RACER: Microbial processes in the northern Gerlache Strait, 1989–1990

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Based upon the results of the 1986–1987 RACER pilot program (Nawrocki and Karl 1989; Karl et al. in press a, in press b; Bird and Karl in press; Bailiff and Karl in press), we formulated several hypotheses that collectively served as the conceptual framework for our 1989–1990 field research program. Our studies focused on determining the role of microheterotrophs (both procaryotic and eucaryotic) in the initiation and maintenance of the regional spring bloom and on the quantitative relationships between euphotic zone carbon production, biomass accumulation, organic-matter decomposition, and particle flux from the surface waters. To accomplish these objectives, we defined a field program that included measurements of:

- temporal and spatial variations in microbial biomass and production as determined by the analyses of several water-column parameters (ATP, LPS, bacterial and protozoan cell numbers, heterotrophic activity and dissolved organic nutrients, inorganic carbon and oxygen concentrations);
- water-column measurements of heterotrophic activity and microbial carbon production;
- field experiments on microbial grazing rates and particle consumption; and
- rates of particle flux (total mass, carbon, nitrogen, phosphorus, ATP, biogenic silicon, etc.).

The comprehensive database resulting from this "Microbiology and Vertical Flux" component of RACER has not yet been completed although selected results are presented in this issue, *Antarctic Journal* (Bird and Karl; Karl and Asper; Karl and Hebel). In this article, we present data on the spatial and temporal changes in total (<202 micrometer) and nanoplankton (<20 micrometer) biomass during the initiation of the 1989 austral spring bloom in the Gerlache Strait, Antarctica.

As during the RACER pilot program in 1986–1987, we collected water samples during fast-grid surveys to define the regional distribution of environmental variables (bucket samples of surface water at 33–37 stations during a 3-day period). We also had four 3-day occupations of a single station before each fast-grid survey (station "A") where more comprehensive measurements and experiments were conducted, including conductivity-temperature-depth (CTD)-Niskin bottle rosette hydrocasts to 200 meters (Huntley et al., *Antarctic Journal*, this issue). For ATP determinations, water samples were first screened through 202-micrometer Nitex mesh to remove large particles and zooplankton. This water, without further treatment, represented the total microbial biomass component (TOTAL). A subsample of the TOTAL seawater was further screened through 20-micrometer Nitex mesh; this fraction represented the nanoplankton portion of the biomass (NANO). Particulate materials from these respective subsamples were concentrated, by vacuum filtration, onto glass-fiber filters (Whatman GF/F), extracted in phosphate buffer, and stored at −20 °C for subsequent analysis by firefly bioluminescence (Karl and Holm-Hansen 1978).

TOTAL ATP increased in the euphotic zone at station A during the 4-week observation period which coincided with the initiation and progression of the spring bloom (figure 1 and the table). At the start of our field study (30 October 1989), both TOTAL ATP and NANO ATP concentrations were low and uniform with depth, indicating a well-mixed, pre-bloom condition. During the subsequent 3-week period, however, ATP increased substantially in the surface layer (figure 1) as a

Depth-integrated microbial biomass inventories (TOTAL ATP) and temporal changes at the RACER program station A (Gerlache Strait) during the initiation of the 1989 spring bloom

Date	Depth interval (in meters)	TOTAL ATP (in milligrams per square meter)	NANO ATP (in milligrams per square meter)	Estimated biomass—carbon[a] (in grams per square meter)
31 Oct 1989	0–10	1.8	1.2	0.4
	0–50	8.1	5.3	2.0
	50–200	4.4	3.4	1.1
7 Nov 1989	0–10	10.4	4.1	2.6
	0–50	31.7	14.3	7.9
	50–200	5.5	4.4	1.4
15 Nov 1989	0–10	13.2	6.0	3.3
	0–50	36.2	19.0	9.1
	50–200	10.8	9.8	2.7
19 Nov 1989	0–10	12.8	9.3	3.2
	0–50	25.5	18.1	6.4
	50–200	8.0	6.8	2.0

[a] Based on relationship, C = ATP × 250 (Holm-Hansen 1973).

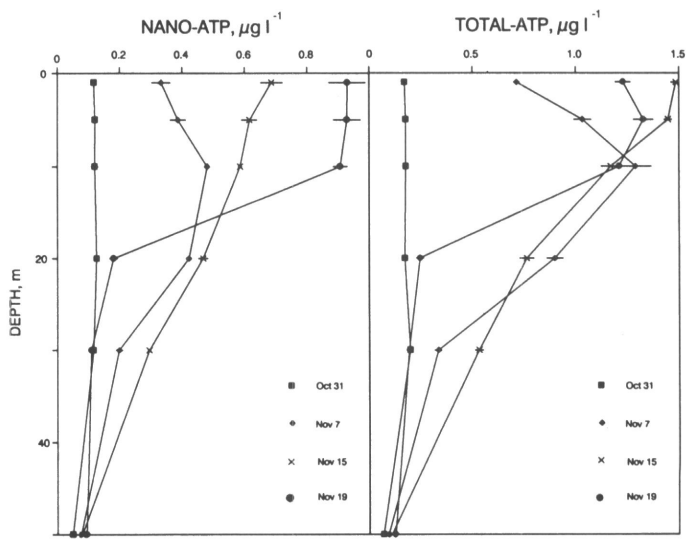

Figure 1. Concentrations of NANO-ATP (left) and TOTAL-ATP (right) versus water depth for the four independent occupations of station A. Data presented are mean (n = 3) ± 1 standard deviation. For certain samples, the ± 1 standard deviation estimate is less than the size of the symbol. (m denotes meter. μg l^{-1} denotes micrograms per liter.)

dramatic manifestation of the austral spring bloom. Depth-integrated (0–50 meters) TOTAL ATP concentrations indicate a sustained net microbial biomass production rate equivalent to 840 milligrams of carbon per square meter per day for the period 31 October to 7 November (table) which is similar to the net production estimated by seasonal oxygen accumulation (Karl and Hebel, *Antarctic Journal*, this issue). These results indicate that phytoplankton removal processes (death, grazing, sinking, etc.) must be minimal during the initiation of the bloom. Below 50 meters, TOTAL and NANO ATP concentrations were relatively constant, demonstrating that the effect of the bloom on microbial biomass accumulation was primarily restricted to the uppermost portion of the water column.

The mean depth-integrated (0–10 meters) NANO ATP:TOTAL ATP ratio ranged from a low of 0.40 on 7 November to a high of 0.73 on 19 November. Though TOTAL ATP values changed little after the second sampling period (7 November), the proportion of NANO increased with time suggesting a shift from larger to smaller plankton cells during the bloom development.

The RACER study region included stations reflecting a variety of ecological conditions. At one extreme was the Bransfield Strait portion of our study area (see map in Huntley et al., *Antarctic Journal*, this issue) which, because of intense mixing, was not expected to show evidence of a spring bloom. At the other extreme were enclosed bays and protected coastal areas of the Antarctic Peninsula where, we hypothesized, the bloom might be most well-developed due to increased surface water temperature and water column stability. The regional ATP distribution for the period 2–4 November indicates that the 1989–1990 austral spring bloom began near station A in the center of the Gerlache Strait and, independently, in several coastal embayments (figure 2). By the third fast grid survey (16–19 November 1989), the surface water microbial biomass distribution revealed a well-defined and steep concentration gradient across the Gerlache Strait with highest concentrations to the east along the Antarctic Peninsula (figure 3). Similar

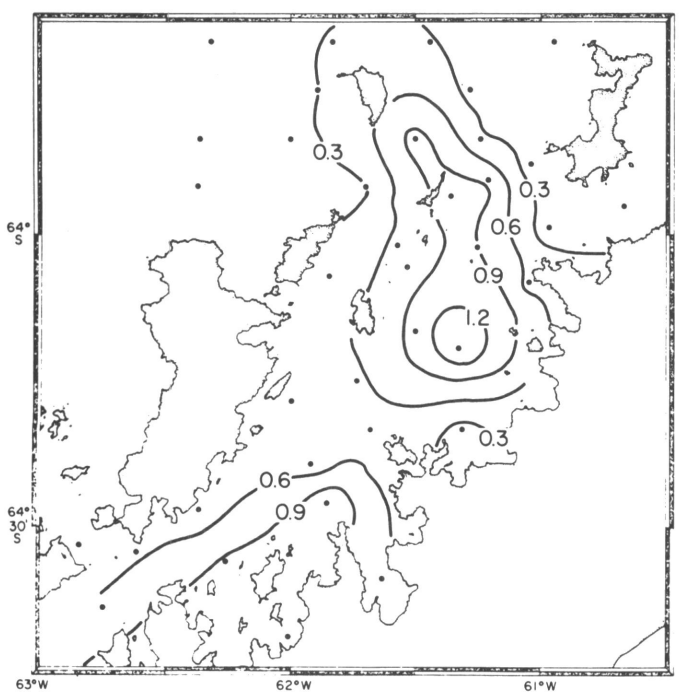

Figure 2. Regional distribution of surface water (0–1 meter) TOTAL-ATP (in micrograms per liter) over the RACER study area for the sampling period 2–5 November 1989.

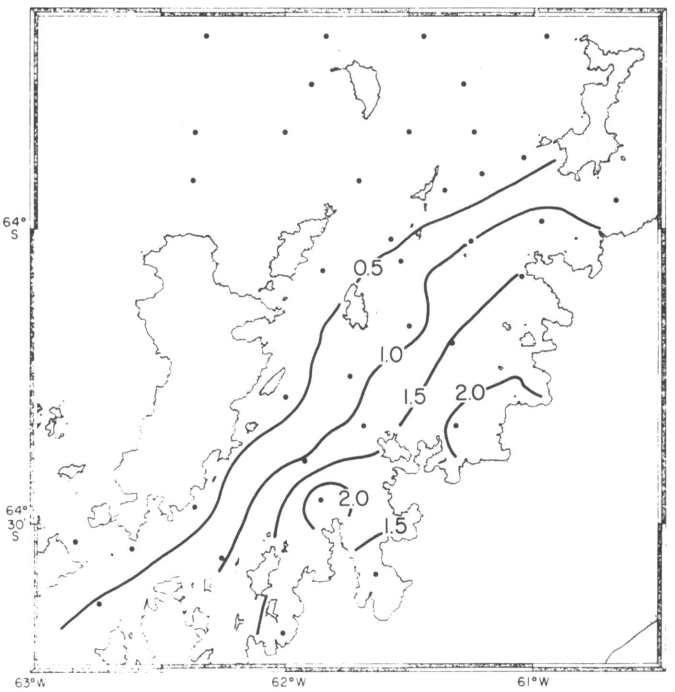

Figure 3. Regional distribution of surface water (0–1 meter) TOTAL-ATP (in micrograms per liter) over the RACER study area for the sampling period 16–19 November 1989.

spatial gradients were also observed for chlorophyll *a* and dissolved oxygen. TOTAL ATP and NANO ATP concentrations for surface water samples increased in concentration over the duration of the cruise, ranging from 100–4,600 nanograms per liter and 100–2,900 nanograms per liter, respectively. The NANO ATP:TOTAL ATP ratio ranged from a mean value of 0.52 during early November to 0.81 at the end of our field experiment which, again, provides evidence for a shift toward a smaller mean cell size as the spring bloom develops and matures.

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RACER: Microbial uptake and regeneration of ammonium during the austral spring bloom

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Recent studies on the dynamics of heterotrophic marine bacteria have shown the importance of this component of the microbial food web on the uptake of dissolved organic nitrogen and ammonium (Wheeler and Kirchman 1986; Kirchman, Keil, and Wheeler 1989). Since marine bacteria are capable of regenerating ammonium through dissolved organic nitrogen mineralization as well, their activity in the water column would influence the cycling of ammonium and, consequently, other nitrogen substrates. As part of the Research on Antarctic Coastal Ecosystem Rates (RACER) study on nitrogen dynamics, the uptake and regeneration of ammonium by bacterial heterotrophs were examined to determine the extent of their role as consumers or mineralizers of ammonium and to assess their possible impact on nutrient dynamics in this highly productive area.

Surface seawater from the RACER stations (see Huntley et al., *Antarctic Journal*, this issue) was collected by a clean bucket, filtered through a 0.8 micrometer Millipore filter, and dispensed into 2-liter polycarbonate bottles. The 0.8-micrometer filter greatly reduced the concentration of chlorophyll-*a*-containing organisms (approximately 1 percent or less of the total chlorophyll *a* was found in the filtrates) but allowed about 95 percent of the original bacterial population to pass through. The number of protozoans after filtration was less than 3 per milliliter so the filtered seawater was essentially composed of the bacterial population. The number of bacteria of the different waters sampled in the Gerlache Strait ranged from 4.0 to 6.0×10^5 bacteria per milliliter.

To five pairs of bottles were added individually nitrogen-15 labeled glutamate (0.040 micromole of nitrogen per liter), glycine (0.050 micromole of nitrogen per liter), leucine (0.040 micromole of nitrogen per liter), lysine (0.050 micromole of nitrogen per liter), and ammonium (0.2 micromole of nitrogen per liter). One bottle from each pair was filtered through a glass fiber filter (Whatman, GF/F) and the filter and filtrate frozen for initial time measurements. The remaining five bottles were placed in black paper bags and incubated for 24 hours on the ship's deck in a tank continuously supplied by surface seawater. Incubation temperature was generally 0 °C. After incubation, the water from each bottle was filtered as above, and the filter and filtrate frozen for storage.

Chlorophyll *a* concentrations were determined using the method of Holm-Hansen and Riemann (1978). Bacteria and protozoan numbers were estimated by epifluorescence microscopy using 4,6-diamidino-2-phenylindole (DAPI) (Porter and Feig 1980). A quadropole mass spectrometer was used to measure the particulate nitrogen and nitrogen isotope ratios according to the method of Ohtsuki, Ino, and Fujii (1983) and Saino (1982). The nitrogen isotope ratio of the ammonium in the filtrate was determined after steam distillation (Keeney and Nelson 1982). Dissolved free amino acid concentrations were determined by reverse phase high-performance liquid chromatography using a modification of the method of Lindroth and Mopper (1979). Ammonium concentrations were measured according to Koroleff (1976) on a Technicon autoanalyzer. Uptake estimates of ammonium and amino acids were calculated according to Dugdale and Goering (1967) and normalized to a 24-hour day. Ammonium regeneration was estimated using the model of Blackburn (1979).

Estimates of ammonium uptake and mineralization from station A301 are shown in the table. An independent estimate of ammonium uptake (u) was derived from the isotope dilution model (value in parenthesis below ammonium uptake value p). The close agreement of these two values shows that the ammonium consumed from the seawater used in the incubation was largely converted into bacterial biomass. This implies that there were no other significant pathways of ammonium metabolism except that of assimilation into particulate organic nitrogen.

According to previous concepts of nitrogen uptake by bacterial heterotrophs, the active assimilation of ammonium implies an insufficiency of organic nitrogen substrates in their environment, as a consequence, there is no regeneration of ammonium (Goldman, Caron, and Dennot 1987). As was seen from the dilution of the nitrogen-15 isotope in the ammonium substrate, however, there was active production of ammonium from the mineralization of dissolved organic nitrogen by the bacterial population. These results show that the microbial