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Regulation of Bacterial Growth Rates by Dissolved Organic Carbon and Temperature in the Equatorial Pacific Ocean

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Abstract

The effect of dissolved organic matter (DOM) and temperature on bacterial production was examined in the equatorial Pacific Ocean. Addition of glucose, glucose plus ammonium, or free amino acids stimulated bacterial production ([^{3}H]thymidine incorporation), whereas changes in bacterial abundance were either negligible or much less than changes in bacterial production. The average bacterial growth rate also greatly increased following DOM additions, whereas in contrast, addition of ammonium alone never affected production, bacterial abundance, or growth rates. Since the large glucose effect was not observed in previous studies of cold oceanic waters, several experiments were conducted to examine DOM-temperature interactions. These experiments suggest that bacteria respond more quickly and to a greater extent to DOM additions at higher temperatures, which may explain apparently conflicting results from previous studies. We also examined how temperate affects the kinetic parameters of sugar uptake. Maximum uptake rates (V_{max}) of glucose and mannose increased with temperature (Q_{10} = 2.4), although the half-saturation constant (K_m) was unaffected; K_m + S was roughly equal to glucose concentrations (S) measured by a high pressure liquid chromographic technique. Bacterial production and growth rates appear to be limited by DOM in the equatorial Pacific, and thus bacterial production follows primary production over large spatial and temporal scales in this oceanic regime, as has been observed in other aquatic systems. Although temperature may not limit bacterial growth rates in the equatorial Pacific and similar warm waters, it could still affect how bacteria respond to changes in DOM supply and help set steady-state DOM concentrations.

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Introduction

Bacterial production is often said to be regulated at least in part by dissolved organic matter (DOM), a hypothesis supported by two types of evidence. First, bacterial production often correlates with primary production, especially when large spatial and temporal scales are examined [4, 13]. Usually, this correlation has been interpreted as indicating that primary production, directly or indirectly, governs the supply of DOM, which in turn determines bacterial production. The second type of evidence is that additions of DOM frequently stimulate bacterial production, although rarely is bacterial abundance enhanced [16, 17, 19, 22, 28, 29]. The stimulation of production without an increase in biomass implies that the average growth rate of the entire bacterial assemblage increases following DOM additions, suggesting that DOM limits or at least partially determines growth rates of some components of the bacterial assemblage. These addition experiments also suggest that organic C rather than inorganic N limits bacterial production and growth rates, since glucose alone frequently stimulates bacterial growth rates while addition of ammonium usually has no effect. Furthermore, the lack of large changes in bacterial biomass, even when bacterial production increases greatly after a DOM addition, points to the importance of grazing and viral attack in controlling bacterial abundance.

The generality of DOM controlling bacterial growth has been challenged by two sets of studies. First, there is evidence that phosphate can limit bacterial growth in lakes [20, 22, 32], the Gulf of Mexico [25], and the Mediterranean Sea [37]. The evidence for P limitation is based mainly on enrichment experiments. If phosphate is hypothesized to limit bacterial growth, then the correlation between bacterial production and primary production would have to be explained by covariance with phosphate. This covariance has been shown for bacterial abundance [6, 20], but similar evidence for bacterial production is not available. The second challenge to the hypothesis that DOM limitation is most important comes from those studies examining temperature effects. Bacterial production and average growth rates frequently correlate with temperature [15, 29, 30, 33], and raising the water temperature experimentally usually stimulates bacterial growth [10, 18, 29].

Of course it is possible that different factors limit bacterial growth in different aquatic systems or at different times in the same system [22, 28]. However, a more complete model for what limits bacterial growth seems necessary because the effects of DOM supply, inorganic nutrients, temperature, and bacterial mortality, on bacterial biomass and biomass production are dependent on each other. Egli [9] has examined colimitation by carbon and nitrogen, and Wiebe et al. [34] discussed how temperature may affect control by DOM of bacterial growth rates. In addition to these pure culture studies, field studies with natural assemblages have addressed questions of colimitation [10, 19, 22], but we need more studies.

The purpose of this study was to examine what controls bacterial production in the equatorial Pacific Ocean. This environment is important because of its high new production, and it is interesting because of its low phytoplankton biomass but high nutrient concentrations [23]. Kirchman et al. [18] suggested that bacterial growth rates in this oceanic region were limited by the DOM supply even though temperature-shift experiments indicated that significant changes in bacterial production should have been measurable when water temperatures changed due to the 1992 El Niño. They concluded that the DOM supply was more important than temperature because bacterial production increased with primary production even though water column temperatures decreased as El Niño waned during 1992. Here we show with addition experiments that DOM does limit bacterial growth, but temperature also has a role in controlling bacterial growth by affecting the response time of bacteria to changes in DOM concentrations.

Material and Methods

The experiments reported on here were from two cruises on transects from 12°N to 12°S along 140°W. This work was part of the JGOFS Equatorial Pacific Program. A detailed description of the sampling procedures is given by Kirchman et al. [18]. That study and another [8] describe bacterial biomass and production in the equatorial Pacific.

The basic experimental design was to place subsurface (ca. 20 m) seawater into 1-liter polycarbonate bottles with and without various additions, such as amino acids, ammonium, and glucose. The final concentration of these additions was 1 μM for all compounds; total concentration of the amino acids, which is the Pierce standard mixture containing all protein amino acids, was also 1 μM. The bottles were incubated in the dark at the in situ temperature. Over time, subsamples were removed to measure bacterial abundance (acridine orange direct counts; [14]) and [^{3}H]thymidine incorporation (duplicate subsamples) [11], which is a measure of bacterial production. We measured [^{3}H]thymidine (TdR) incorporation into the cold trichloroacetic acid (TCA) insoluble fraction as described in more detail elsewhere [18]. We used thymidine incorporation rates per cell as an index of bacterial growth rates. This index is not perfect because it includes nongrowing cells which may be numerous (e.g., ref [36]).

To compare changes in TdR incorporation and cell abundance, we estimated gross and net cell production during the enrichment experiments. Incorporation rates of TdR (pmol l^{-1} h^{-1}) were converted to cell production (cells l^{-1} h^{-1}) using the factor 1.74×10^{18} cells mol^{-1} [18] and then integrated over time up to the period of maximum cell abundance in the enriched samples. Net cell production is simply the difference in abundance at time zero and the time of maximum abundance in the enriched samples.

Uptake kinetics for glucose and mannose were measured following the approach of Wright and Hobbie [35]. Briefly, uptake of either D-[6-^{3}H]glucose or D-[2-^{3}H(N)]mannose (DuPont NEN, Wilmington, DE) was measured in duplicate for each sample. The added, final concentration of the radioactive sugars was 0.5 nM. After 0.5- to 1-h incubations, uptake was stopped by filtration through 0.45-μm Millipore or 0.22-μm Sartorius filters (rates determined with these two filter types did not differ), which were then rinsed twice (3 ml) with cold filtered seawater. The filters were dissolved in ethyl acetate and radioassayed. The scintillation cocktail was Ultima-Gold (Packard Instruments Meriden, CT). Controls were run at the same time in which samples were killed with TCA prior to incubation. Radioactivity from these killed samples was subtracted from the other samples.

Uptake rates at different unlabeled concentrations of either glucose or mannose were measured by first adding the unlabeled sugars, and then the procedure just described was followed. There was no preincubation period. Uptake kinetics (V_{max} and K_{max} + S) were estimated with data from the lowest added concentrations following a linear relationship, as derived by Wright and Hobbie [35]. Data on glucose and mannose concentrations were taken from Rich et al. [26]. Concentrations were measured by a high pressure liquid chromatograph (HPLC) method with pulse amperometric detection [21].

Results

Various compounds were added to otherwise unaltered samples of surface waters in order to examine the controls of heterotrophic bacterial growth rates in the equatorial Pacific. Addition of dissolved free amino acids (DFAA) (1 μM total) or a mixture of glucose and ammonium (both 1 μM) stimulated bacterial production (TdR incorporation) substantially (>tenfold) by 24 h; addition of glucose and ammonium (glucose + NH_4^+) resulted in the earliest, measurable stimulation (Fig. 1A). Bacterial abundance increased by much smaller amounts, in most cases not much more than the measurement errors (Fig. 1B). Addition of glucose + NH_4^+ caused the largest change (0.6×10^9 cells l^{-1} at 22 h), although this large increase is suspicious as it is greater than can be accounted for by the measured bacterial production (see below). In any case, TdR incorporation per cell, which is a measure of the average bacterial growth rate, increased greatly with addition of DFAA or glucose + NH_4^+ (Fig. 1C).

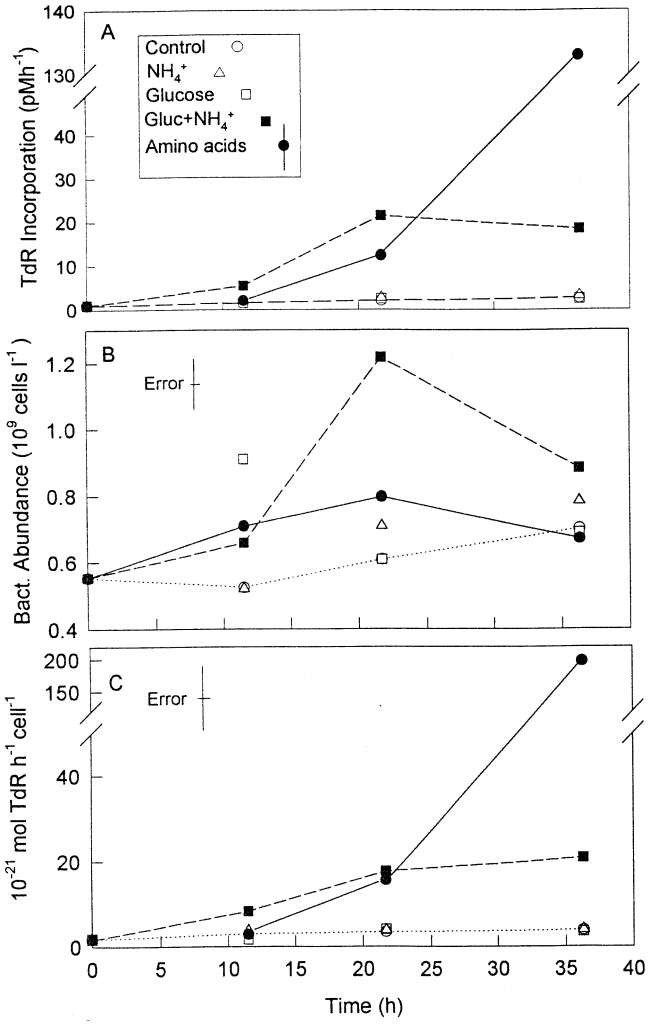

Fig. 1. Effect of various additions on **A.** bacterial production (TdR incorporation); **B.** bacterial abundance; and **C.** average bacterial growth rate (TdR incorporation per cell). The experiment was conducted on 17 August 1992 at 9°N 140°W. The *vertical bar* is the standard deviation for the largest value of each measurement. The coefficients of variation (CV) were 10%, 15%, and 25% for bacterial production, bacterial abundance, and the average bacterial growth rate, respectively.

Noteworthy is the lack of much of an effect on bacterial production or growth rates by additions of glucose or NH_4^+ (Fig. 1).

In two other enrichment experiments, addition of glucose + NH_4^+ greatly stimulated bacterial production (TdR incorporation) (Figs. 2A and 3A). The effect of adding amino acids was not examined. Unlike the first experiment, addition of glucose alone also stimulated bacterial production (Figs. 2A and 3A). During the 1°S experiment, abundance increased by 0.3 to 0.4 $\times 10^9$ cells l^{-1} (compared to measurement errors of 0.2 $\times 10^9$ cells l^{-1}) with the addition of

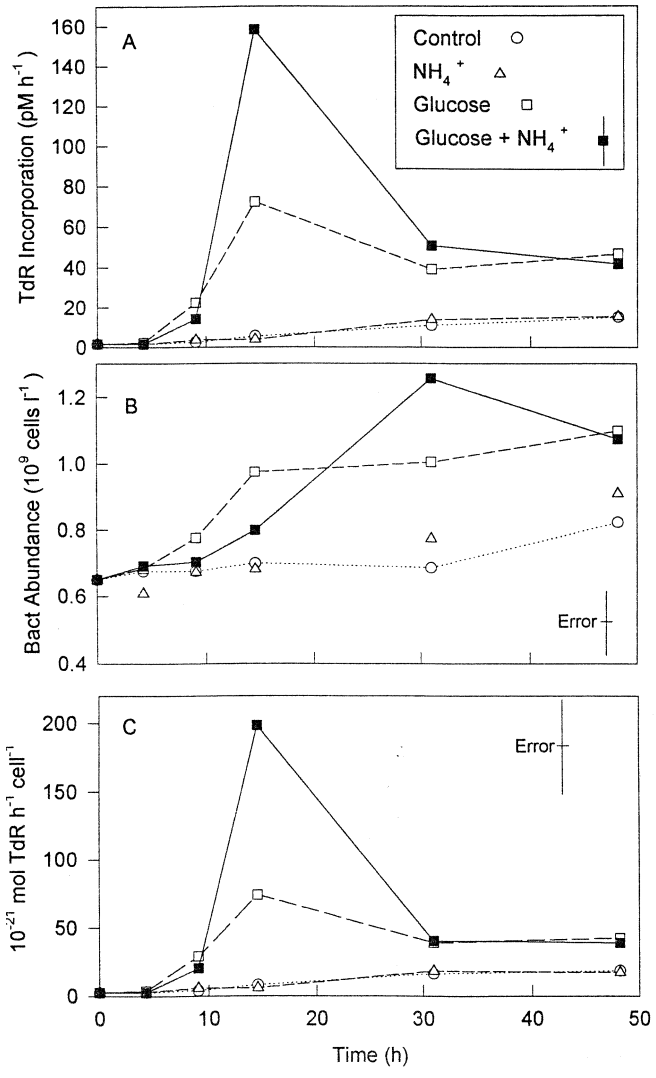

Fig. 2. Effect of various additions on **A.** bacterial production (TdR incorporation); **B.** bacterial abundance; and **C.** average bacterial growth rate (TdR incorporation per cell). The experiment was conducted on 27 February 1992 at 1°S 140°W. See Fig. 1 for explanation of error and *error bar.*

glucose + NH_4^+ or glucose alone (Fig. 2B). During the 5°S experiment, addition of glucose + NH_4^+ or glucose alone again stimulated bacterial abundance, although the increase was only about 0.2 to 0.3 × 10^9 cells l^{-1} over the initial level of 0.75 × 10^9 cells l^{-1} (Fig. 3B). Bacterial abundance increased, but because production increased much more so, the index of bacterial growth rates increased substantially with the addition of glucose + NH_4^+ or glucose alone during experiments at both 1°S and 5°S (Figs. 2C and 3C). As in the first experiment, addition of NH_4^+ alone had no effect on bacterial production, abundance, or growth rates (Figs. 2 and 3). In short, DOM additions (glucose or amino acids) stimulated bacterial production and growth rates in three experiments whereas NH_4^+ additions alone had no effect.

Another approach for examining the effect of DOM additions is to compare gross cell production, which is the TdR-based production integrated over time, with net cell production, which is the observed change in bacterial abundance over time. Table 1 gives gross and net production up to the time of maximum bacterial abundance in the enriched samples; the entire time course of all experiments was analyzed by this approach, but the single time point in Table 1 illustrates adequately the important conclusions. Since net cell production is greatest when bacterial abundance is highest, this choice of time point is best for examining alternative

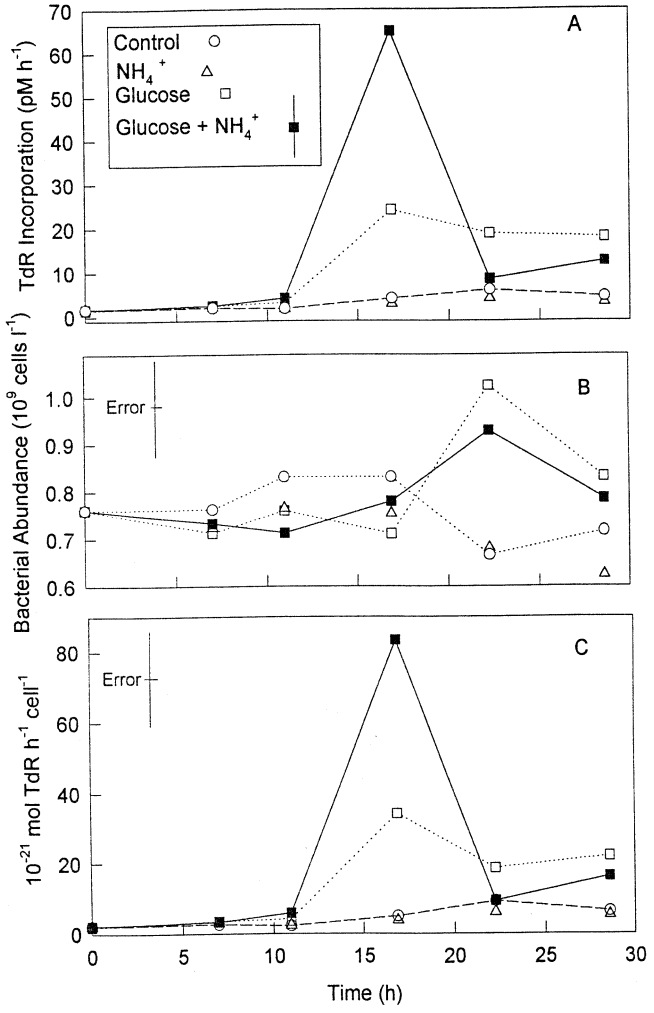

Fig. 3. Effect of various additions on **A.** bacterial production (TdR incorporation); **B.** bacterial abundance; and **C.** average bacterial growth rate (TdR incorporation per cell). The experiment was conducted on 4 March 1992 at 5°S 140°W. See Fig. 1 for explanation of error and *error bar.*

Table 1. Comparison of gross cell production (TdR-based cell production integrated over time) and net cell production (difference in cell abundance over time) during the enrichment experiments. The errors associated with gross and net production are about 0.001 and 0.2 × 10^9 cells l^{-1}, respectively. This corresponds to 20% errors in the original measurements

Figure[a]	Time (h)[b]	Treatment[c]	Cell production (10^9 cells l^{-1}) Gross	Net
1	22	Control	0.072	0.1
1	22	NH_4^+	0.089	0.2
1	22	Glucose	0.078	0.1
1	22	Glucose + NH_4^+	0.377	0.7
1	22	Amino acids	0.198	0.2
2	31	Control	0.383	0.0
2	31	NH_4^+	0.409	0.1
2	31	Glucose	2.690	0.4
2	31	Glucose + NH_4^+	4.834	0.6
3	22	Control	1.187	−0.1
3	22	NH_4^+	0.117	−0.1
3	22	Glucose	0.480	0.3
3	22	Glucose + NH_4^+	0.928	0.2
5	20	Control, 15°C	0.009	0.2
5	20	Glucose + NH_4^+, 15°C	0.012	0.1
5	20	Control, 25°C	0.093	0.3
5	20	Glucose + NH_4^+, 25°C	0.472	0.0

[a] All data from the complete time course are given in the indicated figure
[b] Gross cell production was calculated from TdR incorporation integrated to the indicated time during the enrichment experiment when bacterial abundance had reached a maximum. Net production was the difference between bacterial abundance at the indicated time and the initial abundance
[c] The different treatments are described in the text and in the figures

hypotheses about why bacterial production increased due to the DOM additions.

In nearly all cases, bacterial production was matched or nearly so by bacterial mortality. In 2 out of 17 cases (the glucose + NH_4^+ enrichment in Fig. 2 and the control 25°C treatment discussed below), however, net production exceeded gross production (Table 1), which is not possible and indicates some methodological problem. In seven cases net production was not different from zero, even though gross production was measurable (Table 1). In the remaining nine cases, net production was measurable ($\geq$0.2 × 10^9 cells l^{-1}), but it was still much less than gross production (Table 1). In short, in nearly all cases, bacterial mortality due to grazing and viral lysis balanced bacterial growth and thus prevented large increases in bacterial abundance and kept net production near zero even when DOM was added. These data also indicate that the DOM stimulation of bacterial production was not because DOM limits bacterial biomass levels that the system can sustain ("Liebig type" limitation [5]). Rather, DOM additions stimulate bacterial production apparently because DOM limits bacterial growth rates ("Blackman type" limitation [5]).

Effect of Temperature on Bacterial Production and DOM Uptake

To compare our results with previous studies conducted in colder oceanic waters, we examined whether temperature affects DOM uptake differently than bacterial production and growth rates. Glucose uptake and TdR incorporation in surface waters (ca. 25°C) decreased the same when the temperature was dropped to as low as 10°C (Fig. 4). The Q_{10} was nearly the same (Q_{10} = 5) for glucose uptake and TdR incorporation. It is not clear why this Q_{10} is so much higher than usually observed for biological processes (Q_{10} = 2); it is also much higher than the average for TdR incorporation in equatorial Pacific waters (Q_{10} = 2.5; [18]). In any case, these results suggest that DOM uptake and bacterial production are affected equally by temperature.

We next tested how temperature affects uptake kinetics for surface water assemblages. A 10°C drop in temperature

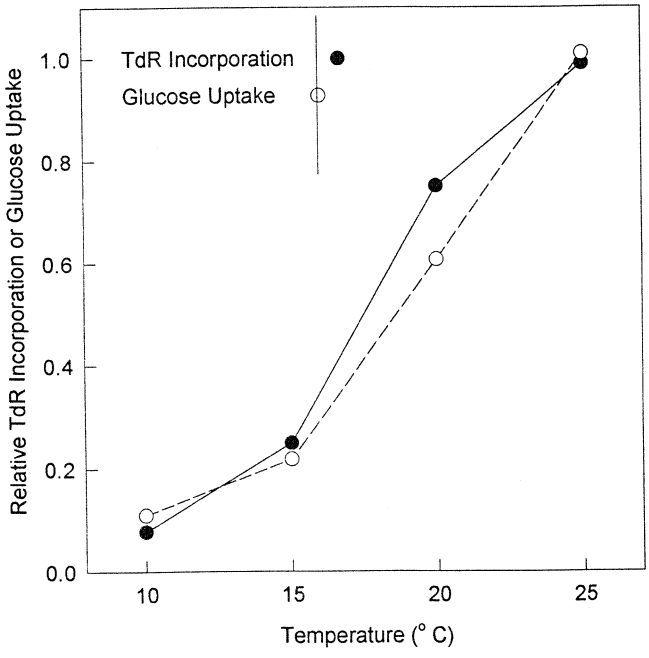

Fig. 4. Relative thymidine incorporation and glucose uptake at various temperatures. Rates at the various temperatures were divided by the rate at the highest temperature. The in situ temperature was about 27°C. The experiment was conducted on 27 August 1992 at 1°N 140°W. The *vertical bar* is an estimate of the standard deviation. The CV was about 20% for both ratios.

Table 2. Summary of uptake kinetics for glucose and mannose (8 September only) at two different temperatures in the equatorial Pacific Ocean in 1992. In situ concentrations for glucose and mannose are also given

Date	V_{max} (pM h^{-1})		V_{max} 25°/V_{max} 15°	K_m + S (nM)		N	In situ conc. (nM)
	15°C	25°C		15°C	25°C		
22 August	35.5 ± 1.3	47.8 ± 5.6	1.3	3.8 ± 0.6	8.4 ± 3.0	4	10
3 September	20.0 ± 2.1	54.6 ± 7.7	2.7	4.6 ± 1.6	4.2 ± 2.2	4	3
8 September[a]	3.3 ± 0.4	12.2 ± 2.1	3.7	1.4 ± 1.7	6.7 ± 2.8	4	<2[b]
13 September	16.6 ± 2.8	31.1 ± 2.5	1.9	9.9 ± 4.4	3.3 ± 2.0	5	10

[a] Uptake kinetics of mannose.
[b] In situ mannose concentration was below the limit of detection, which was 2 nM.

decreased V_{max} of glucose and mannose uptake by the expected amount (Table 2). The average Q_{10} for V_{max} in these experiments was 2.4 ± 1.0, which is similar to that observed for TdR incorporation (Q_{10} = 2.5 ± 1.4) [18]. Temperature did not affect K_m + S significantly ($P > 0.05$; Student's *t*-test) and there was no consistent change in K_m + S with temperature (Table 2).

Although not directly relevant to the main goals of this study, it is important to note that the measured concentrations of glucose were roughly equal to the K_m + S; mannose concentrations were below detection limits [26] (Table 2). Of course K_m + S should be greater than the in situ glucose concentration (S), but given the experimental uncertainties, the differences are not large. This agreement gives us some confidence about the validity of estimates for both the kinetic parameters and the glucose concentration. Fuhrman and Ferguson [12] found a similar agreement between K_m + S for DFAA uptake and DFAA concentrations measured by HPLC.

Response Time of Bacterial Production

The main conclusion of our enrichment experiments is that DOM additions stimulated bacterial production and growth rates whereas NH_4^+ additions had no effect. These results have been observed previously in other oceanic systems (e.g., [16, 17]). What differs is that addition of glucose or glucose + NH_4^+ had a much greater effect in the equatorial Pacific than in the subarctic Pacific and North Atlantic Oceans [16, 17]. We hypothesized that the warm waters of the equatorial Pacific allowed bacteria to respond more quickly to the glucose + NH_4^+ additions than possible in the other oceanic sites examined to date.

To test this hypothesis, glucose + NH_4^+ were added to samples of surface waters held at either 15°C or the in situ temperature of 25°C. As observed in the other experiments, at 25°C the glucose + NH_4^+ addition stimulated bacterial production (TdR incorporation) twofold by 6 h and over 30-fold after 20 h (Fig. 5A). At 15°C, stimulation of production was measurable only after 27 h into the experiment, i.e., more than 20 h later than in 25°C waters (Fig. 5A). Bacterial abundance did not change much nor in a consistent pattern (Fig. 5B). As observed before, bacterial growth rates (TdR incorporation per cell) were stimulated by the glucose + NH_4^+ addition at both temperatures (Fig. 5C).

The interesting point is that the apparent bacterial growth rate changed faster at 25°C than at 15°C with the glucose + NH_4^+ addition (Fig. 5C). Both bacterial production and growth rates peaked at 20 h for the enriched sample at 25°C compared with 50 h for the 15°C enriched sample. This difference in response time (50/20 = 2.5) is the same as the average Q_{10} for TdR incorporation in these waters [18].

The peaks in bacterial production (Fig. 5A) and growth rates (Fig. 5C) were only slightly greater (40%) for the 25°C enriched sample compared with the 15°C enriched sample. However, it is more important to examine the total amount of bacterial production supported by the added compounds, not just the maximum rates. To subtract out the production based on the in situ DOM, TdR incorporation in the control samples was subtracted from the incorporation in the enriched samples. This difference was then integrated over time to estimate the total production supported by the added compounds, i.e., the production enhanced by the glucose + NH_4^+ addition.

If the lower temperature resulted simply in delaying the same bacterial response, then the integrated, enhanced production at the lower temperature should lag behind, but eventually should equal the integrated enhanced production at 25°C. In fact, the enhanced production (TdR incorporation) increased much more quickly at 25°C than at 15°C, and

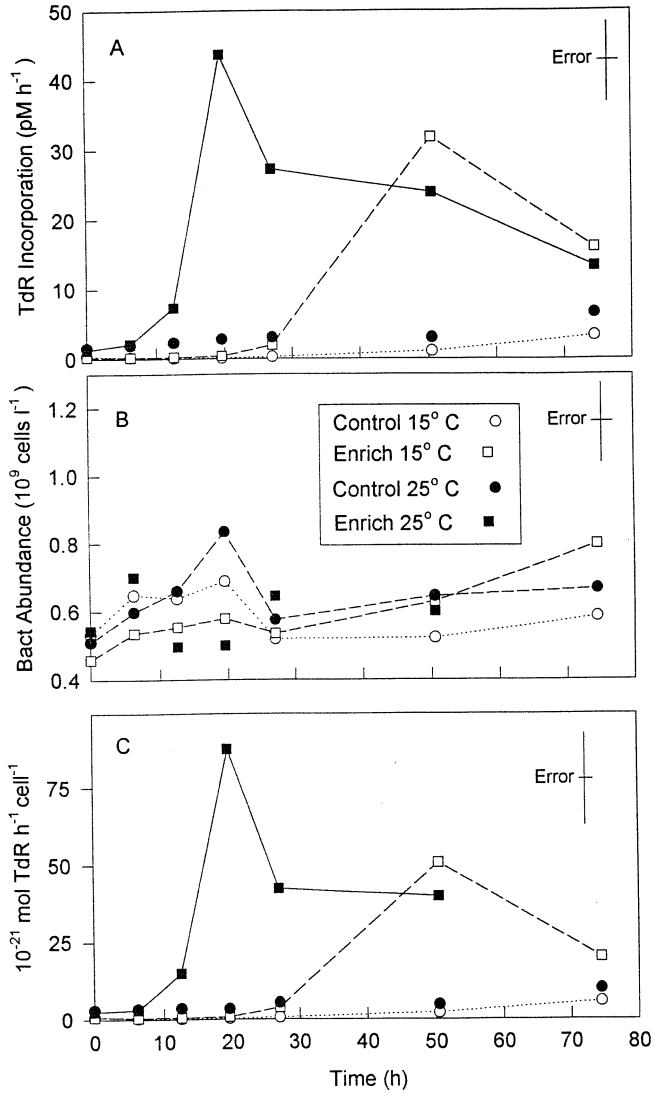

Fig. 5. Response of bacteria to glucose plus ammonium enrichment at two temperatures. **A.** bacterial production; **B.** bacterial abundance; and **C.** TdR incorporation per cell. The experiment was conducted on 24 August 1992 at 2°N 140°W. See Fig. 1 for explanation of error and *error bar.*

the integrated enhanced productions at the two temperatures were not equal even after 75 h (Fig. 6). This analysis suggests that, at least up to 75 h, more bacterial production was supported by the added glucose + NH_4^+ at 25°C than at 15°C.

Discussion

Dissolved organic matter appears to be the main factor limiting bacterial production and growth rates in the equatorial Pacific, implying that DOM also controls the flow of energy and material through the microbial loop. Ammonium and probably phosphate do not have a role in limiting bacterial growth rates in the equatorial Pacific, as DOM additions alone were sufficient to stimulate TdR incorporation, and NH_4^+ additions had no effect. We did not expect NH_4^+ or phosphate to have an impact because this oceanic region is well known to have high levels of all inorganic nutrients, with the exception of iron [23].

Limitation by DOM seems much more likely than temperature limitation of bacterial production in the warm waters of the equatorial Pacific. Still, temperature-shift experiments indicate that the increase in water temperature (5°C at some depths) due to El Niño should have resulted in a measurable increase in bacterial production, if temperature had any role in controlling bacterial production in these waters [18]. However, bacterial production appeared to follow primary production, rather than temperature, during the waning of the 1992 El Niño [18], which points to the importance of DOM in controlling bacterial growth rates. This study supports that hypothesis. Temperature, however, still could have an impact, even in the warm waters of the equatorial Pacific.

One obvious effect of temperature is that bacteria respond more slowly at colder temperatures to DOM additions and presumably to in situ changes in the DOM supply. This effect of temperature on the response time of bacteria

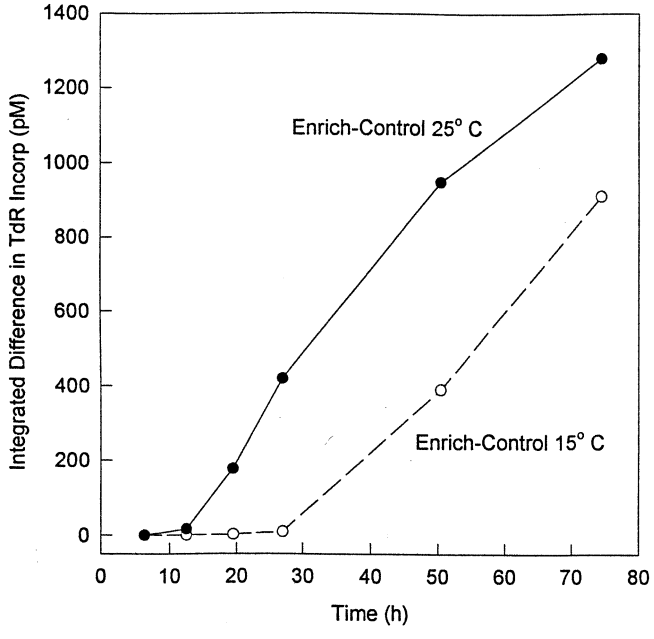

Fig. 6. Bacterial production enhanced by glucose plus ammonium enrichment at two temperatures. The raw data are presented in Fig. 5.

has implications for understanding the coupling of bacterial and primary production and for interpreting enrichment experiments. We probably need longer incubations to observe stimulation by DOM additions at colder temperatures. This may explain why Kirchman [16] observed relatively low enhancement of production by glucose or glucose + NH_4^+ additions in the subarctic Pacific. A similar explanation may apply to the study of Felip et al. [10]; these authors did not observe any stimulation of bacterial production by DOM additions at low temperature (4°C) during their 48-h incubations.

The other conclusion we tried to draw from the temperature-enrichment experiments is that more bacteria were produced at higher temperatures, suggesting that growth yields increase with temperature. This speculation is one possible explanation for the observations of Wiebe et al. [34] who concluded that isolated bacterial strains required more DOM to grow at low temperatures (10 and 15°C) than at high temperatures (>20°C). However, the few other pertinent studies are contradictory or inconclusive about whether or not growth yield changes with temperature. Nedwell and Rutter [24] found no consistent trend with temperature in yields of two Antarctic bacterial isolates growing between 2 and 25°C. Barillier and Garnier [1] also found no correlation between growth yields and temperature for natural assemblages from the Seine River (temperature range of 7 to 25°C). In a study of estuarine bacterial assemblages, Bjørnsen [3] found a negative correlation between growth yield and temperatures, although only three temperatures were examined (6, 10, and 15°C). With more samples, Daneri et al. [7] also observed a week negative correlation (6 to 23°C). T. Toolan (unpublished data), on the other hand, has observed a positive correlation between growth yield and temperature in Massachusetts Bay. We obviously need more work on how growth yield varies with temperature.

Our observations on how temperature affects the kinetics of DOM uptake are consistent with previous studies. Roberts et al. [27] also found that maximum uptake rates of glucose and amino acids increased with temperature. Likewise, Nedwell and Rutter [24] found a similar effect in their pure culture study, but like us they did not observe any temperature effect on half-saturation constants. Consequently, the calculated affinity (V_{max}/K_m) of bacteria for DOM increases with temperature in pure cultures [24] and in natural assemblages (this study). This temperature effect is another explanation for Wiebe et al.'s [34] observation that bacteria need more DOM to grow at low temperatures. A decrease in uptake affinity with temperature implies that bacteria would need higher DOM concentrations in cold water to grow at the same rate as in warm waters.

Temperature effects on DOM uptake have important implications for understanding competition among bacterial species and for examining the biogeochemistry of DOM in carbon cycling. Competition questions are discussed by Nedwell and Rutter [24]. Of course temperature will impact DOM concentrations, if it affects uptake differently than production. Based on the model of Billen et al. [2] and assuming we can generalize to compounds less labile than the simple sugars examined here, we hypothesize that steady-state DOM concentrations would decrease due to a temperature-enhanced V_{max} when water temperatures increase. Although the exact impact varies depending on other parameters (most importantly, the growth yield), the decrease in concentrations could be quite large (as much as 50%), even if the temperature increase is rather small (2°C) and $Q_{10} = 2$. This impact could be important for understanding the amount of carbon stored in the aquatic DOM pool and perhaps should be considered in global climate change models.

It can be difficult to extrapolate from bottle experiments such as described here to in situ processes even on small scales, much less global scales. Selected manipulations in bottle experiments may be necessary to answer specific questions, but some run the risk of disrupting normal relationships among microorganisms and the dissolved compounds they use. For example, techniques such as size fractionation that isolate bacteria from other organisms force bacteria to use DOM and inorganic nutrients in the existing dissolved pools. Consequently, we may learn something about what compound or element is missing from those pools, but we cannot say anything about what component of the dissolved flux is limiting. Given that concentrations of labile DOM and inorganic nutrients are usually low, bacteria probably depend more on released DOM or regenerated nutrients than on the existing dissolved pools. For this reason perhaps we need to reconsider those studies, especially those suggesting phosphate limitation, examining limitation of bacterial growth in experiments without other organisms present.

In contrast, addition experiments with whole water, similar to those performed by this study, may tell us something about what is missing from the dissolved compounds supplied by grazer excretion and phytoplankton release. These experiments indicate that organic carbon is the first component to become limiting. Presumably when organic carbon is added, heterotrophic bacteria outcompete phytoplankton [31] and take up additional inorganic nutrients in order to

grow on the added organic carbon. Perhaps stimulation by a free amino acid addition is the greatest in some waters (e.g., [16]) simply because these compounds supply C and N in forms not usable by phytoplankton. Our experimental design was not flawless as our bottles were incubated in the dark; this can be useful for examining heterotrophic processes, but it may affect release of compounds by phytoplankton.

Our main point of this study is that even when DOM is the main factor limiting growth rates, temperature still may have a role in regulating bacterial activity, but not the straightforward one suggested by temperature-shift experiments. Those experiments showed that an increase in the incubation temperature speeds up TdR incorporation, but the predicted temperature effect failed to materialize in situ during the 1992 El Niño [18]. However, temperature apparently does modulate the control by DOM of bacterial growth by affecting the response time and the affinity of bacteria for DOM. The latter point is also supported by pure culture studies [24] and is one explanation for the results of Wiebe et al. [34], the other being temperature effects on growth yield. These studies illustrate how temperature and DOM may interact to control bacterial production and growth rates in natural aquatic ecosystems.

Acknowledgments

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