Temperature thus in some cases can only act as an indicator for other more effective impacts. Contrarywise, according to POMEROY & DEIBEL (1986), temperature plays the main role in the functioning of arctic aquatic ecosystems. In the opinion of these researchers, primary production was suppressed to a lesser extent than bacterial growth and respiration at water temperatures of +1.00 °C to -1.80 °C. However, French microbiologists have shown that the temperature had only a limited effect on microbial populations in Antarctic (Adelie Land region) water (DELILLE & MALLARD 1991). But only a slight decrease in temperature occurred in this case, because the maximum water temperature in summer did not exceed +0.5 °C in Adelie Land region. The summer temperature in Ardley Cove was higher (about +1.4 °C). A significant decrease in TBN (similar in extent to that in Ardley Cove) occurred during the summer-winter transitional period in another Antarctic marine ecosystem, the coastal waters of Signi Island (South Orkney Islands), located to the east from King George Island (CLARKE & LEAKEY 1996), but no significant seasonal changes have been observed for bacterio-plankton biomass and production in the marginal ice-edge zone of the Weddell-Scotia Seas (MORDY et al. 1995). Some recent data indicate that in the surface waters of the Antarctic circumpolar current, in the marginal ice zone and at the shelf ice edge, psychrophilic communities with growth optima of 11 and 4-8 °C, respectively, were present and bacterial growth at the ambient temperature (0 °C or slightly below) was reduced (SIMON et al. 1999). Therefore, we can assume that temperature changes during the transitional period from autumn to winter can produce a direct effect on TBN in Ardley Cove too.

The transitional period from autumn to winter finished in June, and the bacterial number at marine station (St. 1) started to grow slowly thereafter (Fig. 6A, 6B). It is clear that the changes in the microbial community associated with the beginning of the winter were complete in June. Only one peak value of TBN (both ABN and FLBN) was revealed in the middle of September at 1 m (St. 1) and 20 m (St. 2) depths. The most probable reason for these peaks to appear was the ice cover destruction in Ardley Cove.

Other important abiotic factors, such as mineral nutrients ($NO_3^-$ and $PO_4^{3-}$), also markedly changed during the transitional period from autumn to winter, but remained high (Fig. 2). Since we found numerous relationships between abiotic factor values and numbers of bacteria at St. 1 (Tab. 3), it seems likely that they all follow a common seasonal pattern. From the correlation matrix for abiotic parameters it is evident that there are strong correlations between all of them, except for the nitrite concentration in water. The main source of nitrite in surface marine water is ammonium that is oxidized by bacteria via nitrification processes (RHEINHEIMER 1992). The products of phytoplankton degradation and krill vital activity may serve as sources of ammonium (JOHNSON et al. 1984). In Ardley Cove a marked increase in nitrite concentrations took place soon after mass destruction of phytoplankton (Fig. 3A, 5A).

The chlorophyll a concentration drastically decreased during this period (Fig. 5A). After the end of June, the nitrite concentrations decreased, especially in the nearshore (St. 2) water (Fig. 3B). Presumably, active oxidation processes also decreased in Ardley Cove after June. The winter increase in nitrite and ammonium concentrations had been observed earlier in the surface mixed water layer at some stations along the ice edge in Weddell-Scotia Sea (GORDON & NELSON 1989). Denitrification of organic matter by associated bacteria is possible too. The oxygen concentration inside of particles might go down easily below 4-5 mg/l, a switch that allows nitrate respiration (JANNASCH 1960). But we have to take into account that the associated bacteria number in Ardley Cove is much lower in comparison with free-living bacteria number and denitrification processes may feasible have only limited significance in our case.

There is a good relationship between the characteristics of coastal and open waters in Ardley Cove. The strong correlations between the changes in abiotic parameters at St. 1 and those at St. 2 confirm this suggestion (Tab. 4), but the levels of these parameters may be different. For example, the water temperature was usually lower at nearshore St. 2 than at open water St. 1. We did not observe any significant correlations between FLBN changes at St. 1 and at St. 2. The same pattern also occurred with ABN. This is possibly due to a reaction of microbial communities to the differences in the environmental conditions in coastal and open waters in Ardley Cove.

The data obtained confirm close relationships between free-living and associated bacteria. These relationships are relatively stable during most part of the Antarctic year. Free-living bacteria dominated during the whole observation period, but the share of associated bacteria never dropped below 10 %. Taking into account the comparatively high activity of associated bacteria, they seem to play an important role in the Antarctic ecosystem. Both, biotic and abiotic parameters demonstrate a strong seasonal trend that masks their real mutual influence. Further studies taking account of other important factors (e.g., concentrations of organic matter and grazing pressure) will be necessary to elucidate the actual controlling factors responsible the abundance of free-living and associated bacteria in the nearshore waters of the Antarctic Islands.

## ACKNOWLEDGMENT

The final stage of the project was funded by the Russian Fund Fundamental Research (grant RFFR 03-05-64505). The authors are grateful to Prof. Manfred Bölter for very valuable comments on an earlier version of this manuscript and two anonymous referees for valuable comments on the manuscript.

### *References*

*Azam, F., Fenchel, T., Field, J.G., Gray, J.S. & Meyer-Reil, L.A.* (1983): The ecological role of water-column microbes in the sea.- MEPS 10: 257-263.

*Albright, L.J., McCrae, S.K. & May, B.E.* (1986): Attached and free-floating bacterioplankton in Howe Sound, British Columbia, a coastal marine fjord-embayment.- Appl. Environ. Microbiol. 51: 614-623.

*Anesio, A.M., Abreua, P.C. & Biddanda, B.A.* (2003): The role of free and attached microorganisms in the decomposition of estuarine macrophyte detritus.- Estuarine, Coastal Shelf Sci. 56: 196-201.

*Clarke, A. & Leakey, R.J.G.* (1996): The seasonal cycle of phytoplankton, macronutrients and microbial community in the nearshore Antarctic marine ecosystem.- Limnol. Oceanogr. 41: 1281-1294.

*Dawson, R., Schramm, W. & Bölter, M.* (1985): Factors influencing the production and distribution of organic and inorganic matter in Admiralty Bay, King George Island.- In: W.R. SIEGFRIED, P.R. CONDY & R.M. LAWS (eds.), Antarctic nutrient cycles and food webs, Springer-Verlag, Berlin, 109-114.

*Delille, D.* (1987): Spatial distribution of coastal Antarctic seawater bacteria: relationship with avifauna.- Polar. Biol. 8: 55-60.

*Delille, D. & Mallard, L.* (1991): Seasonal changes of Antarctic marine bacterioplankton.- Kieler Meeresforsch. 8: 213-218.

*Delille, D.* (2003): Seasonal and inter-annual variability of bacterioplankton biomass at station Kerfix, off Kerguelen Islands, Antarctica.- Oceanol. Acta 26: 225-229.

*SCOR* (1966): Determination of photosynthetic pigments in sea water.- Rep. of SCOR-UNESCO Working Group 17, UNESCO, Paris, 9-18.

*Fukami, K.* (1995): Contribution of particle-associated bacteria to the abundance and the activity of total bacterial assamblage in the Antarctic.- Bull. Mar. Sci. Fish. Kochi Univ. 15: 33-38.

*Garritt, D.E. & Carpenter, J.H.* (1966): Comparison and evaluation of currently employed modifications of the Winkler method for determination dissolved oxygen in seawater. A NASCO report.- J. Mar. Res. 24: 286-318.

*Gordon, L.J. & Nelson, D.M.* (1989): Ameriez 1988: Nutrient distribution and variability along Weddell-Scotia confluence and marginal ice zone during austral winter.- Antarct. J. US 24: 150-152.

*Hodson, R.E., Azam, F., Carlucci, A.F., Fuhrman, J.A., Karl, D.M. & Holm-Hansen, O.* (1981): Microbial uptake of dissolved organic matter in McMurdo Sound, Antarctica.- Mar. Biol. 61: 89-94.

*Hong, G.H., Kim, D.Y., Chung, H. & Pae, S.* (1989): Coastal and inshore water interaction, mixing and primary productivity in the Bransfield Strait, Antarctica during austral summer 1989/90.- Korean J. Polar Res. 2: 43-59.

*Il'inskii, V.V.* (1995): Bacterioplankton of the surface water layer in the Central Arctic Region in spring.- Microbiol. 64: 696-704.

*Iriberri, J., Unanue, M., Barcina, I. & Egea, L.* (1987): Seasonal variation in population density and heterotrophic activity of attached and free-living bacteria in coastal waters.- Appl. Environ. Microbiol. 53: 2308-2314.

*Jacq, E., Quequiner, B., Prieur, D. & Treguer, P.* (1985): Modification de l'aspect des peuplements bacteriens en relation avec un bloom phytoplanctonique en rade de Brest.- Oceanis 11: 213-222.

*Jannasch, H.W.* (1960): Versuche über die Denitrifikation und die Verfügbarkeit des Sauerstoffs in Wasser und Schlamm.- Arch. Hydrobiol. 56: 355-369.

*Johnson, M.A., Macaulay, M.C. & Biggs, D.C.* (1984): Respiration and excretion within mass aggregation of Euphausia superba: implication for krill distribution.- Crust. Biol. 4: 174-184.

*Karl, D.M.* (1993): Microbial processes in the southern oceans- In: E.I.

FRIEDMANN (ed.), Antarctic microbiology, Wiley Inc. NY, 1-63.

*Moran, X.A.G., Gasol, J.M., Pedros-Alio, C. & Estrada, M.* (2001): Dissolved and particulate primary production and bacterial production in offshore Antarctic waters during austral summer: coupled or uncoupled?- MEPS 222: 25-39.

*Mordy, C.W., Penny, D.M. & Sullivan, C.W.* (1995): Spatial distribution of bacterioplankton biomass and production in the marginal ice-edge zone of the Weddell-Scotia Sea during austral winter.- MEPS 122: 9-19.

*Mulling, B.W. & Priddle, J.* (1987): Relationships between bacteria and phytoplankton in the Bransfield Strait and southern Drake Passage.- Brit. Antarct. Surv. Bull. 76: 51-64.

*Oradovskii, S.G. (ed.)* (1977): A manual on methods for chemical analysis of marine water.- Gidrometeoizdat, Leningrad, pp. 208 (in Russian).

*Painting, S.J., Lucas, M.I. & Stenton-Dozey, J.M.E.* (1985): The South African SIBEX I Cruise to the Prydz Bay region, 1984: X. Biomass and production of bacterioplankton in Prydz Bay, Antarctica, and phytoplankton, detritus and bacterial relationships.- S. Afr. J. Antarct. Res. 15: 42-52.

*Pomeroy, L.R. & Deibel, D.* (1986): Temperature regulation of bacterial activity during the spring bloom in Newfoundland coastal waters.- Science 233: 359-361.

*Rheinheimer, G.* (1992): Aquatic Microbiology, 4th ed., John Wiley, Chichester, New York, Brisbane, Toronto, Singapore.

*Sajin, A.F. & Kopilov, A.I.* (1989): Ecological role of bacteria, located on detrite and aggregates, in southeast part Pacific Ocean waters.- Zh. Obshey Biologii 50: 682-692, (in Russian).

*Simon, M., Glöckner, F.O. & Amann, R.* (1999): Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean.- AME 18: 275-284.

*Simon, M. & Grossart, H.-P., Schweitzer, B. & Ploug, H.* (2002): Microbial ecology of organic aggregates in aquatic ecosystems.- AME 28: 175-211.