

## Microbial glucose uptake and growth along a horizontal nutrient gradient in the North Pacific

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### Abstract

The quantitative role of carbon-rich compounds for bacterial carbon demand can vary with nutrient availability. This study is the first to investigate the effect of nutrient concentrations on bacterial uptake of a carbon-rich compound (glucose) along a naturally occurring nutrient gradient. Seawater was collected from varying depths at six stations across the nutrient gradient between the nutrient-poor North Pacific gyre and the nutrient-rich sub-Arctic gyre. Ambient concentrations of free glucose, glucose uptake, bacterial production, and nutrient concentrations were determined across the gradient. Incubations were carried out to determine the effect of nutrient additions on glucose uptake and bacterial production. Samples from the northern, nutrient-rich end of the transect had high glucose uptake rates and high bacterial production in surface waters. The northern stations also showed rapid turnover of the glucose pool. At the southern stations, glucose uptake rates, bacterial growth rates, and fractional glucose turnover were lower. At these stations, glucose uptake increased when inorganic nutrients and amino acids were added. The variations in glucose uptake indicate that microbial uptake of nitrogen- and phosphorus-poor organic material depends on the availability of nitrogen and phosphorus from other sources.

Carbohydrates are common biological products and bacterial substrates in the ocean (e.g., Pakulski and Benner 1994; Skoog and Benner 1997). Large changes in total combined carbohydrate concentrations with depth in the ocean indicate carbohydrates are highly reactive (e.g., Pakulski and Benner 1994; Skoog and Benner 1997), pointing to a potentially important role of carbohydrates in marine bacterial growth and respiration. Recent studies indicate that dissolved aldoses (a group of sugars with no charged groups) are important substrates for marine bacteria (Jørgensen et al. 1993; Rich et al. 1996; Skoog et al. 1999). Glucose has been shown to be the most abundant free neutral aldose in seawater (Rich et al. 1996; Skoog and Benner 1997). Little is known about the quantitative role of aldoses in supporting bacterial production. The few studies made to date, however, have shown that glucose can support a substantial fraction of bacterial production in the equatorial Pacific (Rich et al. 1996) and the Arctic Ocean (Rich et al. 1997) and as much as individual amino acids in other environments (Skoog et al. 1999).

Dissolved organic material (DOM) supports the major fraction of heterotrophic bacterial metabolism in the ocean (e.g., Azam et al. 1983; Kirchman et al. 1991). In the surface ocean, DOM in general and high-molecular-weight (HMW) DOM in particular have been found to contain a high frac-

tion of carbohydrates and to have C:N ratios of 10–30 (Benner et al. 1997). Bacterial biomass has C:N ratios in the range of 4.1–9.8 (Goldman et al. 1987; Fukuda et al. 1998), suggesting that the DOM ideal for bacterial growth would have a much lower C:N ratio. An additional nitrogen source would therefore be necessary for bacterial growth when nitrogen-poor DOM is the substrate.

It has been suggested that direct additions of inorganic nitrogen can enhance heterotrophic bacterial growth in environments with low dissolved nitrogen concentrations (e.g., Wheeler and Kirchman 1986; Horrigan et al. 1988; Skoog et al. 1999). Bacterial utilization of inorganic nitrogen during uptake and growth on nitrogen-poor DOM suggests that the availability of inorganic nutrients in the surface ocean can have a profound influence on DOM cycling.

This study investigates bacterial production, bacterial glucose uptake, and the effect on those parameters of inorganic nutrient additions on a transect across the surface nutrient gradient in the North Pacific. We hypothesize that the quantitative role of neutral sugars in bacterial production can vary along a nutrient gradient, such as the horizontal nutrient gradient in the North Pacific. Specifically, that the fraction of bacterial production supported by neutral sugars will decrease from nutrient-rich to nutrient-poor waters.

### Methods

*Sampling*—All samples were collected along 152°W in the North Pacific in November 1997 (Fig. 1). Niskin-type samplers with Teflon-coated springs were used for water collection. Samples for carbohydrate analyses were carefully drawn directly from the Niskin bottles with gloved hands into combusted 20-ml glass scintillation vials with Teflon-lined caps. Samples for free neutral aldoses were frozen, and

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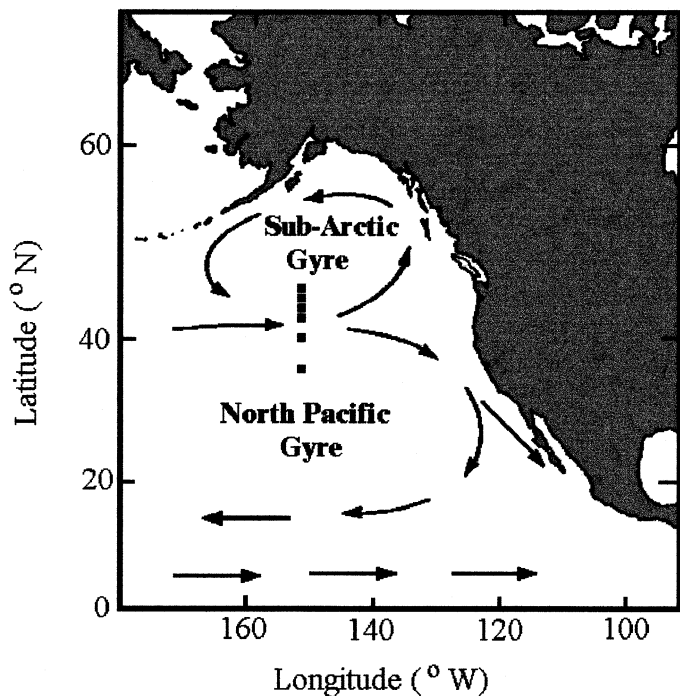


Fig. 1. Map of the northwest Pacific Ocean with the major current systems and the locations of our sampling stations on a transect along 152°W. The northernmost stations along the transect were located in the sub-Arctic gyre with nutrient-rich surface waters, whereas the southernmost stations along the transect were located in the North Pacific gyre with nutrient-poor surface waters.

processing was done on shore. Samples for bacterial incubations were collected in 1-liter acid-washed polycarbonate bottles. Incubation experiments were conducted at sea within 20 min of collection. Samples were kept at in situ temperatures during sample handling and incubations.

**Glucose uptake**—Incubations were conducted in combusted (500°C, 6 h) 30-ml glass sample tubes for 30 min in the dark at in situ temperatures. [ $^3\text{H}$ ]glucose (15 Ci mmol $^{-1}$ ) was added at a final concentration of 1 nM. Incubations were terminated by passing the sample through 0.2- $\mu\text{m}$  pore size MF Nuclepore membrane filters, followed by a 10-ml rinse with ice-cold filtered (0.2  $\mu\text{m}$ ) seawater. One formaldehyde-killed (4% final concentration) control was run corresponding to every three live incubations. Filters were transferred to 7-ml scintillation vials and dissolved by adding 0.6 ml Solvable (New England Nuclear) and heated in a heating block at 50°C, and the solution was then neutralized with 0.1 ml 5 M HCl before 5 ml Scintiverse II scintillation liquid was added. Sample activity was determined in a liquid scintillation counter (Beckman). Uptake of glucose was linear over 60 min.

**Bacterial abundance**—Samples (10 ml) for bacterial abundance were preserved with filtered formaldehyde (2% final concentration) and stored at 4°C. All samples were analyzed within a month of collection. Bacterial abundances were determined by epifluorescence microscopy at  $\times 1,260$

magnification of DAPI-stained samples collected on 0.2- $\mu\text{m}$  black Nuclepore filters (Porter and Feig 1980). Duplicate filters were prepared for each sample, and 25–50 fields of view were examined.

**Bacterial production**—Bacterial production (BP) was estimated from rates of protein synthesis with [ $^3\text{H}$ ]leucine (Kirchman et al. 1985). Triplicate 10-ml water samples were incubated in combusted (500°C, 6 h) glass containers in the dark at in situ temperatures for 30 min with 10 nM of leucine ( $2.52 \times 10^{12}$  Bq mmol $^{-1}$ ). One formaldehyde-killed (4% final concentration) control was run corresponding to every three live incubations. Incubations were terminated by filtration through 0.2- $\mu\text{m}$  pore size MF Nuclepore membrane filters and extraction in 3 ml of ice-cold 5% trichloroacetic acid (TCA) for 3 min. This treatment was followed by a 3-ml rinse with ice-cold 5% TCA and an additional rinse with 3 ml of ice-cold distilled water. The filters were stored in scintillation vials. Filter dissolution and determination of sample activity were carried out in the same way as for glucose utilization incubations. Uptake of [ $^3\text{H}$ ]leucine was linear over 60 min. Killed controls accounted for 0.5–6% of the radiolabel found in live samples. Rates of [ $^3\text{H}$ ]leucine incorporation were converted to bacterial carbon production values with the conversion factor of 0.108 cells mol $^{-1}$  leucine incorporated (Kirchman 1992). This conversion factor was determined empirically in the subarctic Pacific (Kirchman 1992). Cell production was converted to biomass production by assuming 12 fg C cell $^{-1}$  (Fukuda et al. 1998).

**Nutrient addition experiments**—Samples were incubated in triplicate for determination of leucine and glucose uptake (see above). Seawater samples were incubated at ambient nutrient concentrations or with the addition of either NaNO $_3$  (30  $\mu\text{M}$  final concentration), NH $_4\text{Cl}$  (1  $\mu\text{M}$  final concentration), or Na $_2\text{HPO}_4$  (1  $\mu\text{M}$  final concentration). Three killed controls were run with Na $_2\text{HPO}_4$  + NH $_4\text{Cl}$  (1  $\mu\text{M}$  final concentration each). All nutrients were cell culture grade (Sigma).

**Concentrations of nutrients in nutrient addition experiments**—The nutrient concentrations chosen for the incubation experiments enhanced the ambient nutrient concentration level 2–10-fold. Nutrient concentrations were chosen to mimic concentrations found right below the surface mixed layer (SML) in this area of the North Pacific (e.g., the nitrate concentration right below the SML is  $\sim 20$ –30  $\mu\text{M}$ ). In addition, we wanted to significantly enhance the nutrient concentrations in samples from both the nutrient-rich environment and from the nutrient-poor environment while keeping the final nutrient concentration roughly similar in incubations with samples from the two different environments.

**Determination of free neutral aldoses**—Sample work-up was carried out according to Skoog and Benner (1997). Immediately before analysis, samples were deionized by passing aliquots through a 3-ml mixed bed of equal volumes of anion (AG 2-X8, 20–50 mesh, Biorad) and cation (AG 50W-X8, 100–200 mesh, Biorad) exchange resins. The resin bed was rinsed three times with Milli-UV+ water and then with

Table 1. Concentrations of free glucose, fractional turnover time, and glucose uptake rates at various depths at stations along 150°W. Surface mixed layer depth was ~60 m for stations at 45, 43, 42.1, and 40°N, whereas stations at 37 and 33°N had a surface mixed layer depth of ~50 m.

Latitude (°N)	Depth (m)	[Glucose] (nM)	Turnover time (d)	Glucose-uptake (nM C d <sup>-1</sup> )
45	0	55	13	4.2
45	20	12	8	1.6
45	60	29	8	3.6
45	80	24	63	0.4
45	200	8	146	0.1
43	0	7.5	6	1.3
43	20	3.5	5	0.7
43	60	6	6	1.1
43	80	1.3	36	0.04
43	200	5	141	0.04
42.1	0	3.2	21	0.2
42.1	20	3.3	27	0.1
42.1	60	3.2	24	0.1
42.1	80	5.1	52	0.1
42.1	180	7.8	179	0.04
40	0	5.8	24	0.2
40	40	11.6	19	0.6
40	80	10	17	0.6
40	100	5	46	0.1
40	180	6.1	606	0.01
37	0	1.8	13	0.1
37	40	7.1	9	0.8
37	80	8.3	17	0.5
37	100	4.6	43	0.1
37	200	3.4	112	0.03
33	4	2.4	27	0.1
33	40	12.8	60	0.2
33	62	6.6	34	0.2
33	101	8.8	733	0.01
33	163	4.2	307	0.01

~1 ml of sample, which was discarded. A sample volume (2–3 ml) barely covering the resin was added. When CO<sub>2</sub> stopped evolving (ca. 5 min), the sample was drained into sample vials under vacuum. Aldoses were separated with an isocratic 28-mM NaOH elution using a PA-10 column in a Dionex 500 Ion Chromatography system with pulsed amperometric detection (PAD) employing a gold working electrode and an Ag/AgCl reference electrode. Procedural blanks were run using Milli-UV+ water. Relative standard deviations were in the range of 5–30%. For details of the chromatographic separation see Skoog and Benner (1997).

## Results

**Free glucose concentrations**—Glucose was the most abundant free neutral aldose and accounted for 50–100% of detectable free aldoses. Mannose and xylose were also detected at low concentrations. We did not determine fructose concentrations, and no galactose was detected. The free glucose concentration varied between 1.5 and 55 nM in the upper 200 m of the water column (Table 1). The highest

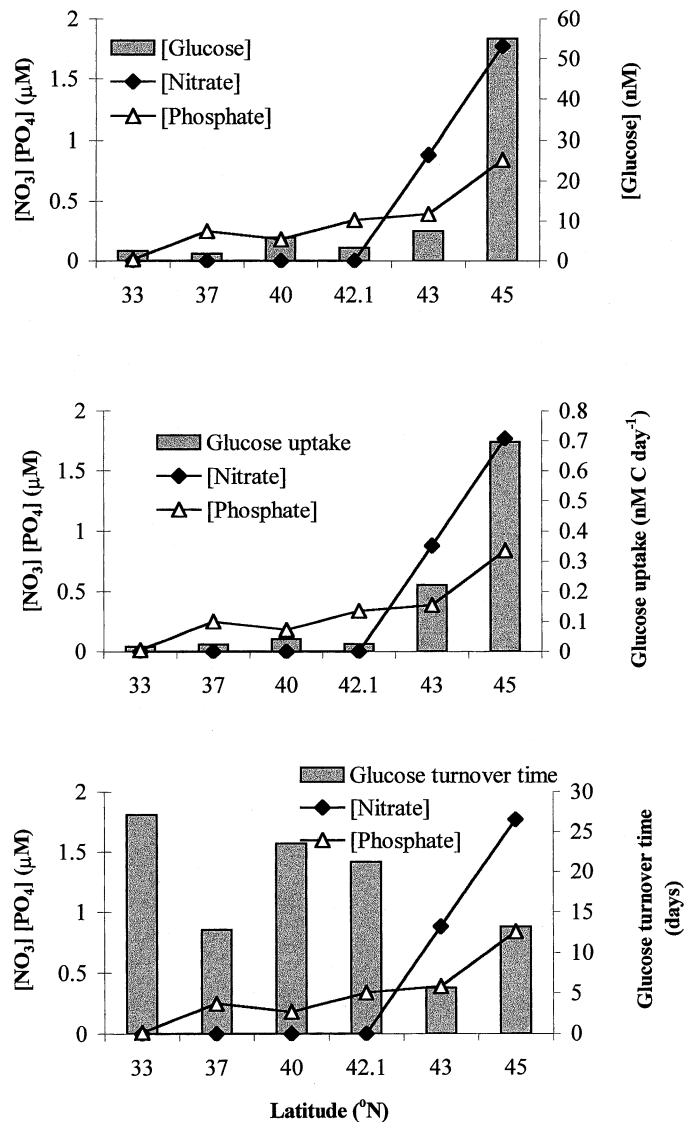


Fig. 2. Surface water values for glucose concentrations ([glucose]), glucose uptake, glucose turnover time, and nutrient concentrations along the station transect. The interface between the nutrient-rich surface waters of the sub-Arctic gyre and the nutrient-poor surface waters of the North Pacific gyre was located at 42.1°N. Glucose concentrations and uptake are higher in the nutrient-rich surface water of the northern stations, whereas the glucose turnover time is longer in the surface waters of the nutrient-poor North Pacific gyre.

concentrations were found the farthest north on the transect (Fig. 2).

**Uptake and fractional turnover of glucose**—Glucose uptake rates found in this study were in the range of <0.01 to 4.2 nM d<sup>-1</sup> (Table 1). Glucose uptake rates were higher in the SML than below it (Table 1). The average glucose uptake rates were higher in the SML of stations in the nutrient-rich sub-Arctic gyre (latitude > 42.1°N) than in the SML of stations in the nutrient-poor North Pacific gyre (latitude < 42.1°N; Table 1). Glucose uptake rates in surface samples

Table 2. Bacterial abundance, bacterial production (BP), growth rate and fraction of bacterial production supported by glucose uptake at various depths at stations along 152°W. Surface mixed layer depth was ~60 m for stations at 45, 43, 42.1, and 40°N, whereas stations at 37 and 33°N had a surface mixed layer depth of ~50 m.

Latitude (°N)	Depth (m)	No. of bacteria ( $\times 10^8 \text{ L}^{-1}$ )	BP (nM C d <sup>-1</sup> )	Growth rate (d <sup>-1</sup> )	Glucose supported BP (%)
45	0	5.60	30	0.053	14
45	20	3.61	19	0.053	8.3
45	60	6.75	17	0.024	22
45	80	3.44	3.7	0.011	10
45	200	3.45	1.6	0.004	3.5
43	0	6.28	30	0.047	4.5
43	20	9.70	24	0.025	3.1
43	60	1.08	25	0.023	4.3
43	80	4.30	17	0.006	1.3
43	200	1.45	3.4	0.004	6.3
42.1	0	4.81	21	0.043	0.73
42.1	20	5.80	21	0.037	0.57
42.1	60	5.76	19	0.033	0.69
42.1	80	4.37	3.4	0.008	2.8
42.1	180	1.63	0.27	0.002	16
40	0	6.15	22	0.036	1.1
40	40	4.44	24	0.053	2.6
40	80	5.07	13	0.027	4.3
40	100	5.36	3.9	0.007	2.8
40	180	2.50	—	—	—
37	0	6.87	21	0.030	0.68
37	40	6.51	24	0.036	3.2
37	80	6.29	7.6	0.012	6.3
37	100	1.18	3.7	0.031	2.9
37	200	1.84	0.25	0.001	12.2
33	4	4.37	11	0.024	0.8
33	40	5.57	11	0.014	2.0
33	62	4.92	12.4	0.025	1.6
33	101	1.52	—	—	—
33	163	3.79	0.37	0.001	3.7

showed a clear trend with latitude and with nitrate concentration (Fig. 2).

Turnover time of the glucose pool ranged from 5 to 733 d (Table 1), with a range of 5–60 d in the SML. At any given station (with the exception of 80 m at 40°N), turnover time was shorter in the SML than in deeper water. Below the SML, the mean turnover time for glucose increased to 63–733 d.

In the SML of the northern stations, the turnover time of glucose was in the range of 6–13 d (Table 1), whereas in the SML of the southern stations, the turnover time of glucose was in the range of 9–60 d. The average turnover time of glucose was significantly ( $p < 0.1$ ) shorter in the SML of stations in the nutrient-rich sub-Arctic gyre than in the SML of stations in the nutrient-poor North Pacific gyre (Fig. 2).

**Bacterial production**—BP ranged from unmeasurable below the SML to as much as 30 nM C d<sup>-1</sup> in the SML (Table 2; Fig. 3). The range in the SML was 11–64 nM C d<sup>-1</sup>. The growth rates of bacteria were in the range of 0.001–0.053 d<sup>-1</sup> (Table 2; Fig. 3). Bacterial growth rates in surface waters

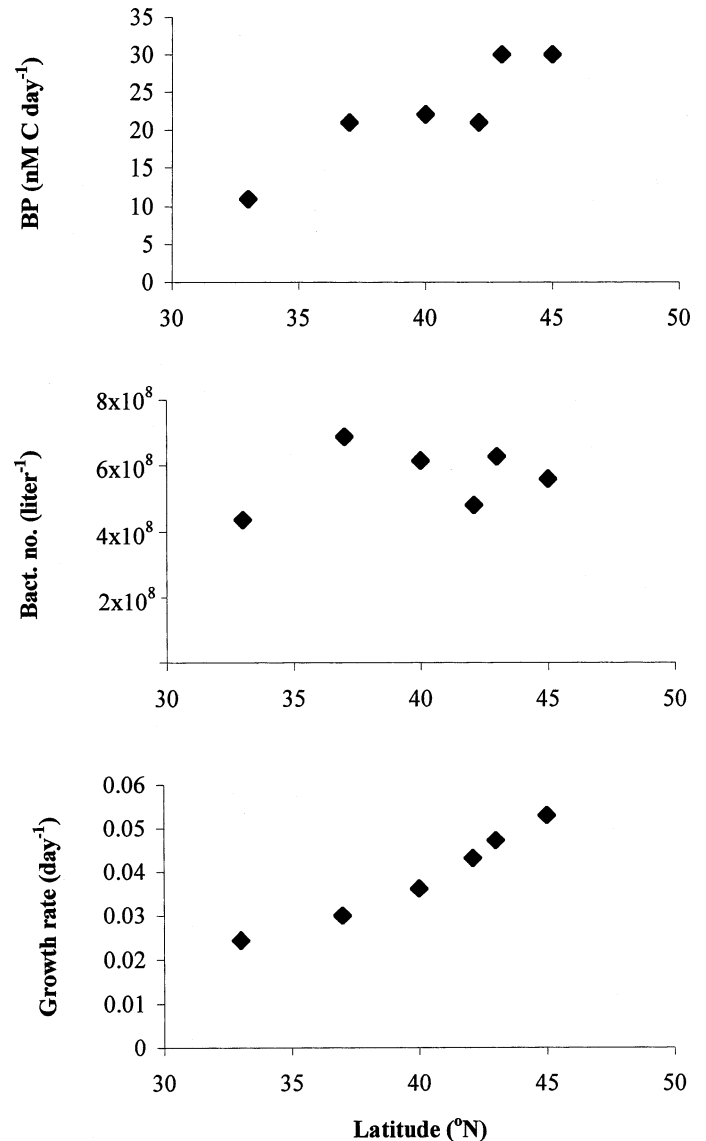


Fig. 3. Bacterial production (BP), bacterial numbers (Bact. no.), and growth rate in surface waters along the transect. Bacterial growth rate increased from south to north along the transect; that is, bacterial growth rates were higher in the colder but more nutrient-rich waters of the sub-Arctic gyre than in the warmer but nutrient-poorer waters of the North Pacific gyre.

decreased from north to south along the nutrient gradient. Bacterial growth rates were higher in surface waters of the northern stations, which had higher surface water inorganic nutrient concentrations.

Glucose uptake supported 0.57–22% of BP. The highest fraction of glucose-supported BP was found at the northern stations (Fig. 4). For comparison, Rich et al. (1996) found that as much as 40% of BP in surface waters can be supported by glucose uptake in the equatorial Pacific.

**Effect of nutrient addition on glucose uptake and bacterial production**—The relatively low glucose assimilation rate in surface waters of the southern stations of the transect occurred in nutrient-limited areas (Fig. 5). Glucose uptake was

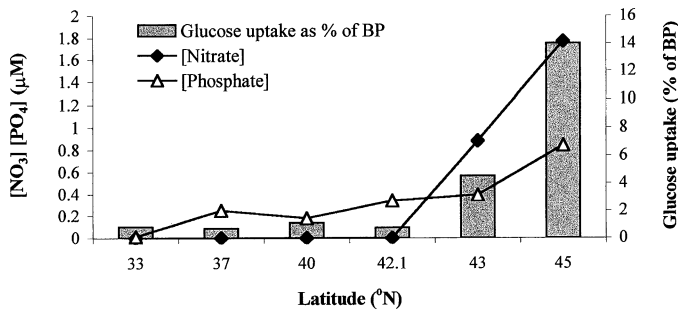


Fig. 4. Glucose-supported bacterial production (BP) and nutrient concentrations in surface waters along the transect. Glucose constituted a larger fraction of bacterial carbon demand in the nutrient-rich waters of the sub-Arctic gyre than in the nutrient-poor waters of the North Pacific gyre.

stimulated by the addition of NO<sub>3</sub>, NH<sub>4</sub>, PO<sub>4</sub>, or amino acids ( $p < 0.1$  when comparing incubations with nutrient additions to controls; Fig. 5). In contrast, no statistically significant effect of NO<sub>3</sub>, NH<sub>4</sub>, PO<sub>4</sub>, or amino acid additions on glucose uptake was observed ( $p > 0.1$ ) in samples from the northern stations (Fig. 5). Nutrient additions had no statistically significant effect on BP (Fig. 5) in surface water incubations.

## Discussion

**Free glucose concentrations**—Free glucose concentrations in this study are in the range of 1.3–55 nM (Table 1) and are similar to glucose concentrations found in other studies using similar methods. Rich et al. (1996) found concentrations in the equatorial Pacific surface waters in the approximate range of 5–110 nM, with averages of 15 nM in late summer and 38 nM in winter. Skoog and Benner (1997) found concentrations in the range of 2–13 nM in the Gulf of Mexico in summer.

Glucose is released to the water column by phytoplankton (e.g., Mopper et al. 1995) and by zooplankton grazing on plankton (e.g., Cowie and Hedges 1996; Strom et al. 1997). Chlorophyll fluorescence can be used as a proxy for phytoplankton abundance, whereas phaeopigment concentration can be used as an indicator of pigments that have been modified in zooplankton guts (i.e., as an indicator of grazing by zooplankton). A weak correlation ( $r = 0.38$ ,  $p < 0.05$ ) between glucose concentrations and chlorophyll fluorescence (data not shown) was found. This correlation indicates that no more than 10–12% of the free glucose concentration distribution could be explained by chlorophyll fluorescence. We also looked for a correlation with phaeopigments, which was even weaker ( $r = 0.18$ ,  $p < 0.05$ ). These results indicate that the amount of free glucose in the water column was not directly determined by release from phytoplankton cells. The alternative flux controlling the glucose concentration would therefore have to be uptake of glucose, indicating that the rate-limiting step is release of glucose.

**Uptake and fractional turnover of glucose**—Glucose uptake rates ( $<0.01$ – $4.2$  nM d<sup>-1</sup>; Table 1) are lower than the rates found in the tropical Pacific where Rich et al. (1996)

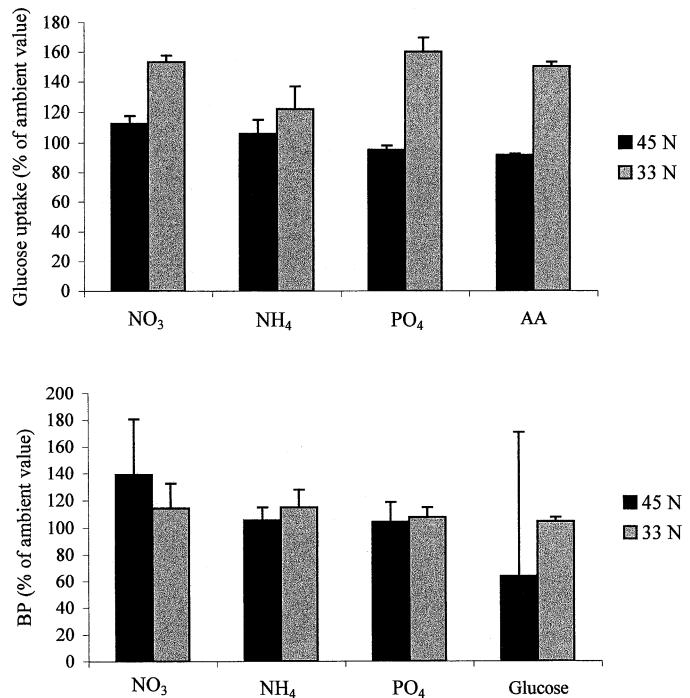


Fig. 5. Glucose uptake and bacterial production (BP) in the  $>0.22$ - $\mu$ m size fraction after additions of various nutrients in the surface waters of the sub-Arctic gyre at 45°N and the surface waters of the North Pacific gyre at 33°N. Glucose uptake and BP are normalized to incubations with ambient values of nutrients (i.e., no additions of nutrients) and are expressed as percentages of the ambient value. Glucose uptake increased in surface waters of the nutrient-poor North Pacific gyre, whereas no effect of nutrient additions on glucose uptake could be found in incubations with waters from the nutrient-rich sub-Arctic gyre. BP showed no statistically significant trend.

measured glucose uptake rates of 3–148 nM d<sup>-1</sup>. Turnover time of the glucose pool ranged from 5 to 733 d (Table 1) with a range of 5–60 d in the SML. These results encompass the range found in other studies (e.g., Gocke 1977; Rich et al. 1996; Skoog and Benner 1997).

There are very few studies available where glucose uptake rates have been determined, and it is therefore not possible to make any large-scale comparisons. Based on the results from this study and the results of Rich et al. (1996), it appears glucose uptake rates in the Pacific are higher than glucose uptake rates found in the Gulf of Mexico ( $<1$ – $3$  nM d<sup>-1</sup>; Skoog et al. 1999).

**Bacterial production**—BP and growth rate data from our study agree well with data from other studies. BP ranged from unmeasurable below the SML to as much as 64 nM C d<sup>-1</sup> in the surface mixed layer (Table 1; Fig. 4). Kirchman et al. (1993) found a range of 0.1–1.5 mg C m<sup>-3</sup>, corresponding to 3.3–125 nM C d<sup>-1</sup>, in the subarctic Pacific in May. Our data were collected later in the year than the cited study, which could explain the lower range of BP we found. The range of BP in the SML was 6.7–64 nM C d<sup>-1</sup>. These values are lower than data from the equatorial Pacific (Rich

et al. 1996), where the range for BP in the SML during February–April and August–October was 51–158 nM C d<sup>-1</sup>.

Growth rates of bacteria were in the range 0.002–0.053 d<sup>-1</sup> (Table 2; Fig. 4). These values are similar to another data set from the subarctic Pacific in the fall—Kirchman et al. (1993) found bacterial growth rates in the range of <0.01 to ~0.09 d<sup>-1</sup> in the subarctic Pacific in September. When comparing their results with studies from other ocean areas, Kirchman et al. (1993) suggested that bacterial growth rates in the subarctic Pacific are lower than in other ocean areas. Likewise, a review by Ducklow (1999) shows that the bacterial growth rates in the subarctic Pacific are lower than most other ocean regions, with the exception of Bermuda. Our data support the low values for bacterial growth rates in the sub-Arctic Pacific and, hence, the suggestion that this area might have lower growth rates than other ocean areas.

In our study, glucose uptake supported 0.3–16% of BP. The highest fraction of glucose-supported BP was found at the northern stations (Fig. 5). For comparison, Rich et al. (1996) found that as much as 40% of BP in surface waters can be supported by glucose uptake in the equatorial Pacific. The fraction of BP supported by glucose varies with ambient nutrient concentrations in our study (Fig. 5), suggesting that the importance of dissolved organic carbon (DOC) as a bacterial substrate might vary with nutrient availability.

*Effect of nutrient additions on glucose uptake and bacterial production*—The stimulation of glucose uptake by inorganic nutrient additions indicate N and P limitation for carbon uptake at the southern station in our transect (Fig. 5). In contrast, no statistically significant effect of NO<sub>3</sub>, NH<sub>4</sub>, PO<sub>4</sub>, or amino acid additions on glucose uptake was observed ( $p > 0.1$ ) in samples from the northern station (Fig. 5), indicating that heterotrophic carbon uptake in surface water was not nitrogen or phosphorus limited at these stations. There was no statistically significant difference in BP after nutrient additions ( $p > 0.1$ ) at each station; neither was there any statistically significant difference ( $p > 0.1$ ) between 45° and 33°N ( $p > 0.1$ ) after nutrient additions (Fig. 5).

The finding that both N and P additions stimulate uptake of glucose in the northern section of the transect indicates some form of dual limitation of carbon uptake in this area. Goldman et al. (1987) established that ammonium would be incorporated into bacterial cells to meet nitrogen biomass requirements if the substrate utilized for growth had a C:N ratio greater than that of the bacteria. Goldman et al. (1987) found C:N ratios of bacterial biomass in the range of 4.8:1–5.9:1 in bacteria grown on a variety of substrates. Fukuda et al. (1998) found C:N values of open-ocean bacterial assemblages of 6.8. Estimates from data in Abell et al. (2000) of the organic matter C:N ratio in surface waters across our transect range from ~9 in the northern section of the transect to 13 in the southern section of the transect. Estimates from data in Abell et al. (2000) of the organic matter C:P ratio in surface waters across our transect range from ~400 in the northern section of the transect to ~430 in the southern section of the transect. For comparison, the C:P ratio of bacteria is in the range of 40:1–50:1 (Goldman et al. 1987). Heterotrophic bacteria growing on the ambient organic ma-

terial along the transect would therefore need additional nitrogen and phosphorus.

The largest effects on glucose uptake were found in incubations with additions of phosphate. The N:P ratio of organic matter in the surface waters of our transect was ~43 in the northern section and ~41 in the southern section (estimated from data in Abell et al. 2000). Goldman et al. (1987) found that the N:P ratios of bacterial cells are in the range of 6:1–27:1, with an average of ~11:1, clearly lower than the N:P ratio of the ambient organic matter in our study area. It therefore appears that the limiting element for organic matter uptake was P. The N:P ratio of inorganic nutrients in the northern section of the transect was 2.2 (from Fig. 4), showing that there was an excess of inorganic nitrogen in comparison with inorganic phosphorus, both when compared with the Redfield ratio (16:1) and with the N:P ratio of bacterial cells (11:1).

The large increase in glucose uptake after addition of amino acids (Fig. 5) indicate that the bacterial uptake of carbon-containing compounds is highly stimulated by nitrogen present in a form that can be directly incorporated into cell material, such as amino acids. A similar effect has been found for BP (Cherrier et al. 1996), where the stimulation in BP by amino acids was interpreted as energy limitation. Cherrier et al. (1996) suggested that bacterioplankton biomass production in the eastern North Pacific is energy limited.

Nitrogen can be present in seawater as nitrate, nitrite, ammonium, and dissolved organic nitrogen (DON), with a reduction of N from nitrate to DON. Nitrate requires reduction to ammonia and incorporation into an organic molecule before it can be incorporated into cell material. The basic understanding of bacterial N uptake is that bacteria will most readily take up the most energy-efficient form of nitrogen. Both ammonium and amino acids should therefore be preferred to nitrate. In our study, we found the largest increases in N-stimulated glucose uptake after amino acid addition, which would agree with the above idea. However, ammonia gave lower increases in glucose uptake than did nitrate, which does not agree with the above idea, and we have no explanation for this result. Assuming that bacteria will most readily assimilate DON and that DON availability might therefore affect DOC uptake, it would be important to correlate bacterial uptake of DOC with DON uptake.

*Was there a contamination problem?*—The stimulation of glucose uptake by all additions in surface samples from the nutrient-poor North Pacific gyre raises the question of whether we somehow contaminated our samples. Because we used controls and because all data is normalized to the controls, any contamination would have had to occur only in the treatments or be manifested the most in the treatments. Possible candidates for contamination are organic components, bacteria, and micronutrients such as iron or cobalt.

The potential for organic contamination, chemical or biological, was low. We used acid-washed bottles for the collection of samples, freshly muffled glassware for the incubations, muffled glassware for the storage of frozen nutrient solutions, and gloves during all sample manipulations. All

incubations were carried out in an enclosed space used only by the two people involved in the incubations.

The possibility of adding micronutrients, such as iron, together with the additions of macronutrients, amino acids, or glucose is more difficult to dismiss. All chemicals used for preparations of nutrient solutions were of the highest possible purity, but we made no determinations of, for example, trace metal concentrations in any of the inorganic nutrient solutions. However, a line of evidence suggesting that iron contamination was not a problem comes from the incubation results. It has been suggested that high-nutrient, low-chlorophyll (HNLC) regions, such as the subarctic Pacific, are iron limited (Tortell et al. 1996). If we contaminated our experiments when adding the nutrient solutions, it seems we should expect a larger effect of the addition in the northern, iron-limited section of the transect than in the southern section. Our results were the opposite and could therefore indicate that iron contamination was not a problem in our incubations.

*Implications for cycling of carbon-rich material*—It has been shown that marine DOM in general and surface HMW DOM especially are rich in carbon. The C:N ratio of HMW DOM in the Pacific and Atlantic Oceans is, on the average, 16, and a large fraction of HMW DOM is carbohydrate (Benner et al. 1997). The organic material along our transect had C:N ratios in the range of 9–16 (Abell et al. 2000). Goldman et al. (1987) established that dissolved inorganic nitrogen (DIN) would be incorporated into bacterial cells to meet nitrogen biomass requirements if the substrate used for growth had a C:N ratio greater than did the bacterial cell. Heterotrophs growing on organic material with a C:N ratio of 9–16 would therefore need additional nitrogen. The increased uptake of glucose after inorganic N and amino acid additions indicates that variations in N availability might cause variations in carbon uptake in the surface ocean. Incubation studies of natural bacterial assemblages have demonstrated DIN uptake when the C:N ratio of the consumed DOM was high (Kirchman et al. 1991; Amon and Benner 1994).

We hypothesize a geographical variation in bacterial DOC degradation: Ocean regions with high N availability could have higher heterotrophic carbon uptake than ocean regions with low N availability. Nitrogen for bacterial uptake of DOC could come from ambient high concentrations or from bacterial fixation of inorganic N (e.g., Dugdale et al. 1961; Zehr et al. 2001). Areas with high N concentrations or high activity of N-fixing bacteria could therefore be areas of high DOC degradation and turnover. Furthermore, based on the accepted ideas of preferred uptake of reduced nitrogen by bacteria, we suggest that variations in general N availability, as well as the reductive state of N, could cause seasonal variations in relative heterotrophic carbon uptake.

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