Bacterial abundance and production and their relation to primary production in Funka Bay

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Abstract: Abundances of bacteria, phototrophic picoplankton, and heterotrophic and phototrophic nanoflagellates in Funka Bay were measured on five occasions (from June to November) in a water column with a summer thermocline. Nutrient concentrations (nitrate, nitrite, ammonium and phosphate) in the euphotic layer were low whereas chlorophyll-a concentrations fluctuated from 0.3 to 1.2 µg l⁻¹. Bacterial abundance ranged from 3.1 x 10⁵ to 1.1 x 10⁶ cells ml⁻¹, whereas the heterotrophic nanoflagellate (HNF) abundance ranged from 4.2 x 10² to 3.5 x 10³ cells ml⁻¹. In the euphotic layer, cyanobacterial abundance ranged from 1.3 x 10⁴ to 2.9 x 10⁵ cells ml⁻¹. Bacterial abundance correlated significantly with both chlorophyll-a concentration and HNF. Bacterial growth rates in the euphotic layer were estimated using a dilution culture method, and ranged from 0.024 to 0.048 h⁻¹. Bacterial net production ranged from 8.7 to 23.9 mg C m⁻³ d⁻¹. Net primary production measured in this study ranged from 0.024 to 0.048 h⁻¹. Bacterial net production ranged from 8.7 to 23.9 mg C m⁻³ d⁻¹. Net primary production measured in this study ranged from 1.1 to 52.5 mg C m⁻³ d⁻¹. We found that for the euphotic layer, integrated bacterial net production (460–780 mg C m⁻³ d⁻¹) was higher than integrated net primary production (220–610 mg C m⁻³ d⁻¹). The disparity between bacterial net production and net primary production allowed us to suggest that primary production may not be adequate to support bacterial carbon demand, and that other sources of organic matter were also used.

Key words: bacterial production, picoplankton, nanoflagellate, primary production, Funka Bay

Introduction

Bacteria are recognised as an important link between detritus, dissolved organic matter (Ducklow & Carlson 1992; Simon et al. 1992), and higher trophic organisms, especially heterotrophic nanoflagellates (Sherr & Sherr 1984; Porter et al. 1985). When temperature increases, both bacterial abundance and production are highest (Shiah & Ducklow 1994), and a higher portion of primary production is utilised by bacteria (Andersen 1988). Recently, events where bacterial production is 150% or more of phytoplankton production (i.e. the ecosystem becomes net-heterotrophic) have been reported (Goosen et al. 1997; Amon & Benner 1998).

Funka Bay is located in the subarctic region, south-west of Hokkaido, Japan. It has an area of about 2300 km² and a maximum depth of about 100 m (Fig. 1). In Funka Bay, the spring phytoplankton bloom occurs every March or April (Odate 1992), and nutrients (nitrate and silicate) in the euphotic layer are usually exhausted during the bloom (Kudo et al. 2000). Thermal stratification that occurs in summer restricts the nutrient supply from the deeper layer and the euphotic layer becomes oligotrophic. At this time, the primary producers are dominated by cyanobacteria and eukaryotic phototrophic picoplankton (Odate 1992).

In this study, we measured the seasonal fluctuations in abundance of bacteria, phototrophic picoplankton, and heterotrophic and phototrophic nanoflagellates from June until November 1998. We also investigated the importance of bacterial production in the euphotic layer in relation to primary production during this period. Bacterial growth and net production rates were measured using a dilution culture technique. This study was a part of a research project to understand the influence of bacteria on the carbon budget of Funka Bay, and moreover, few studies on the seasonal changes in microbial parameters exist for offshore waters.

The sampling period covered the summer season of
1998, and we were able to detect the cyanobacterial bloom that is typical of Funka Bay in summer (Odate 1989). We showed that in the euphotic layer, bacterial net production was more than net phytoplankton production. We also suggested that contemporary phytoplankton production may not be adequate to support bacterial carbon demand, and that other sources of organic matter were also used.

**Materials and Methods**

Sampling was carried out at Stn 30 (average depth, 92 m) in Funka Bay (42°16.2'N, 140°36.0’E) (Fig. 1) on five occasions from June until November 1998. Water samples were collected at intervals of 10 m down to 90-m depth using 5-litre Niskin samplers. Subsamples for the determination of microbial abundance were preserved with filtered (Nuclepore filter, 0.2 μm pore size) 1% glutaraldehyde (final concentration). The rest of the samples were kept in a cooler box for no more than 5 h until processing in the laboratory. Samples for nutrient determination were filtered through Whatman GF/F filters and stored at -30°C until analysis.

The temperature–salinity profile at the time of sampling was obtained with a CTD profiler (SeaBird SBE 19) whereas light attenuation (PAR) was measured with an underwater quantum sensor (Li-Cor LI-193SB). Chlorophyll a (Chl a) was extracted with N,N-dimethylformamide (Suzuki & Ishimaru 1990), and measured with a Turner fluorometer. Chlorophyll-a concentrations were measured in duplicates, and the coefficient of variation (CV) ranged from 3 to 6%. Nitrate (NO₃), nitrite (NO₂), ammonium (NH₄), and phosphate (PO₄) were measured using a Technicon AutoAnalyzer II (Strickland & Parsons 1972). Inorganic nutrient concentrations were not measured in duplicates but several samples were replicated to determine the CV of the analyses, which ranged from 3 to 5%.

To determine bacterial abundance, samples were stained with 4'6-diamidino-2-phenylindole (DAPI) (0.1 μg L⁻¹ final concentration) for 7 min (Kepner & Pratt 1994). Prepared filters were examined using a Nikon Optiphot XF microscope equipped with an epifluorescent illuminator system (Nikon EDF, 100 W super pressure mercury lamp) and Nikon filter cassette. UV (ultra violet excitation: excitor 330–380 nm, dichroic mirror 400 nm, barrier 420 nm). More than 200 cells were counted from each sample. Two replicates were read for each sample, and the CV ranged from 3 to 5%. The length and width of more than 90 bacterial cells from the upper layer were measured from photomicrographs, and their volumes (V) calculated as spheres or cylinders with two half spheres. The bacterial volume ranged from 0.013 to 0.300 μm³, and the average was 0.102 ± 0.013 μm³ (±95% CL). The equation used to convert biovolume to biomass, followed that of Simon & Azam (1989):

\[
\text{fg C cell}^{-1} = 89.6 (\mu m^3 \text{ cell}^{-1})^{0.50}.
\]

Fig. 1. Location of the sampling station (Stn 30: 42°16.2’N, 140°26.0’E) in Funka Bay, south-western Hokkaido, Japan. Iso-baths for the 40-m and 80-m depths are shown as broken lines. Inset: location of Funka Bay in Hokkaido.
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cleporc polycarbonate membrane-filter, and then 30 ml of the filtrate was filtered onto dyed 0.2 μm Nuclepore polycarbonate filters. Filters for phototrophic picoplankton counts were examined with the Nikon filter cassette B (blue excitation; excitor 420–490 nm, dichroic mirror 510 nm, barrier 520 nm). A minimum of 200 cells were counted on each filter, but for samples containing extremely low cell numbers (60 m depth and below), a minimum of 15 fields were counted. Bright yellow cells were counted as phycocerythrin rich cyanobacteria (PEC), whereas red ones were counted as eukaryotic phototrophic picoplankton (EPP). The CV for both PEC and EPP ranged from 3 to 23%, and from 10 to 33%, respectively.

For nanoflagellates, 20 ml of sample was filtered onto a dyed 1.0-μm pore-size Nuclepore filter, and then stained with the fluorochrome primulin (Bloem et al. 1986). Fields were viewed first for primulin fluorescence in nanoflagellates using the UV filter cassette, and then for Chl-a fluorescence in phototrophic nanoflagellates (PNF) using the B filter cassette. Nanoflagellates without Chl-a fluorescence were counted as heterotrophic nanoflagellates (HNF). About 50 cells were counted for each sample, and the CV for both HNF and PNF ranged from 1 to 6%, and 9 to 29%, respectively.

To measure net primary production, water samples were taken at 2, 5 and 10-m depths. The samples were spiked with NaH14CO3, and were incubated for 3 h at in situ temperatures (±1°C) in both light (in situ light intensity) and dark conditions in the laboratory. After incubation, these samples were filtered (Millipore filter Type HA, 0.45-μm pore size), and the filters were then placed in scintillation vials, and their 14C uptake rates were measured. Photosynthetic net carbon fixation rates (mg C m⁻² h⁻¹) were then calculated according to the method of Parsons et al. (1984):

\[
\text{Photosynthesis} = (R_s - R_b)W/RN
\]

where \( R \) is the total activity (dpm) of NaH14CO3 added, \( R_s \) is the sample count, \( R_b \) is the dark bottle count, \( W \) is the weight of total CO₂ present (calculated from salinity), and \( N \) is the number of hours of incubation. Details of the methodology for the determination of primary production methodology will be described elsewhere.

**Results**

The heating of the surface water by both the increasing solar radiation energy and longer daylight duration raised the surface water temperature from 13°C in June to 20°C in August. After August, surface water temperature decreased to less than 15°C in November (Fig. 2A). Salinity ranged from 31.2 to 33.9 throughout the study period (Fig. 2B). Salinity increased with season, beginning in August in the intermediate layer (40 to 60-m depth), due to the influence of the intruding Tsugaru Warm Water (salinity >33.8; Ohtani 1971), a branch of the Kuroshio. The hydrographic structure observed in this study is typical of Funka Bay (Ohtani & Kido 1980).

Both dissolved inorganic nitrogen (DIN: NO3+NO2+NH4) (Fig. 3A) and PO4 (Fig. 3B) concentrations were relatively low in the upper 40-m layer (<3.0 μM and <1.0 μM, respectively) when compared with nutrient concentrations in the 60 to 90-m layer (DIN: 0.6–23.9 μM, PO4: 0.7–2.1 μM). When integrated over the whole water column (up to 90-m depth), DIN concentration increased with season, from 360 to 650 mmole m⁻² whereas PO4 concentration ranged from 56 to 71 mmole m⁻², and was lowest in October.

In this study, the depth where light level (PAR) reached 1% of the surface value ranged from 26 to 50 m depth (mean=36 m) and for purposes of discussion, the upper 40 m was defined as the euphotic layer (Fig. 4A). Chlorophyll-a concentration ranged from 0.3 to 1.2 μg l⁻¹ in the euphotic layer, and was less than 0.1 μg l⁻¹ in the 70 to 90-m layer (Fig. 4A). Chlorophyll-a concentrations integrated over the whole water column ranged from 31 to 44 mg m⁻², and were lowest in July.

Bacterial abundance ranged from \( 3.1 \times 10^5 \) to \( 1.1 \times 10^6 \) cells ml⁻¹ (Fig. 4B), and abundance in the euphotic layer...
Fig. 3. Dissolved inorganic nitrogen (A, µM), and phosphate (B, µM) concentrations observed at Stn 30 on each sampling date.

(3.3 × 10^5 to 11.3 × 10^5 cells ml^-1), was higher than in the 60 to 90-m layer (3.1 × 10^5 to 6.0 × 10^5 cells ml^-1). When integrated over the whole water column, bacterial standing stock fluctuated within a small range (5.0 × 10^13 to 6.2 × 10^13 cells m^-2).

The distributions of both phycoerythrin rich cyanobacteria (PEC) and eukaryotic phototrophic picoplankton (EPP) were limited to the upper 60 m of the water column at Stn 30 (Fig. 5). Both PEC and EPP abundances, measured in the euphotic layer ranged from 1.1 × 10^4 to 29 × 10^4 cells ml^-1 and from 1.6 × 10^2 to 1.0 × 10^3 cells ml^-1, respectively. This was an order or two orders of magnitude lower than bacteria. When integrated over the whole water column, PEC standing stock ranged from 1.1 × 10^12 to 4.6 × 10^12 cells m^-2, whereas EPP standing stock ranged from 8.9 × 10^10 to 2.3 × 10^11 cells m^-2.

In this study, heterotrophic nanoflagellate (HNF) abundance ranged from 4.0 × 10^2 to 3.5 × 10^3 cells ml^-1, whereas phototrophic nanoflagellate (PNF) abundance was less than 1.8 × 10^2 cells ml^-1 (Fig. 6). Nanoflagellate abundance was 40% higher in the euphotic layer than in the 60 to 90-m layer. The abundances of both HNF and PNF increased towards the end of summer.

Bacterial specific growth rates (µ) ranged from 0.025 to 0.048 h^-1 for the 10-m-depth samples, and 0.024 to 0.044 h^-1 for the 40-m-depth samples (Table 1). The regression coefficients for all slopes at the exponential growth phases were above 0.7. In the euphotic layer, the average water temperature was 15°C, and correlation analysis between µ and water temperature was not significant (p > 0.10, n = 10). Shiah & Ducklow (1994) also found that bacterial growth rates were not correlated with temperature in the summer season, and suggested that the effect of water temperature...
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Abundance (x $10^5$ cells ml$^{-1}$)

Abundance (x $10^3$ cells ml$^{-1}$)

Fig. 5. Eukaryotic phototrophic picoplankton (EPP) and phycoerythrin rich cyanobacteria (PEC) abundance ($\times 10^5$ cells ml$^{-1}$) observed at Stn 30 on each sampling date.

Fig. 6. Phototrophic nanoflagellate (PNF) and heterotrophic nanoflagellate (HNF) abundance ($\times 10^3$ cells ml$^{-1}$) observed at Stn 30 on each sampling date.

Table 1. The regression slope of bacterial growth rate ($\mu$, h$^{-1}$) obtained in this study. Abbreviations: $n$ is the number of points in the regression analysis; $b\pm SE$ is the standard error in the regression slope ($b$) based on replicate measurements over time; $r^2$ is the coefficient of determination; $p$ is the significance level for the null hypothesis for $b$.

<table>
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<tr>
<th>Sampling date</th>
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<th>$n$</th>
<th>$b\pm SE$ ($\mu$, h$^{-1}$)</th>
<th>$r^2$</th>
<th>$p$</th>
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<td>22 June 1998</td>
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<tr>
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<tr>
<td>9 November 1998</td>
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<td>0.033$\pm$0.010</td>
<td>0.79</td>
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<tr>
<td></td>
<td>40</td>
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<td>0.038$\pm$0.013</td>
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was not significant towards the high end of the temperature range (in Chesapeake Bay at $>20^\circ$C). Bacterial net production (BNP) in the euphotic layer ranged from 8.7 to 23.9 mg C m$^{-3}$ d$^{-1}$. In this study, net primary production (NPP) was also measured, and ranged from 1.1 to 52.5 mg C m$^{-3}$ d$^{-1}$ (Fig. 7). The integrated NPP over the euphotic layer ranged from 220 to 610 mg C m$^{-2}$ d$^{-1}$. To integrate BNP in the euphotic layer, we used an average value of $\mu$ obtained from the 10-m and 40-m-depth-sample incubations as the $\mu$ for both depths were not significantly different (Student's $t$-test, $p>0.25$). Integrated BNP ranged from 460 to 780 mg C m$^{-2}$ d$^{-1}$, and was higher than inte-
In this study, bacterial growth rates ($\mu$) were measured by observing changes in cell numbers over time. Concerns about the 'wall-growth' effect have been reported to not be a major problem in unenriched seawater incubations (Ammerman et al. 1984). From morphological studies, Ammerman et al. (1984) also inferred that none of the major bacterial strains present at the start of the incubation overwhelmed the incubation culture. At present we know that the presence of active and inactive cells in a bacterial population might pose a problem in the evaluation of bacterial growth rates. The bacterial growth rates could be underestimated if the observed increase in cell counts were 'diluted' by the inactive bacteria. However, even if the bacterial growth rates in this study were underestimated, it would not change the conclusion that the bacterial net production (BNP) was higher than the net primary production (NPP). The growth curve can also appear to have a lag period if there is a gradual overgrowth of the inactive cells by the active cells (Zweifel & Hagström 1995). Although a lag phase was not observed in any of the observations, sub-sampling was not frequent enough to confirm this with certainty. Both $\mu$ and BNP obtained in this study were within the range reported for Ise Bay (Naganuma 1997), and in the lower range of that reported for the Seto Inland Sea (Naganuma & Miura 1997). Moreover the growth rates obtained in this study were also within the range of those obtained using the thymidine incorporation method (10 nM $^3$H-thymidine, 1-h incubation) at the same station (Lee, unpublished data).

In the euphotic layer, the ratio of integrated BNP (intBNP) to integrated net primary production (intNPP) ranged from 1.0 to 3.0, and for the whole study period the average was 1.6 (Table 2). This was high when compared to the recent suggestion by Ducklow (1999) that bacterial production is usually 20% of primary production. The BNP could be overestimated if the constant carbon conversion factor used was higher than the actual value. If we used a 'constrained' conversion factor of 15 fg C cell$^{-1}$ (Caron et al. 1995), which is similar to the lowest value obtained by Fukuda et al. (1998) for coastal samples, the integrated BNP (intBNP$_{15}$) ranged from 300 to 510 mg C m$^{-2}$ d$^{-1}$. The intBNP$_{15}$: intNPP remained high (0.7 to 1.9). However, BNP does not take into account carbon loss through bacterial respiration. To account for this respiration loss, we calculated bacterial carbon demand (BCD) according to the following equation: BCD = BNP/BGE, where BGE is bacterial growth efficiency.

Across different aquatic systems, BGE could range from less than 5% to more than 60% (del Giorgio et al. 1997). Although recent publications favour a lower growth efficiency of 10–30% (Amon & Benner 1998; Ducklow 1999), we assumed a growth efficiency of 50%, as has been used in bacterial studies in the subarctic Pacific (Simon et al. 1992; Kirchman et al. 1993), to avoid overestimating the BCD. The integrated BCD (intBCD) for the euphotic layer ranged from 930 to 1570 mg C m$^{-2}$ d$^{-1}$ and from 610 to 1020 mg C m$^{-2}$ d$^{-1}$ when the 'constrained' conversion factor was used (intBCD$_{15}$). The intBCD : intNPP ratios were always more than two (2.0 to 5.8; mean = 3.1) whereas the intBCD$_{15}$ : intNPP ratios ranged from 1.3 to 3.8 (mean =
2.0).

The NPP rates observed in this study were similar to the range of NPP (or 14C uptake rate) at Stn 30 previously reported by Odate (1992). However only the particulate primary production (net) was measured. As it is usually too difficult to measure the amount of organic matter excreted extracellularly by phytoplankton, BCD is an alternative way to guess the total primary production rates. As the BCD in this study was two to three times the measured value for net primary production, phytoplankton exudation rates of 70 and 50%, respectively, would be required to meet the BCD. However, the mean exudation rates compiled by Baines & Pace (1991) are always <40%. Moreover, studies in Funka Bay by Odate (1992) have shown that about 53% of net primary production is consumed by micro and net-zooplankton in the summer season. HNF have also been shown to utilise high molecular weight dissolved organic matter, and could compete with bacteria for it (Marchant & Scott 1993; Tranvik et al. 1993).

Although primary production drives the microbial loop in many ecosystems, conditions where seasonal bacterial carbon demand was more than phytoplankton production have been reported in lakes (Benner et al. 1988; Tranvik 1988), and both estuarine and coastal ecosystems (Goosen et al. 1997; Amon & Benner 1998). As the amount of primary production available for bacteria in the euphotic layer was insufficient, an additional source was probably needed. An important source of nutrients for coastal systems could be terrigenous dissolved organic matter (Rivkin et al. 1996; Opsahl & Benner 1997). Another probable source could be from kelp (Phaeophyta), which is widely distributed over the southern part of the mouth of the bay. Blum & Mills (1991) have shown that bacterial transformation of plant matter from seagrass plays a role in carbon transfer in aquatic food webs. Moreover, the high water temperatures observed in the euphotic layer could promote the efficiency with which bacteria utilise dissolved organic matter (Kirchman et al. 1993). The uncoupling between heterotrophic bacterial activity and primary production at times of high primary production relative to bacterial activity has also been suggested to fuel bacterial respiration and production at a later date or in another location (del Giorgio et al. 1997).

In this study, bacterial abundance was significantly correlated with Chl $a$ ($r=0.74$, $p<0.001$, $n=50$) even when a large imbalance between both BNP and NPP existed. This was previously suggested by Naganuma & Miura (1997) to also occur in the Seto Inland Sea. The log–log relationship between bacteria (BA: cells ml$^{-1}$) and Chl $a$ (Chl: $\mu$g l$^{-1}$) was examined further using linear regression analysis, and the equation generated ($\pm 95\%$ CL) was log BA$=0.219$ ($\pm 0.058$) log Chl$+5.900$ ($\pm 0.047$), ($p<0.001$, $n=50$) (Fig. 8A). The slope was within the range reported by Cota et al. (1990) (slope between 0.11 to 0.39), and showed that the relationship between bacteria and phytoplankton in Funka Bay was not fundamentally different to that found in other regions.

Bacterial abundance which reflected the balance between growth and loss, showed some correlation with HNF ($r=0.36$, $p<0.05$, $n=48$). Log–log regression analysis between bacteria (BA: cells ml$^{-1}$) and HNF (cells ml$^{-1}$) yielded the following equation ($\pm 95\%$ CL): log HNF$=0.513$ ($\pm 0.393$) log BA$+0.123$ ($\pm 2.272$), ($p<0.05$, $n=48$) (Fig. 8B). Sanders et al. (1992) also found a positive correlation between heterotrophic nanoplanckton (primarily HNF) and bacterial abundance using published data sets from a variety of planktonic environments (log heterotrophic nanoplanckton$=0.90$ log bacteria$-2.4$, $r=0.71$, $p<0.001$). In this study, HNF abundance was also correlated with PEC ($r=0.49$, $p<0.001$, $n=48$). HNF utilisation of phototrophic picoplankton (Caron et al. 1991) could have occurred and caused the lower regression slope and weak correlation between bacteria and HNF. In positive correlations, Honde-veld et al. (1994) suggest that either bacterial population density limits HNF density or HNF graze on bacteria to threshold values.

In this study, the monthly sampling strategy only gave ‘snap shots’ of the system at each date. However the period from June until November is relatively stable with respect to Chl$-a$ concentrations which remain at less than 1 $\mu$g l$^{-1}$ (Odate 1992). Except for rare storm events that upwell nutrients from below the thermocline, we were still able to evaluate the ecological significance of bacterial production in this period. The bacterial activity was high relative to the level of primary production. Other sources of organic matter were probably also utilised, and bacteria are inferred to have functioned as an important component in the food web by repackaging and transferring organic matter otherwise unavailable, to higher trophic organisms.

<table>
<thead>
<tr>
<th>Date</th>
<th>Integrated BNP (mg C m$^{-2}$ d$^{-1}$)</th>
<th>Integrated NPP (mg C m$^{-2}$ d$^{-1}$)</th>
<th>Ratio of intBNP : intNPP</th>
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<td>450</td>
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<tr>
<td>22 July 1998</td>
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</tr>
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<td>480</td>
<td>1.6</td>
</tr>
<tr>
<td>9 November 1998</td>
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<td>610</td>
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Fig. 8. Relationship between chlorophyll a (log Chl a, µg l⁻¹) and bacteria (log cells ml⁻¹) (A), and bacteria (log cells ml⁻¹) and heterotrophic nanoflagellates (HNF, log cells ml⁻¹) (B) at Stn 30 from 22 June until 9 November 1998.

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