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# Growth rates and production of heterotrophic bacteria and phytoplankton in the North Pacific subtropical gyre

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**Abstract**—In field work conducted at 26°N, 155°W, in the North Pacific subtropical gyre, phytoplankton growth rates $\mu_p$ estimated from $^{14}C$ labeling of chlorophyll *a* (chl *a*) averaged approximately one doubling per day in the euphotic zone (0–150 m). Microbial (microalgal plus heterotrophic bacterial) growth rates $\mu_m$ calculated from the incorporation of $^{3}H$-adenine into DNA were comparable to or exceeded phytoplankton growth rates at most depths in the euphotic zone. Photosynthetic rates averaged 727 mg C $m^{-2}$ $day^{-1}$. Phytoplankton carbon biomass, calculated from $^{14}C$ labeling of chl *a*, averaged 7.2 mg $m^{-3}$ in the euphotic zone. Vertical profiles of particulate DNA and ATP suggested that no more than 15% of particulate DNA was associated with actively growing cells. Heterotrophic bacterial carbon biomass was estimated from a two-year average at station ALOHA (22°45'N, 158°W) of flow cytometric counts of unpigmented, bacteria-size particles which bound DAPI on the assumption that 15% of the particles were actively growing cells and that heterotrophic bacterial cells contained 20 fg C $cell^{-1}$. The heterotrophic bacterial carbon so calculated averaged 1.1 mg $m^{-3}$ in the euphotic zone. Heterotrophic bacterial production was estimated to be 164 mg C $m^{-2}$ $day^{-1}$, or 23% of the calculated photosynthetic rate. Estimated heterotrophic bacterial growth rates averaged 0.97 $day^{-1}$ in the euphotic zone and reached 4.7 $day^{-1}$ at a depth of 20 m. Most heterotrophic bacterial production occurred in the upper 40 m of the euphotic zone, suggesting that direct excretion by phytoplankton, perhaps due to photorespiration or ultraviolet light effects, was a significant source of dissolved organic carbon for the bacteria. Copyright © 1996 Elsevier Science Ltd

## INTRODUCTION

The oligotrophic realm constitutes more than 75% of the surface waters of the world's oceans. The contribution of this vast area to global carbon fixation and new production (Dugdale and Goering, 1967) has been a matter of contention for more than 50 years (Riley, 1939; Rabinowitch, 1945; Steemann Nielsen, 1952; Peterson, 1980; Eppley, 1989). Although the use of clean sampling and incubation techniques (Fitzwater *et al.*, 1982; Williams and Robertson, 1989) has eliminated the causes of bias in some earlier production estimates, there remain unresolved issues concerned with the fate of photosynthetically fixed carbon and the role of heterotrophic bacteria and protozoans in the marine microbial loop (Pomeroy, 1974; Ducklow, 1983; Williams, 1984; Ducklow *et al.*, 1986; Cho and Azam, 1988; Fuhrman *et al.*, 1989). Ducklow (1983), for example, has estimated that heterotrophic

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bacterial production amounts to about 20% of primary production in the oceans' surface waters. Cho and Azam (1990), however, have estimated that in oligotrophic marine waters, heterotrophic bacterial biomass is 2–3 times greater than that of phytoplankton. These two estimates are incompatible unless the phytoplankton are growing roughly 10 times faster than the heterotrophic bacteria. Most estimates of heterotrophic bacterial growth rates in the open ocean, however, fall in the range 2–10 $day^{-1}$ (Ducklow, 1983), while phytoplankton in the same areas appear to be growing at rates of no more than 1–2 $day^{-1}$ (Laws *et al.*, 1984, 1987; Taguchi *et al.*, 1988). The resolution of this paradox has important implications for estimates of new production and the role of microorganisms in marine food webs (Williams, 1984; Peinert *et al.*, 1989). The field work reported here, carried out near the center of the North Pacific subtropical gyre, was intended in part to help clarify the relative importance of heterotrophic bacteria and autotrophic phytoplankton as producers of particulate organic carbon in the oligotrophic open ocean and to determine whether the growth rate of the phytoplankton community differs substantially from that of the heterotrophic bacteria.

## MATERIALS AND METHODS

The experiments reported here were carried out during two research cruises to 26°N, 155°W in the North Pacific subtropical gyre aboard the R.V. *Moana Wave* during March–April and September–October of 1986. The two cruises were designated ADIOS (Asian Dust Input to the Oceanic System) I and II, respectively (DiTullio and Laws, 1991; Young *et al.*, 1991).

The field samples were collected using 30 l Niskin Go-Flo bottles and trace-metal-free sampling techniques as recommended by Fitzwater *et al.* (1982). Kevlar line was used instead of standard hydro-wire and all metal components of the sampling string (blocks, sheaves, weights, messengers) were replaced with metal-free substitutes, either polyvinyl chloride, composite or teflon®-coated. Samples were collected from depths corresponding to irradiances of 100%, 35%, 18%, 3.5%, 1% and 0.1% of the irradiance at a depth of 0.5 m. The samples were incubated in on-deck incubators designed to reproduce the light intensity and spectral characteristics at each sampling depth. Details of the light measurements and incubation procedures are given by DiTullio and Laws (1991).

Water samples for use in nucleic acid synthesis rate experiments were collected before dawn to avoid light shock. Because of the labor-intensive nature of time-course experiments, no more than two time-course experiments were attempted simultaneously. Experiments involving only end-point measurements were performed six at a time. For time-course incubations, a sample of approximately 9 l taken from a given depth was dispensed into a 10 l polycarbonate carboy. $^{3}$H-adenine was added to radiochemical and molar concentrations of about $3.7 \times 10^{6}$ Bq $l^{-1}$ and 6.7 nM. The sample was then mixed, divided into two 4.5 l polycarbonate bottles and the two bottles transferred to the appropriate on-deck incubator. For end-point incubations, samples were dispensed directly into a single 4.5 l polycarbonate bottle, which was then spiked with $^{3}$H-adenine and incubated in the same manner as the time-course incubation bottles. Microbial growth rates were estimated from the incorporation of $^{3}$H-adenine into DNA using the basic techniques of Karl (1979) with modifications described by Karl (1981) and Karl *et al.* (1981a,b) and Karl and Winn (1984).

Photosynthetic rates were estimated from the incorporation of $^{14}$C-labeled inorganic

carbon into particulate carbon after an incubation of 24 h as described by DiTullio and Laws (1991). Phytoplankton growth rates and carbon biomass were estimated using the chlorophyll *a*-labeling technique of Redalje and Laws (1981). Samples were collected on 2.4 cm diameter Whatman GF/F glass-fiber filters. Both chlorophyll *a* concentrations and the specific activity of the chlorophyll *a* at the end of the 24 h incubation were determined by isolating the chlorophyll *a* using a high-pressure liquid chromatograph as described by DiTullio and Laws (1991). Particulate carbon (PC) concentrations were determined on samples collected on 2.4 cm GF/F filters using a Hewlett-Packard model 185B CHN analyzer.

Samples for particulate DNA and ATP analyses were drawn on deck into 4.5 l polycarbonate bottles, which were transferred inside the ship's laboratory. Subsamples for ATP were filtered through 2.4 cm GF/F filters, placed in boiling 60 mM $PO_4$ buffer for 5 min, and then frozen for subsequent measurement by the firefly bioluminescence assay (Holm-Hansen and Booth, 1966) using peak height analysis (Karl and Holm-Hansen, 1976). Subsamples for particulate DNA analysis were filtered through 2.5 cm Millipore HA filters and processed using the DABA technique (Kissane and Robins, 1958) modified for oceanographic applications by Holm-Hansen *et al.* (1968).

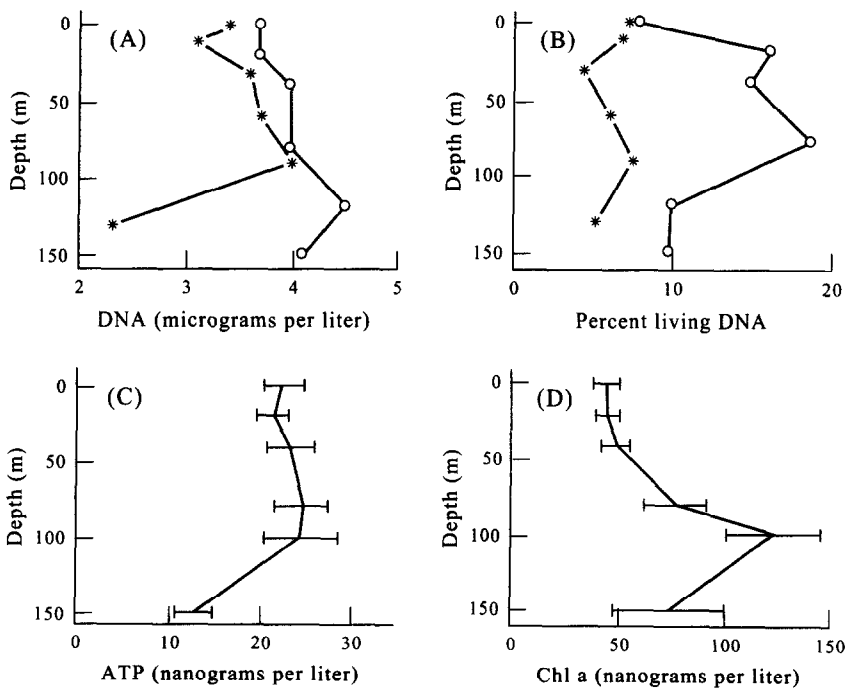



Fig. 1. (A) Particulate DNA concentrations measured on 25 March 1986 (*) and 29 March 1986 (○) at 26°N, 155°W during ADIOS I. (B) Percentage of living DNA estimated from profiles in (A) by multiplying ATP concentrations by 25 and dividing by the corresponding DNA concentration. (C) and (D) Average ATP and chlorophyll *a* concentrations measured during ADIOS I and II. Error bars are standard errors of the mean based on 10 (ATP) and 7 (chl *a*) depth profiles.

## RESULTS

Particulate DNA concentrations, which were measured only during ADIOS I, were, with one exception, in the range 3.0–4.5 $\mu g\, l^{-1}$ (Fig. 1A). Jones *et al.* (1995) found that the DNA/ATP ratio in log phase laboratory cultures of marine phytoplankton and heterotrophic bacteria averaged about 17 and 34, respectively, by weight, with almost all values lying between 10 and 40. We estimated the DNA of actively growing microorganisms in our field samples by multiplying particulate ATP concentrations by 25, the mean of the DNA/ATP ratios for phytoplankton and bacteria reported by Jones *et al.* (1995). The results, expressed as a percentage of particulate DNA, almost all fell within the range 5–15% (Fig. 1B). ATP concentrations (Fig. 1C) were rather uniform in the upper 100 m, where they averaged $23.1 \pm 1.4$ ng $l^{-1}$. Chlorophyll *a* concentrations (Fig. 1D) were uniform in the upper 40 m ($46.0 \pm 2.6$ ng $l^{-1}$) but increased to a maximum of 123 ng $l^{-1}$ at a depth of 100 m. There was a sharp decline in both ATP and chl *a* concentrations between 100 and 150 m.

Analysis of the time series describing the incorporation of $^3$H-adenine into DNA requires a theoretical framework for interpreting the results. A general equation describing the rate of change of activity in the DNA is

$$\frac{d(DNA^*(t))}{dt} = \mu DNA(t) SA(t) - g' DNA^*(t) \tag{1}$$

where DNA($t$) and DNA$^*$($t$) are the concentration (mol $l^{-1}$) and activity (Bq $l^{-1}$) of deoxyadenylic acid in living microbial (phytoplankton and bacterial) DNA at time $t$, $\mu$ is the growth rate ($t^{-1}$) of the microbial DNA, SA($t$) is the specific activity of microbial dATP (Bq mol$^{-1}$) at time $t$, and $g'$ is the rate of loss of label from particulate DNA due to grazing. Experience has shown that SA($t$) is, for all intents and purposes, identical to the specific activity of cellular ATP after incubations lasting more than 15% of an organism's doubling time (Winn and Karl, 1984). Our field data indicated that $^3$H was incorporated into particulate ATP at a rate corresponding to a turnover time of about 3 h for the adenine moiety (Fig. 2). Hence SA($t$) could be rather well approximated by an equation of the form

$$SA(t) = SA_a(1 - e^{-t/3}) \tag{2}$$

where $SA_a$ is the asymptotic value of SA($t$) as $t \to \infty$ and $t$ is the duration of the incubation in hours. If the bacterial and phytoplankton microbial community is grazed at a rate $g$, then

$$DNA(t) = DNA(0)e^{(\mu - g)t} \tag{3}$$

Substituting the right-hand sides of equations (2) and (3) for SA($t$) and DNA($t$), respectively, in equation (1) and solving the differential equation gives

$$DNA^*(t) = \frac{\mu KATP^*(t)}{1 - e^{-t/3}} \left( \frac{1 - e^{-(\mu + g' - g)t}}{\mu + g' - g} - \frac{e^{-t/3} - e^{-(\mu + g' - g)t}}{\mu + g' - g - 1/3} \right) \tag{4}$$

where ATP$^*$($t$) is the activity (Bq $l^{-1}$) of $^3$H in particulate ATP at time $t$ and $K$ is the molar ratio of D-adenylic acid in DNA to ATP in living cells. On the assumption that DNA is synthesized from equimolar amounts of D-adenylic, D-cytidylic, D-guanylic and thymidylic acid, the appropriate value of $K$ is $K'(507/1235)$, where $K'$ is the ratio by weight of DNA:ATP in the cells, 507 is the molecular weight of ATP and 1235 is the corresponding

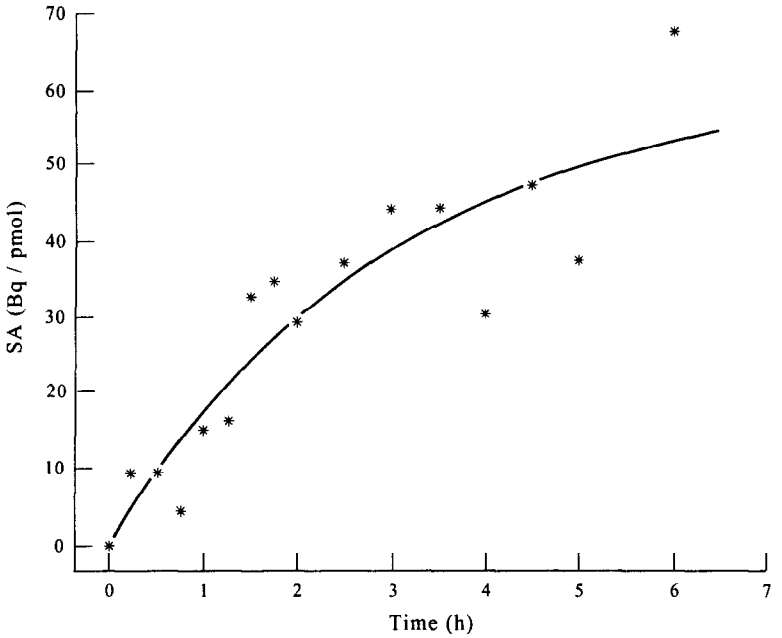



Fig. 2. Time course of ATP specific activity (SA) for an experiment during ADIOS I using water collected from a depth of 20 m. The incubation was begun at 10 p.m. Hawaii standard time on 15 April 1986. The smooth curve is a least-squares fit of equation (2) to the data.

weight of DNA synthesized from exactly 1 mol each of D-adenylic, D-cytidylic, D-guanylic and thymidylic acid. Given the assumptions of the model, it is reasonable to assume that $0 \leq g' \leq g$. It is therefore possible to set bounds on the possible values of $\mu$ consistent with a given $\mathrm{DNA}^*(t)$ and $\mathrm{ATP}^*(t)$ by considering the following two cases:

$$\mathrm{I}: \; g = g'$$

$$\mathrm{II}: \; g' = 0, g > 0.$$

In the first case, the calculated value of $\mu$ is independent of $g$, and $\mathrm{DNA}^*(t)$ is given by

$$\mathrm{DNA}^*(t) = \frac{\mathrm{KATP}^*(t)}{\mu - 1/3}\left(\mu - \frac{1 - \mathrm{e}^{-\mu t}}{3(1 - \mathrm{e}^{-t/3})}\right) \tag{5}$$

In the second case $\mathrm{DNA}^*(t)$ is given by the equation

$$\mathrm{DNA}^*(t) = \frac{\mu \mathrm{KATP}^*(t)}{1 - \mathrm{e}^{-t/3}}\left(\frac{1 - \mathrm{e}^{-(\mu - g)t}}{\mu - g} - \frac{\mathrm{e}^{-t/3} - \mathrm{e}^{-(\mu - g)t}}{\mu - g - 1/3}\right) \tag{6}$$

Equation (6) reduces to equation (5) in the case $g = 0$. If $g = \mu$, equation (6) becomes

$$\mathrm{DNA}^*(t) = \mu \mathrm{KATP}^*(t)\left(\frac{t}{1 - \mathrm{e}^{-t/3}} - 3\right) \tag{7}$$

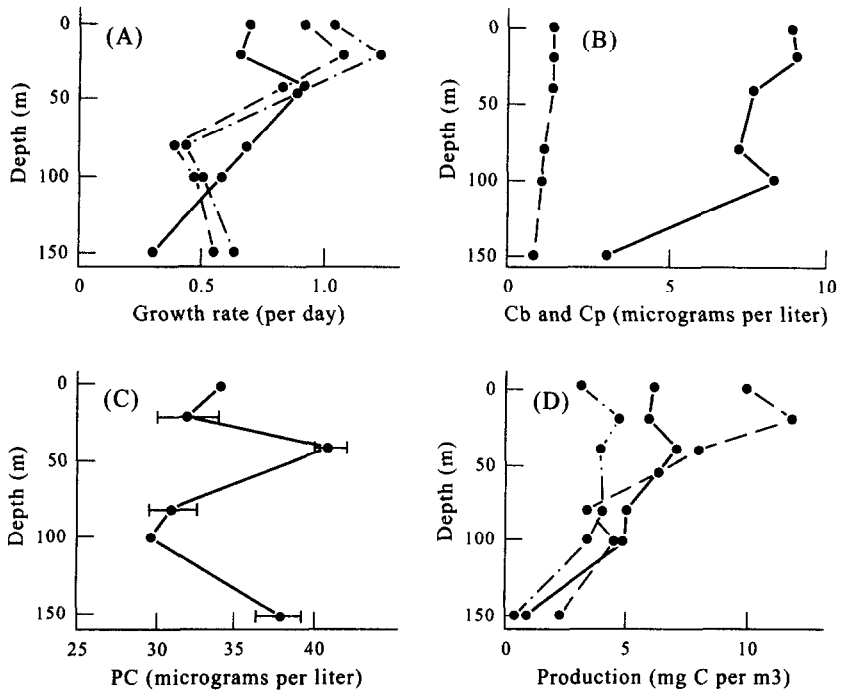



Fig. 3. (A) Phytoplankton growth rates $\mu_p$ (solid line) and microbial growth rates $\mu_m$ estimated using model I (dash–dot line) and model II (dashed line). (B) Concentration of phytoplankton carbon $C_p$ (solid line) and heterotrophic bacterial carbon $C_b$ (dashed line). The latter were estimated from data collected at station ALOHA. (C) Concentrations of particulate carbon PC measured on 29 March 1986 and 11 October 1986. Error bars indicate range of duplicate values. One value of 83 $\mu g\, l^{-1}$ at a depth of 100 m has been ignored. (D) Microbial production (dashed line) estimated from $\mu_m(C_b + C_p)$ and photosynthetic rates calculated from uptake of $^{14}C$ after 24 h (dash–dot line) and from $\mu_p C_p$ (solid line).

Equation (5) applies for any value of $g$ as long as $g = g'$. The assumption that $g = g'$ implies that the label in grazed microorganisms is removed from the particulate phase as a result of grazing, i.e. it is not retained in the body of the grazer. Equation (7) applies only in the case of balanced growth and assumes that grazing does not remove labeled particulate DNA, i.e. all labeled DNA is retained in the body of the grazer.

Figure 3A shows total microbial growth rates calculated by methods I and II. In the case of method II, we assumed that $\mu = g$. In other words, we solved either equation (5) (method I) or equation (7) (method II) for $\mu$. For comparison, we have included phytoplankton growth rates estimated by the chl *a* labeling method of Redalje and Laws (1981). An analysis of variance showed that there was no significant depth dependence of the total microbial growth rates in the upper 40 m of the water column or in the depth interval 80–150 m. However, the average rates in the depth intervals 0–40 m and 80–150 m were significantly different at $p < 0.03$. Average rates in the depth intervals 0–40 m and 80–150 m were $1.07 \pm 0.48\ day^{-1}$ ($n = 9$) and $0.52 \pm 0.49\ day^{-1}$ ($n = 9$) by method I and $0.93 \pm 0.40\ day^{-1}$ ($n = 9$) and $0.47 \pm 0.41\ day^{-1}$ ($n = 9$) by method II with $g = \mu$. A similar analysis showed no significant depth dependence of phytoplankton growth rates in the upper 100 m of the water column, the average rate being $0.71 \pm 0.24\ day^{-1}$ ($n = 9$). This average rate was significantly

different ($p = 0.05$) from the average phytoplankton growth rate at 150 m, $0.30 \pm 0.1$ day$^{-1}$ ($n = 2$). Microbial growth rates calculated by method I consistently exceeded the rates calculated by method II with $g = \mu$, but the difference was only 10–20%. With the exception of the results at 80 m, total microbial growth rates were comparable to or exceeded the phytoplankton growth rates.

Total microbial carbon production is the biological conversion of dissolved carbon into particulate organic carbon (POC) and consists of the sum of the transformation of inorganic carbon into POC (primary production) and heterotrophic bacterial conversion of dissolved organic carbon (DOC) into POC. Microbial carbon production rates were calculated from the product of microbial growth rate and the concentration of microbial carbon. Figure 3B shows estimates of phytoplankton carbon ($C_p$) during our sampling based on $^{14}C$ incorporation into chl *a* (Redalje and Laws, 1981; Laws, 1984). For comparison, Fig. 3C shows the particulate carbon (PC) profile. With the exception of one PC value of 83 $\mu$g l$^{-1}$ measured at 100 m, all the PC measurements fell in the range 30–42 $\mu$g l$^{-1}$. The $C_p$ averaged $8.2 \pm 0.75$ $\mu$g l$^{-1}$ in the upper 100 m and declined to 3.1 $\mu$g l$^{-1}$ at 150 m.

Heterotrophic bacterial biomass estimates were not made during the ADIOS cruises, but concentrations of unpigmented DNA-containing particles in the size range 0.3–1.0 $\mu$m have been routinely made since May 1992 by dual-laser flow cytometry (Monger and Landry, 1993) as a part of the Hawaiian Ocean Time Series (HOTS) work at station ALOHA (22°45'N, 158°W). The results of much previous work (Holm-Hansen *et al.*, 1968; Holm-Hansen, 1969; Sutcliffe *et al.*, 1970; Karl and Winn, 1984; Winn and Karl, 1986) as well as the data reported here (Fig. 1B) indicate that a substantial percentage of particulate DNA is often not associated with actively growing microorganisms in marine waters. Our results as well as those of Winn and Karl (1984) imply that the DNA in actively growing cells accounts for only 10–20% of particulate DNA in the North Pacific gyre. Taking 15% as an average and assuming that this percentage applies to cells in the heterotrophic bacterial size fraction, we multiplied the flow cytometric cell counts from the HOTS data by 0.15 to convert to actively growing heterotrophic bacterial cell counts. We converted the heterotrophic bacterial cell counts to bacterial carbon ($C_b$) assuming a heterotrophic bacterial carbon cell quota of 20 fg cell$^{-1}$ (Cho and Azam, 1990). The results (Fig. 3B) show that the calculated $C_b$s average 1.3 $\mu$g l$^{-1}$ in the upper 75 m of the water column and decline with increasing depth to 0.8 $\mu$g l$^{-1}$ at 125–150 m.

Figure 3D shows both autotrophic and total microbial production calculated from our data. The autotrophic production values were calculated in two ways: first using the standard equations from Strickland and Parsons (1972) and second from the product of $C_p$ and $\mu$. The latter method corrects for the loss of label due to the excretion and respiration of labeled organics by grazers (Laws, 1984). Integrated (0–150 m) autotrophic production calculated by these two methods amounted to 490 and 727 mg C m$^{-2}$ day$^{-1}$. These figures are very similar to the estimates of 484 and 692 mg C m$^{-2}$ day$^{-1}$ previously reported by Laws *et al.* (1989) from work conducted during ADIOS I, and the figure of 490 mg C m$^{-2}$ day$^{-1}$ is comparable to the five-year mean (1989–1993) of $463 \pm 156$ mg C m$^{-2}$ day$^{-1}$ calculated in a similar manner at station ALOHA (Karl *et al.*, 1996). Total microbial production, calculated by integrating the product of $C_p + C_b$ and the average microbial growth rates calculated by methods I and II, was 891 mg C m$^{-2}$ day$^{-1}$. Integrated heterotrophic bacterial production is therefore calculated to be $891 - 727 = 164$ mg C m$^{-2}$ day$^{-1}$, about 23% of the corresponding photosynthetic production. The ratio of integrated production to integrated biomass for the phytoplankton and

heterotrophic bacteria was 0.67 $day^{-1}$ and 0.97 $day^{-1}$, respectively. The highest calculated heterotrophic bacterial growth rate was 4.7 $day^{-1}$ at a depth of 20 m.

## DISCUSSION

Assuming that isotope discrimination effects are negligible, the rate of production of microbial DNA during a short time interval $\Delta t$ can be calculated from the equation

$$\frac{\Delta(\text{DNA})}{\Delta t} = \frac{\Delta(\text{DNA}^*)(\text{dATP})}{(\text{dATP}^*)(\Delta t)} = \frac{\Delta(\text{DNA}^*)(\text{ATP})}{(\text{ATP}^*)(\Delta t)} \tag{8}$$

where $\Delta$(DNA) and $\Delta(\text{DNA}^*)$ are the changes in the concentration and activity, respectively, of D-adenylic acid in DNA during the time interval $\Delta t$, (dATP) and $(\text{dATP}^*)$ are the concentration and activity, respectively, of dATP, and (ATP) and $(\text{ATP}^*)$ are the concentration and activity, respectively, of ATP. The last equality in equation (8) assumes that the specific activity of dATP and ATP are identical. The microbial growth rate $\mu_m$ is calculated from the expression

$$\mu_m = \frac{\Delta(\text{DNA})}{(\text{DNA})(\Delta t)} = \frac{\Delta(\text{DNA}^*)\text{ATP}}{(\text{ATP}^*)(\text{DNA})(\Delta t)} \tag{9}$$

If the ratio of D-adenylic acid in DNA to ATP in the microbial cells equals $K$, then equation (9) becomes

$$\mu_m = \frac{\Delta(\text{DNA})}{(\text{DNA})(\Delta t)} = \frac{\Delta(\text{DNA}^*)}{K(\text{ATP}^*)(\Delta t)} \tag{10}$$

Equation (10) is a simplified version of equations (5) and (7). In the latter two equations we have allowed for the fact that the turnover time of the adenine moiety in the ATP is about 3 h, and we have considered grazing effects. An important point about equations (5), (7) and (10) is that calculation of the growth rate does not require one to measure either the concentration or specific activity of ATP. Thus, one does not need to be concerned about whether the ATP pool contains significant contributions from organisms other than microalgae and heterotrophic bacteria and one does not need to be concerned about whether there are large differences in the ATP specific activities of different microorganisms. Neither the concentration nor specific activity of ATP enters into the calculation of microbial growth rates in equations (5) and (7). The calculated growth rates depend only on the ratio $(\text{DNA}^*)/(\text{ATP}^*)$.

One does, however, need to know the ratio of DNA to ATP in the microbial cells. Based on the work of Jones *et al.* (1995), the range of this ratio in actively growing cells is about 10–40 by weight. The variability of this ratio is the principal source of uncertainty in the growth rates calculated using equation (5) or equation (7).

An important point about our microbial growth rate estimates is that they represent the growth rates of cells that take up adenine. Cells that do not take up adenine do not enter into the calculations. Our microbial growth rates are therefore to be considered the growth rates of actively growing cells, i.e. cells which take up adenine and have a DNA/ATP ratio of approximately 25 by weight. Senescent cells and stationary phase cells would likely have a much higher DNA/ATP ratio (Jones *et al.*, 1995). We have assumed that such cells take up little or no adenine.

One criticism of the adenine technique is the evidence cited by Fuhrman *et al.* (1986) that eucaryotic microalgae in coastal waters do not take up adenine in appreciable amounts and that oceanic eucaryotes take it up but incorporate a much lower fraction into DNA than do bacteria. The fact that eucaryotic microalgae take up adenine has been well documented in the literature (Karl and Winn, 1984; Winn and Karl, 1984; Karl and Bossard, 1985) and the issues raised by Fuhrman *et al.* (1986) concerning coastal waters are not relevant to the open ocean studies reported here. With respect to the issue of uptake by open ocean species, it is noteworthy that most (78%) of the microalgal biomass in the upper 75 m of the water column at station ALOHA is accounted for by *Prochlorococcus* and *Synechococcus*. In other words, the phytoplankton biomass is dominated by procaryotes and not by eucaryotes.

The results presented here indicate that the growth rates of phytoplankton and heterotrophic bacteria in the upper 150 m of the water column near the center of the North Pacific subtropical gyre are comparable and equal to 1.0 and 1.5 doubling per day, respectively. The maximum calculated heterotrophic bacterial growth rate, 4.7 $day^{-1}$, was about five times the maximum estimated phytoplankton growth rate of 0.9 $day^{-1}$. These growth rates are generally consistent with previously reported values for marine phytoplankton and heterotrophic bacteria (Laws *et al.*, 1984, 1987; Taguchi *et al.*, 1988; Ducklow, 1983). If the biomass of actively growing heterotrophic bacteria were high compared to phytoplankton in oligotrophic regions of the ocean, as suggested in recent papers (Cho and Azam, 1988, 1990; Fuhrman *et al.*, 1989), the implication would be that heterotrophic bacterial carbon production greatly exceeds photosynthetic carbon fixation.

There are several causes of the discrepancies between the estimates of heterotrophic bacterial biomass and phytoplankton biomass reported by different authors. Certainly one problem has been the difficulty in distinguishing autotrophic and heterotrophic prokaryotes by microscopic methods. As noted by Campbell *et al.* (1994) (p. 954), "Assessments of plankton community structure in the oligotrophic oceans based solely on microscopy may overstate the importance of heterotrophic bacterial biomass." Using dual-laser flow cytometry, they concluded that autotrophic *Prochlorococcus* and *Synechococcus* spp. accounted for 45% of prokaryotic biomass in the euphotic zone at station ALOHA. Another source of error has been the factors used to convert chl *a* and heterotrophic bacterial cell counts into $C_p$ and $C_b$. Cho and Azam (1990) assumed a $C_p$:chl *a* ratio of 50. Our $C_p$:chl *a* ratios, calculated using the chlorophyll *a* labeling technique of Redalje and Laws (1981), averaged 156 g $g^{-1}$ in the upper 80 m of the water column and decreased to 42 g $g^{-1}$ at a depth of 150 m. Our estimates are similar to those of Campbell *et al.* (1994), who concluded that $C_p$:chl *a* ratios at station ALOHA were $128 \pm 10$ g $g^{-1}$ in the surface mixed layer (0–75 m) and $40 \pm 0.5$ g $g^{-1}$ at the deep chlorophyll maximum.

As noted by Fuhrman *et al.* (1989) (p. 211), "Choosing the best conversion factors is problematic, particularly for bacteria". This is especially true in field work, since there is no radioisotopic tracer technique comparable to the chl *a* labeling method of Redalje and Laws (1981) to permit a direct calculation of $C_b$. Both Fuhrman *et al.* (1989) and Cho and Azam (1990) assume a conversion factor of 20 fg $cell^{-1}$, although Fuhrman *et al.* (1989) consider an alternative conversion of 10 fg $cell^{-1}$. Christian and Karl (1994) concluded that the carbon cell quota for heterotrophic bacteria at station ALOHA was closer to 10 than 20 fg and that (p. 14, 275) "The extent of dominance of biomass in the oligotrophic ocean by heterotrophic bacteria has probably been overstated".

Perhaps the greatest source of error in heterotrophic bacterial carbon estimates has been the assumption that all particles containing DNA, as determined, for example, by staining

with DAPI, are living cells. The evidence to the contrary is based on microautoradiographic studies of uptake of $^3$H-labeled substrates (Tabor and Neihof, 1982a), electron transport activity (Tabor and Neihof, 1982b), simultaneous measurements of particulate DNA and ATP concentrations (Holm-Hansen *et al.*, 1968; Holm-Hansen, 1969; Sutcliffe *et al.*, 1970; Karl and Winn, 1984; Winn and Karl, 1986) and calculations based on the specific activities of DNA and ATP during uptake of $^3$H-adenine (Winn and Karl, 1986). As long ago as 1969 Holm-Hansen (1969) (p. 740) commented, "Because of the large amounts of DNA in the detrital fraction, it cannot be used as a biomass indicator". Holm-Hansen (1969) went on to say (p. 745), "The DNA reacting in our test therefore either is associated with detrital material, much of which is in the very small size range (less than 2 $\mu$m diameter), or is in the colloidal or soluble state and is being adsorbed onto the Millipore filter". Given these observations, made over 25 years ago, and comparable results found in several other studies since then, it seems remarkable that all bacterial-size particles which bind DAPI are sometimes assumed to be living cells. Most recently, Zweifel and Hagström (1995) have concluded that only a fraction of the particles that are enumerated as bacteria by DAPI staining are truly nucleoid-containing cells. They conclude that (p. 2185), "In formaldehyde-fixed samples, DAPI-binding most likely occurs on reactive bacterial surfaces that have been created by formaldehyde.... In practice, DAPI staining of bacterial nucleoids was impossible at salt concentrations above 12‰, which is well below the salt concentration in marine waters".

Whether cells that have no detectable electron transport system activity and fail to take up labeled organic substrates are literally dead or dormant, senescent, inactive, non-replicating or viable but non-culturable is unclear. There does, however, seem to be a general consensus that in natural waters only a fraction of the particulate DNA and only a fraction of the bacteria identified by vital stains and microscopic techniques are actively growing. It also seems apparent that the percentage of such actively growing bacteria varies both temporally and spatially. Zweifel and Hagström (1995), for example, found that only 4–6% of the bacteria in the Baltic Sea were nucleoid-containing cells (NUCC) during October, but 12–27% were NUCC in May. Likewise, based on simultaneous measurements of particulate DNA and ATP, Bailiff and Karl (1991) estimated that $87 \pm 21\%$ of particulate DNA was associated with living organisms in the upper 100 m of the water column during a phytoplankton bloom in Gerlache Strait, Antarctica. During the post-bloom period, however, the percentage of living DNA dropped to $9 \pm 2\%$. The subtropical gyres are associated with a much more constant environment than the Baltic Sea or Antarctica and it seems reasonable to assume that the percentage of actively growing heterotrophic bacteria is correspondingly less variable. Both our results and those of Winn and Karl (1984) suggest that 10–20% of particulate DNA is associated with actively growing organisms in the subtropical gyres.

It is perhaps worth pointing out that our calculated total microbial production rates are very insensitive to the percentage of DNA presumed to be associated with actively growing microorganisms. For example, had we assumed that all the DNA and ATP that we measured was associated with actively growing microalgae and heterotrophic bacteria, then it would have been logical to equate $K'$ with the ratio of our measured DNA and ATP concentrations, which was about 300 by weight. This figure is 12 times our assumed ratio of 25 for actively growing microorganisms and the growth rates calculated by equations (5) or (7) would have been correspondingly smaller. Calculated total microbial production, however, would have been virtually unchanged, since our estimate of actively growing

microbial carbon would have increased by a similar amount. The issue then is not so much the magnitude of the microbial production but whether the heterotrophic bacterial production is associated with a small biomass of heterotrophic bacteria growing rapidly or a large biomass of heterotrophic bacteria growing slowly. On this point we are inclined to concur with Zweifel and Hagström (1995) (p. 2180) that, "A much lower number of bacteria that grow at rates higher than those previously estimated must be responsible for the measured bacterial production".

One constraint on calculations based on heterotrophic bacterial biomass and growth rate is the source of the DOC required to support the implied heterotrophic bacterial production. Williams (1984), for example, has estimated that heterotrophic bacterial production may be as much as 40% of net photosynthetic carbon fixation in marine surface waters. His calculation assumes that phytoplankton excrete 30% of the carbon they fix. According to Williams' model, heterotrophic bacterial production would therefore be 40%/0.7 = 57% of the inorganic carbon incorporated by phytoplankton into particulate organic carbon. This is much higher than the figure of 23% derived from our estimates. Williams' (1984) calculations, however, assume a heterotrophic bacterial growth efficiency of 70% based on studies in which DOC was provided to heterotrophic bacteria in the form of glucose or amino acids. Recent work in which heterotrophic bacteria have been grown on natural marine substrates suggests that in nature heterotrophic bacterial growth, efficiencies are much lower than 70%. The results of Bjornsen (1986), Tranvik (1988), Middleboe and Søndergaard (1993), and Carlson and Ducklow (1996) indicate that marine heterotrophic bacterial growth efficiencies fall in the range 20–35%. Linley and Newell (1984) and Kirchman *et al.* (1991) have reported even lower growth efficiencies, roughly 5–10%. Our results are consistent with Williams' (1984) model if we assume a heterotrophic bacterial growth efficiency of (23/57)(70%) = 28%.

Release of DOC by phytoplankton and excretion by protozoans and zooplankton are generally considered to be the most important sources of the organic matter needed for heterotrophic bacterial growth in the ocean. Williams (1984) postulates that somewhat more than half the DOC comes directly from phytoplankton excretion. The pattern of growth rates in Fig. 3A bear on this point. Heterotrophic bacterial growth rates exceeded phytoplankton growth rates in near-surface waters and near the base of the euphotic zone. The fact that $\mu_m > \mu_p$ near the base of the euphotic zone, can easily be rationalized by light limitation of photosynthesis. The depression of photosynthetic rates and $\mu_p$ near the surface is a commonly observed phenomenon and may result from photorespiration or stress caused by ultraviolet light (Jokiel and York, 1984). In any case, it seems plausible that phytoplankton near the surface may be excreting a substantial percentage of the carbon they fix and this supply of DOC may be the explanation for the high heterotrophic bacterial growth rates in this region of the water column. Between 40 and 100 m there was virtually no heterotrophic bacterial production (Fig. 3D). This is a region of the water column where light intensities are adequate to optimal and where phytoplankton excretion is probably minimal. The implication is that excretory products of phytoplankton are indeed a significant source of DOC for marine bacteria, perhaps even more so than envisioned by Williams (1984).

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## REFERENCES

Bailiff M. D. and D. M. Karl (1991) Dissolved and particulate DNA dynamics during a spring bloom in the Antarctic Peninsula region, 1986-1987. *Deep-Sea Research*, **38**, 1077–1095.

Bjornsen P. K. (1986) Bacterioplankton growth yield in continuous seawater cultures. *Marine Ecology Progress Series*, **30**, 191–196.

Campbell L., H. A. Nolla and D. Vaulot (1994) The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. *Limnology and Oceanography*, **39**, 954–961.

Carlson C. A. and H. W. Ducklow (1996) Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. *Aqualic Microbial Ecology*, **10**, 69–85.

Cho B. C. and F. Azam (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature*, **332**, 441–443.

Cho B. C. and F. Azam (1990) Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. *Marine Ecology Progress Series*, **63**, 253–259.

Christian J. R. and D. M. Karl (1994) Microbial community structure at the US Joint Global Ocean Flux Study Station ALOHA: inverse methods for estimating biochemical indicator ratios. *Journal of Geophysical Research*, **99**, 269–276.

DiTullio G. R. and E. A. Laws (1991) Impact of an atmospheric–oceanic disturbance on phytoplankton community dynamics in the North Pacific central gyre. *Deep-Sea Research*, **38**, 1305–1329.

Ducklow H. W. (1983) Production and fate of bacteria in the oceans. *BioScience*, **33**, 494–501.

Ducklow H. W., D. N. Purdie, P. J. Leb. Williams and J. M. Davies (1986) Bacterioplankton: a sink for carbon in a coastal marine plankton community. *Science*, **232**, 865–867.

Dugdale R. C. and J. J. Goering (1967) Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnology and Oceanography*, **12**, 196–206.

Eppley R. W. (1989) New production: history, methods, problems. In: *Productivity of the ocean: past and present*, W. H. Berger, V. S. Smetacek and G. Wefer, editors, John Wiley, NY, pp. 85–97.

Fitzwater S. E., G. A. Knauer and J. H. Martin (1982) Metal contamination and its effect on primary production measurements. *Limnology and Oceanography*, **27**, 544–551.

Fuhrman J. A., H. W. Ducklow, D. L. Kirchman, J. Hudak, G. B. Mcmanus and J. Kramer (1986) Does adenine incorporation into nucleic acids measure total microbial production? *Limnology and Oceanography*, **31**, 627–636.

Fuhrman J. A., T. D. Sleeter, C. A. Carlson and L. M. Proctor (1989) Dominance of bacterial biomass in the Sargasso Sea and its ecological implications. *Marine Ecology Progress Series*, **57**, 207–217.

Holm-Hansen O. (1969) Determination of microbial biomass in ocean profiles. *Limnology and Oceanography*, **14**, 740–747.

Holm-Hansen O. and C. R. Booth (1966) The measurement of adenosine triphosphate in the ocean and its ecological significance. *Limnology and Oceanography*, **13**, 507–514.

Holm-Hansen O., W. H. Sutcliffe and J. Sharp (1968) Measurement of deoxyribonucleic acid in the ocean and its ecological significance. *Limnology and Oceanography*, **13**, 507–514.

Jokiel P. L. and R. H. York (1984) Importance of ultraviolet radiation in photoinhibition of microalgal growth. *Limnology and Oceanography*, **29**, 192–199.

Jones D. R., D. M. Karl and E. A. Laws (1995) DNA:Atp ratios in marine microalgae and bacteria: Implications for growth rate estimates based on rates of DNA synthesis. *Journal of Phycology*, **31**, 215–223.

Karl D. M. (1979) Measurement of microbial activity and growth in the ocean by rates of stable ribonucleic acid synthesis. *Applied and Environmental Microbiology*, **38**, 850–860.

Karl D. M. (1981) Simultaneous rates of ribonucleic acid and deoxyribonucleic acid synthesis for estimating growth and cell division of aquatic microbial communities. *Applied and Environmental Microbiology*, **42**, 802–810.

Karl D. M. and P. Bossard (1985) Measurement of microbial nucleic acid synthesis and specific growth rate by $^{32}PO_4$ and [$^3$H] adenine: field comparison. *Applied and Environmental Microbiology*, **50**, 706–709.

Karl D. M. and O. Holm-Hansen (1976) Effects of luciferin concentration on the quantitative assay of ATP using crude luciferase preparations. *Analytical Biochemistry*, **75**, 100–112.

Karl D. M. and C. D. Winn (1984) Adenine metabolism and nucleic acid synthesis: applications to microbiological oceanography. In: *Heterotrophic activity in the sea*, H. E. Hobbie and P. J. LeB. Williams, editors, Plenum Publishing Co., New York, pp. 197–213.

Karl D. M., C. D. Winn and D. C. L. Wong (1981) RNA synthesis as a measure of microbial growth in aquatic environments. I. Evaluation, verification and optimization of methods. *Marine Biology*, **64**, 1–12.

Karl D. M., C. D. Winn and D. C. L. Wong (1981) RNA synthesis as a measure of microbial growth in aquatic environments. II. Field applications. *Marine Biology*, **64**, 13–21.

Karl D. M., J. R. Christian, J. E. Dore, D. V. Hebel, R. M. Letelier, L. M. Tupas and C. D. Winn (1996) Seasonal and interannual variability in primary production and particle flux at station Aloha. *Deep-Sea Research II*, **43**, 539–568.

Kirchman D. L., Y. Suzuki, C. Garside and H. W. Ducklow (1991) High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. *Nature*, **352**, 612–614.

Kissane J. M. and E. Robins (1958) The fluorometric measurement of deoxyribonucleic acid in animal tissues with special reference to the central nervous system. *Journal of Biological Chemistry*, **233**, 184–188.

Laws E. A. (1984) Improved estimates of phytoplankton carbon based on $^{14}C$ incorporation into chlorophyll *a*. *Journal of Theoretical Biology*, **110**, 425–434.

Laws E. A., D. G. Redalje, L. W. Haas, P. K. Bienfang, R. W. Eppley, W. G. Harrison, D. M. Karl and J. Marra (1984) High phytoplankton growth and production rates in oligotrophic Hawaiian coastal waters. *Limnology and Oceanography*, **29**, 1161–1169.

Laws E. A., G. R. Ditullio and D. G. Redalje (1987) High phytoplankton growth and production rates in the North Pacific subtropical gyre. *Limnology and Oceanography*, **32**, 905–918.

Laws E. A., G. R. Ditullio, P. R. Betzer, D. M. Karl and K. L. Carder (1989) Autotrophic production and elemental fluxes at 26°N, 155°W in the North Pacific subtropical gyre. *Deep-Sea Research*, **36**, 103–120.

Linley E. A. S. and R. C. Newell (1984) Estimates of bacterial growth yields based on plant detritus. *Bulletin of Marine Science*, **35**, 409–425.

Middleboe M. and M. Søndergaard (1993) Bacterioplankton growth yield: seasonal variations and coupling to substrate lability and β-glucosidase activity. *Applied and Environmental Microbiology*, **59**, 3916–3921.

Monger B. C. and M. R. Landry (1993) Flow cytometric analysis of marine bacteria with Hoechst 33342. *Applied and Environmental Microbiology*, **59**, 905–911.

Peinert R., B. Von Bodungen and V. S. Smetacek (1989) Food web structure and loss rate. In: *Productivity of the ocean: past and present*, W. H. Berger, V. S. Smetacek and G. Wefer, editors, John Wiley, NY, pp. 35–48.

Peterson B. J. (1980) Aquatic primary productivity and the $^{14}C$-$CO_2$ method: A history of the productivity problem. *Annual Review of Ecological Systems*, **11**, 359–385.

Pomeroy L. R. (1974) The ocean's food web, a changing paradigm. *Bioscience*, **24**, 499–504.

Rabinowitch E. I. (1945) *Photosynthesis and related processes*, Vol. I. Wiley Interscience, NY.

Redalje D. G. and E. A. Laws (1981) A new method for estimating phytoplankton growth rates and carbon biomass. *Marine Biology*, **62**, 73–79.

Riley G. A. (1939) Plankton studies II. The western North Atlantic, May–June, 1939. *Journal of Marine Research*, **2**, 145–162.

Steemann Nielsen E. (1952) The use of radioactive carbon ($^{14}C$) for measuring organic production in the sea. *Journal du Conseil International pour l'Exploration de la Mer*, **18**, 117–140.

Strickland J. D. H. and T. R. Parsons (1972) A practical handbook of seawater analysis, 2nd edn, *Bulletin of the Fisheries Research Board of Canada*, **167**.

Sutcliffe W. H., R. W. Sheldon and A. Prakash (1970) Certain aspects of production and standing stock of particulate matter in the surface waters of the northwest Atlantic Ocean. *Journal of the Fisheries Research Board of Canada*, **27**, 1917–1926.

Tabor P. S. and R. A. Neihof (1982) Improved method for determination of respiring individual microorganisms in natural waters. *Applied and Environmental Microbiology*, **43**, 1249–1255.

Tabor P. S. and R. A. Neihof (1982) Improved microautoradiographic method to determine individual microorganisms active in substrate uptake in natural waters. *Applied and Environmental Microbiology*, **44**, 945–953.

Taguchi S., G. R. Ditullio and E. A. Laws (1988) Physiological characteristics and production of mixed layer and chlorophyll maximum phytoplankton populations in the Caribbean Sea and western Atlantic Ocean. *Deep-Sea Research*, **35**, 1363–1377.

Tranvik L. (1988) Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of different humic content. *Microbial Ecology*, **16**, 311–332.

Williams P. J. LeB. (1984) Bacterial production in the marine food chain: the emperor's new suit of clothes? In: *Flows of energy and materials in marine ecosystems, theory and practice*, M. J. R. Fasham, editor, Plenum Press, New York, pp. 271–299.

Williams P.J. LeB. and J. I. Robertson (1989) A serious inhibition problem from a Niskin sampler during plankton productivity studies. *Limnology and Oceanography*, **34**, 1300–1305.

Winn C. D. and D. M. Karl (1984) Laboratory calibration of the [$^3$H] adenine technique for measuring rates of RNA and DNA synthesis in marine microorganisms. *Applied and Environmental Microbiology*, **47**, 835–842.

Winn C. D. and D. M. Karl (1986) Diel nucleic acid synthesis and particulate DNA concentrations: conflicts with division rate estimates by DNA accumulation. *Limnology and Oceanography*, **31**, 637–645.

Young R. W., K. L. Carder, P. R. Betzer, D. K. Costello, R. A. Duce, G. R. Ditullio, N. W. Tindale, E. A. Laws, M. Uematsu, J. T. Merrill and R. A. Feely (1991) Atmospheric iron inputs and primary productivity: phytoplankton responses in the North Pacific. *Global Biochemical Cycles*, **5**, 119–134.

Zweifel U. and A. Hagström (1995) Total counts of marine bacteria include a large fraction of non-nucleoid-containing bacteria (ghosts). *Applied and Environmental Microbiology*, **61**, 2180–2185.