J. Great Lakes Res. 12(3):161-174
Internat. Assoc. Great Lakes Res., 1986

# MICROBIAL RESPONSE TO AMINO ACID ADDITIONS IN LAKE MICHIGAN: GRAZER CONTROL AND SUBSTRATE LIMITATION OF BACTERIAL POPULATIONS

**Wayne S. Gardner, Joann F. Chandler, Gwenyth A. Laird, and Donald Scavia**
*U.S. Department of Commerce*
*Great Lakes Environmental Research Laboratory*
*2300 Washtenaw Avenue*
*Ann Arbor, Michigan 48104*

*ABSTRACT. Removal of low levels (0.3 - 1.3 μM) of primary amines (PA) were examined by direct-injection liquid chromatography in offshore Lake Michigan water to provide insight about potential cycling rates of labile organic compounds and to examine the hypothesis that populations of active bacteria are controlled by organic-substrate limitation. Uptake rates of amino acids in untreated lake water ranged from <0.1 to 3.7 nmole PA $L^{-1}h^{-1}$ (0.01 to 0.34 μg C $L^{-1}h^{-1}$) as compared to rates of up to 9 nmole PA $L^{-1}h^{-1}$ (0.61 μg C $L^{-1}h^{-1}$) in samples fortified with amino acids. Highest PA uptake rates were observed in samples collected in July and August. As expected for Michaelis-Menton kinetics, PA uptake rates increased with increasing amino acid concentrations (up to a maximum velocity), suggesting that bacterial "growth rates" were substrate limited. However, PA removal rates in given lake water samples usually remained relatively constant over periods of several days. The consistency of population uptake rates over time suggested that bacterial abundances did not change greatly in response to amino acid additions. Thus, although bacterial growth rates in Lake Michigan epilimnetic water are apparently controlled by organic substrate supply, our data are consistent with the hypothesis that bacterial abundances, and therefore total-population heterotrophic uptake activity, may often be controlled by grazers. The chemical approach used in this study yields a conservative estimate of the maximum velocity of microbial use of amino acids in natural waters.*
*ADDITIONAL INDEX WORDS: Growth kinetics, plankton, heterotrophic bacteria, limnology.*

## INTRODUCTION

Factors controlling bacterial growth rates and population dynamics in lakes and marine waters are not yet completely understood. For example, the relative role of organic-substrate supply rate vs. grazers as first-order regulators of bacterial populations in low-nutrient waters has not been clarified (Wright 1984). Although planktonic bacteria depend primarily on dissolved organic matter (DOM) for growth, maintenance, and respiration, the flux (F) of carbon from DOM to planktonic bacteria, and the importance of this process to bacterial growth and dynamics, are difficult to assess in aquatic ecosystems. Only a small portion of the total DOM pool in nutrient-poor waters is thought to be readily available to heterotrophs. For example, concentrations of total dissolved organic carbon (DOC) in offshore ocean or large-lake waters are several orders of magnitude higher than those of compounds known to be labile (i.e., available to bacteria) such as dissolved free amino acids, carbohydrates, and organic acids (Lee and Bada 1975, Gardner and Stephens 1978, Ittekkot *et al.* 1981, Pedros-Alio and Brock 1982).

The flux of carbon from DOM to bacteria in aquatic ecosystems has been estimated in a variety of ways, including: assuming it to be a function of measured primary production (Ducklow and Hill 1985); calculating it from bacterial growth rates, e.g., as determined by thymidine uptake (Fuhrman and Azam, 1982, Moriarty, 1984) or frequency of dividing cells (Hagstrom *et al.* 1979); determining short-term uptake kinetics of isotopically-labeled compounds (Wright and Hobbie 1966, Burnison

and Morita 1974, Wright and Burnison 1979); and measuring concentration differences of specific natural organic compounds sampled successively from the same sites (Burney *et al.* 1981, Mopper and Lindroth 1982, Ittekkot *et al.* 1981). Problems associated with these various approaches have recently been summarized (Wright 1984, Ducklow and Hill 1985, Scavia *et al.* 1986).

Another approach that we explore further here is "substrate enrichment" (Vaccaro 1969, Williams and Gray 1970, Hollibaugh 1979). If sufficient measurable substrate is added to saturate bacterial uptake sites, subsequent substrate removal rates should reflect the difference between the "potential" and "actual" bacterial cycling rates of the substrate(s), assuming that natural input rates are constant during an experiment. If the "actual" substrate uptake rates in untreated water are small relative to the "potential" uptake rates of the existing microbial population, the measured net rate of change in concentration should approach the latter and provide an independent chemical estimate of $V_{max}$, the maximum velocity of uptake for the substrate(s) by natural populations (Wright and Hobbie 1966, Burnison and Morita 1974, Williams and Gray 1970).

Observing the kinetics of substrate removal over several days can also provide information about factors controlling active bacterial populations in the water. For example, if all or part of the bacterial population is substrate-limited, and is not significantly affected by predation, the addition of an appropriate substrate should cause an increase in bacterial population and activity, with subsequent increases in substrate-removal rates, over time scales consistent with bacterial growth rates (e.g., ca. 2.4 $day^{-1}$; Scavia *et al.* 1986). Concentration decreases of added labile compounds vs. time over incubation periods of up to several days should then mirror the exponential-growth phase of a normal bacterial growth curve. On the other hand, *constant* substrate removal rates over time would be expected either if added substrates do not provide the appropriate growth-limiting factor(s) or if bacterial growth is balanced by grazing (e.g., Wright and Coffin 1984a). If the latter is true, the magnitude of the slope of substrate concentration vs. time should be a function of the abundance and/or uptake activity of the microbes.

Dissolved free amino acids (DFAA) are examples of typical substrates that occur at low levels in natural waters (Gardner and Stephens 1978, Mopper and Lindroth 1982, Jorgensen 1984), are available to natural populations of aquatic microbes (Crawford *et al.* 1974, Dawson and Gocke 1978, Ferguson and Sunda 1984) and can be measured at low concentrations (Gardner and Miller 1981, Mopper and Lindroth 1982). Following the rationale expressed above, if DFAA are added to water samples at levels that will be measurably affected by the microbial community, subsequent concentration changes over time under approximately *in situ* conditions should provide an estimate of the ability of the microbial population to use or adapt to a new supply of organic substrates (Hollibaugh 1979).

In this study, we enriched unfiltered lake water with amino acids and monitored primary amine (PA) concentrations over time to examine the potential of offshore Lake Michigan microbial populations to take up free amino acids, and to examine the hypothesis that microbial populations are limited by substrate concentrations in these waters.

## METHODS

### Experimental Approach

Sterile tissue culture bottles (Corning or Costar tissue culture flasks 25 or 150 $cm^2$ size) served as experimental containers. The uncapped bottles were tared and rinsed with distilled or unfiltered lake water. The latter was added on a top-loading balance up to a measured net weight (70, 580, or 650 g) to provide a known volume of sample. After measured quantities of amino acids in a standard mixture in 0.1 M HCl (AA-S-18, Sigma Chemical Co.) were added to respective sample bottles with a 10 $\mu$L Hamilton syringe, the bottles were capped, mixed, and placed in an incubator at near *in situ* temperatures. Most experiments were conducted in light (ca. 30 $\mu$Ein $m^{-2}s^{-1}$) to allow photosynthesis to continue. The standard mixture contained 2.5 nmoles $\mu L^{-1}$ of $NH_4Cl$ and of each of the following amino acids: L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-gylcine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-valine, and 1.25 nmole $\mu L^{-1}$ of L-cystine. Duplicate culture bottles were generally run for each sample at each amino acid level. Controls, without added amino acids, were also run with each set of samples.

Amino acid removal rates were quantified by measuring primary amine (PA = those amino

acids that form fluorescent derivatives with o-phthalaldehyde: OPA) levels before and shortly after spiking the samples with amino acids and at intervals for several days after the experiments were begun. After the samples were gently mixed in the culture bottles, a 1-mL sample was removed, with a 3-mL syringe equipped with a clean, but not sterilized, needle. (Between samples, the sampling syringe and needle were rinsed at least five times with PA-free distilled water and dried by repeated rinsing with air.) The sample was passed through a 0.4 $\mu$m pore-size filter, held in a low-dead-volume filter holder equipped with a Leur inlet (Gardner and Vanderploeg 1982), directly into a 0.1-mL sample loop of an ammonium-PA analyzer (Gardner 1978). One-half mL of sample was adequate to rinse the filter and sample loop and to fill the loop with pure filtrate.

The ammonium-PA analyzer automatically separated PA from ammonium by cation exchange chromatography, reacted them with OPA, and quantified the PA as a group fluorometrically. This automated direct-injection measurement technique for PA was preferred to high performance liquid chromatographic analysis of individual amino acids for this study because of its high precision and relative speed. PA less basic than ammonium elute as a single peak before ammonium. The OPA technique does not respond to the prolines and we did not quantify response to amino acids more basic than ammonium, as these compounds are usually below detection in natural waters. Fluorescent response factors are generally similar for OPA derivatives of the remaining 12 amino acids (Gardner and Miller 1980).

Primary amine removal rates were estimated from the change in PA concentration over measured time intervals. When linear decreases with time were observed, PA flux was estimated both by quantifying PA removal rates over the first 20 to 24 h after substrate addition, and by calculating the least-squares slope of the relationship between PA concentration and time over several days.

To estimate carbon flux (F) in these substrate-addition experiments, we assumed that the added non-fluorescing and basic amino acids were removed from solution by microbes at the same rates as the measured primary amines. The ratio of total amino acids to primary amines in the standard mixture was 1.46 on a molar basis. Based on an average molecular weight of 133 and a total percent carbon of 43.6 for the added amino acids, a conversion factor of 84.7 ng C per mole of PA was calculated to convert PA removal rates (nmoles PA $L^{-1}h^{-1}$) to carbon uptake rates (ng C $L^{-1}h^{-1}$).

### Culture Water Samples

We conducted PA uptake experiments using culture water without and with added microbes to differentiate between physical/chemical bottle effects and biological uptake in the experimental system. The culture water (NOPN), prepared to simulate the approximate inorganic composition of lake water, contained 40 mg $CaCl_2.2H_2O$, 20 mg $MgSO_4$, and 40 mg $NaHCO_3$ per liter of distilled water (after Lehman 1980). For the experiment without added bacteria, the NOPN was autoclaved before it was added to sterile 70-mL culture bottles. Portions of amino acid standard solution were added to duplicate bottles of NOPN to give amino acid concentrations of 0.59 and 1.18 $\mu$M. PA levels were measured at intervals over a period of 14 days. To examine amino acid removal patterns in the presence of high numbers of microbes, the NOPN was supplemented with water from an aged *Daphnia* culture and portions of this mixture were added to different culture bottles. Several levels of amino acids (0, 0.59, 1.18, 1.78, and 2.36 $\mu$M) were added to respective duplicate culture bottles (and mixed) to allow examination of different levels of substrate.

### Lake Michigan Samples

Water samples were collected in Niskin bottles at sites in Lake Michigan about 25 km west of Grand Haven, Michigan. Samples were placed in clean insulated water coolers and transferred to the Great Lakes Environmental Research Laboratory in Ann Arbor where experiments were begun within 24 h.

A portion of lake water, collected on 6 June 1984, was enriched with ten times the natural level of zooplankton, concentrated with a 153-$\mu$m mesh plankton net, to observe the potential effects of these animals on PA-removal rates. Five hundred eighty-mL incubation volumes were used for these experiments.

Bacterial abundance was determined on some lake samples, before and after incubation, by the acridine orange direct count method (Hobbie *et al.* 1977).

Short-term containment effects on amino acid uptake rates were examined with standard isotope techniques on 29 October 1985. Duplicate 100-mL

subsamples were poured off at ca. 4 h and at 20 and 40 h after sample collection. $^3$H-labeled amino acids (100 $\mu$Ci L$^{-1}$, NET-250, New England Nuclear) were added to one flask resulting in a 5.9 nM addition of unlabeled carrier substrate. $^3$H-labeled and unlabeled amino acids (AA-S-18), Sigma Chemical Company) were added to the second flask to give a 1.2 $\mu$M addition of unlabeled substrate. Samples were incubated at 14°C in the dark and triplicate 5-mL subsamples were taken over time during the 1-h incubation, and filtered through 25 mm diameter, 0.2 $\mu$m pore size Gelman Metricel GA-8 filters. Filters were rinsed with filtered lake water, transferred to scintillation vials along with 12 mL of Filter Count Cocktail (Packard), and assayed on a TriCarb scintillation spectrometer. Counting efficiency was determined with the external standard method.

## RESULTS

### Physical/Chemical Removal vs. Biological Uptake of Primary Amines in Tissue Culture Bottles

Primary amine removal was undetectable over 14-day incubations in bottles containing autoclaved NOPN with added amino acids (Fig. 1a). This suggested that amino acids were not removed from solution by sorption to the bottle walls or other physical/chemical effects in the simulated lake water.

In contrast to the experiments with autoclaved NOPN, measurable and pronounced PA removal was observed during the first few days of incubation for each of the bottles containing microbes from an aged *Daphnia* culture (Fig. 1b). Removal patterns were typical of those expected for "substrate-limited" systems without grazers and suggested an adaptation (growth or uptake site activation) of the microbes to the presence of amino acids in the bottles. Primary amine removal rates were relatively slow the first day, but increased considerably during the second day in bottles where amino acids were added. Uptake rates during the second day were directly proportional to the concentrations of the added amino acids. Most of the PA were removed by the end of the second day in all treatments, indicating that the microbial activity increased sufficiently to remove the amino acids over a relatively short time period. These results resemble those reported for natural coastal marine bacteria after enrichment with amino acids (Vaccaro 1969, Hollibaugh 1979). Similar results were obtained in a second experiment (data not shown).

Primary amine removal was examined in autoclaved lake water to verify that killing the bacteria would prevent amino acid removal from spiked lake water. Removal was negligible in the autoclaved water (in comparison to an untreated portion of the same sample) during the first week of incubation (Fig. 2). However, PA were removed from some of the autoclaved lake water treatments during the second week indicating probable microbial contamination. Removal rates from these samples were relatively rapid after removal began, possibly because microbial grazers were absent from the autoclaved water.

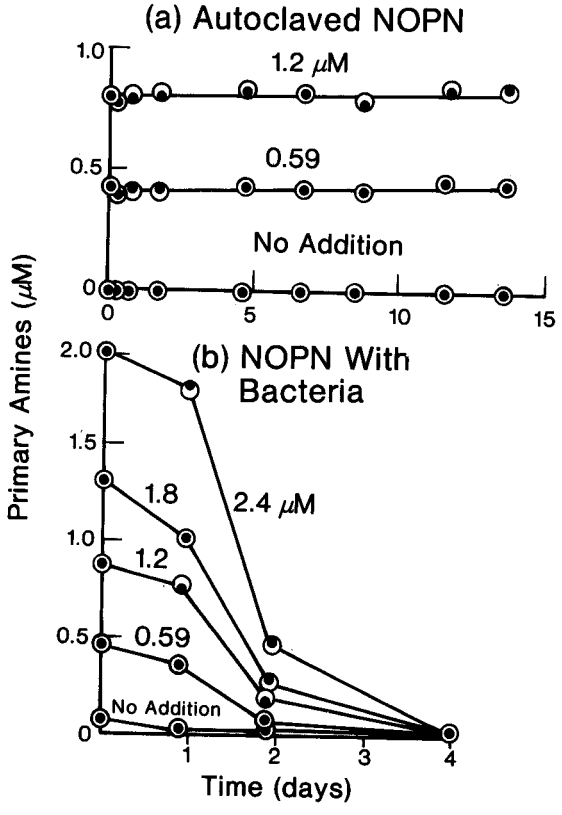


*FIG. 1. Primary amine removal patterns for culture water (NOPN) without (a) and with added microbes (b) and fortified with amino acids. The numbers associated with each line indicate concentrations of added amino acids (μM). Dots and circles represent measurements on duplicate experimental culture bottles. Twelve of the 17 amino acids in the mixture of added amino acids responded analytically as primary amines.*

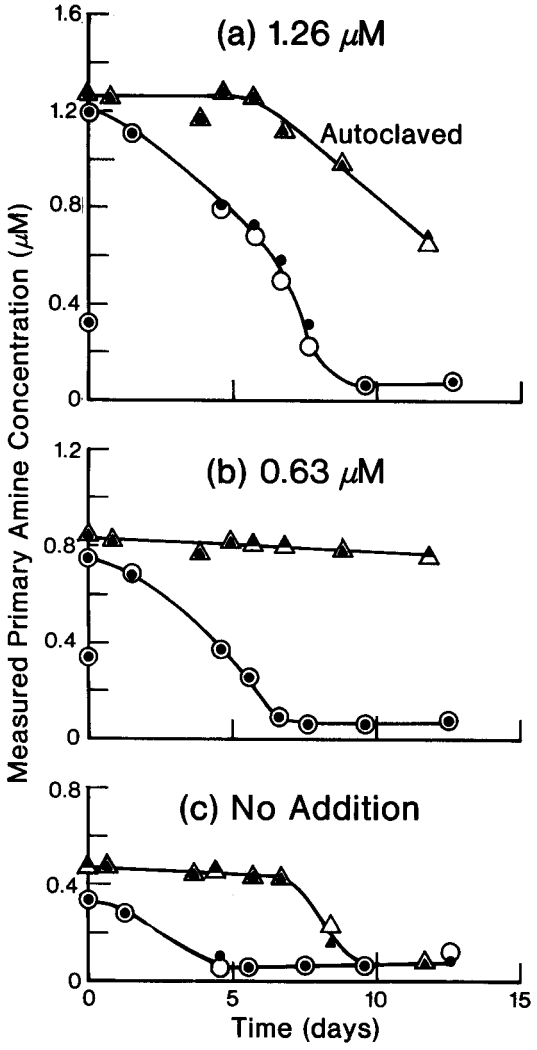


*FIG. 2. Primary amine removal patterns for an untreated (circles) and autoclaved (triangles) Lake Michigan sample collected from a 3-m depth on 31 July 1985. The numbers on each graph represent the concentrations of added amino acids.*

### Primary Amine Concentrations and Uptake Patterns in Lake Michigan Water

The above experiments, with and without microbes, indicated that our "substrate enrichment" method has potential for examining the capability of microbes to take up free amino acids in unfiltered lake water. The ambient PA levels, the measured PA fluxes, and the calculated amino acid carbon flux (F) for Lake Michigan water were examined on several sample dates in 1984 and 1985 for both untreated samples (Table 1) and those fortified with amino acids (Table 2).

Primary amine uptake rates from duplicate bottles generally agreed remarkably well and usually could not be distinguished from each other (eg., Figs. 1, 2, and 3). A comparison of the effects of incubation volumes (70 mL vs. 580 mL) on PA removal rates (Fig. 4) suggested the absence of significant size-related bottle effects. In agreement with the hyperbolic Michaelis-Menton substrate-uptake pattern expected for microbial populations, uptake rates increased with increased levels of added amino acids for a given sample (Tables 1 and 2), but approached an asmptote with higher level additions (Fig. 4; Table 2).

Primary amine concentrations in untreated lake samples ranged from 0.10 to 0.62 μM (Table 1), with the highest levels in sample collected in late July and in August. Background concentrations of PA remained higher after incubations in lake water than in NOPN. Whereas PA concentrations in NOPN decreased to $<0.02\mu$M (Fig. 1b), concentrations in lake water never decreased below the background levels of ca. 0.07 μM even after samples were incubated for several days in the laboratory.

Bacteria abundances were determined for both initial untreated water and final incubation waters in some experiments to determine if bacterial abundance was significantly altered by either containment or amino acid addition. Cell concentrations were within the range of $3 \times 10^5$ to $11 \times 10^5$ cells $mL^{-1}$ in all samples we examined. No relationship was observed between cell numbers and added amino acid concentration (or uptake activity) in these substrate-addition experiments. In a separate experiment, bacterial abundances were monitored over 26 h in lake water held at *in situ* temperature; they did not change significantly with time (Laird and Scavia, unpublished data).

Primary amine uptake rates varied from undetectable (slope not significantly different from zero) to 3.7 nmoles PA $L^{-1}h^{-1}$ (Table 1) in unspiked lake samples and reached as high as 8–9 nmoles PA $L^{-1}h^{-1}$ in one summer sample fortified with 0.64 μM amino acids (Table 2). Strikingly, in most samples, PA uptake rates did not appear to change over time of incubation (several days) in response to additions of these low levels of amino acids. Except for two sampling dates (29 February 1984 and 31 July 1985), removal rates of added amino acids, if measurable, were relatively constant during incuba-

*TABLE 1. Conditions of incubation and amino acid flux in samples of untreated Lake Michigan water. Flux was calculated both as the mean change in primary amine concentration during the first 20–24 h of incubation and as the slope of primary amine concentration vs. time over several days when the relationship was linear. Carbon flux was calculated from primary amine flux using a factor of 84.7 (see text).*

| Sampling date | Sample depth (m) | Initial PA conc. (μM) | Incubation volume (mL) | Incubation conditions | Amino acid flux (nmole PA/L/h) Day 1 | Slope (r) | F (ngC/L/h) Slope |
|---|---|---|---|---|---|---|---|
| 29/2/84 | 3 | 0.38 | 70 | Dark, 4°C | +1.50 | nonlinear decrease | |
| 27/3/84 | 3 | 0.14 | " | " | -0.56 | -0.22(-0.875) | -19 |
| " | " | " | " | Light, 4°C | -0.54 | -0.19(-0.671) | -16 |
| 6/6/84 | " | 0.10 | " | " | -0.31 | -0.01(-0.067) | -1 |
| " | " | " | 580 | ", 10 × Zooplankton | -0.18 | -0.064(-0.443) | -5 |
| 20/6/84 | " | 0.18 | " | Light, 10°C | -1.72 | -0.25(-0.887) | -21 |
| " | 30 | 0.13 | " | " | -1.93 | -0.06(-0.294) | -5 |
| " | " | " | " | " | -1.32 | -0.02(-0.105) | -1 |
| 5/7/84 | 3 | 0.11 | 650 | Light, 16°C | -1.32 | -0.17(-0.456) | -15 |
| " | 30 | 0.13 | " | Light, 8°C | -0.22 | -0.17(-0.846) | -14 |
| 31/7/84 | 3 | 0.49 | " | Light, 21°C | -2.50 | -4.89(-0.995) | -414 |
| " | 30 | 0.62 | " | Light, 12°C | -3.71 | -3.95(-0.998) | -335 |
| 22/8/84 | 3 | 0.27 | " | Light, 23°C | -2.12 | -1.25(-0.964) | -106 |
| " | 30 | 0.35 | " | Light, 8°C | -0.29 | -1.52(-0.978) | -129 |
| 5/6/85 | 3 | 0.10 | " | Light, 4°C | -0.80 | -0.04(-0.193) | -3 |
| 31/7/85 | " | 0.33 | " | Light, 18°C | -1.43 | nonlinear decrease | |

tions, and fit the pattern expected for grazer control rather than that expected for substrate limitation in the absence of significant grazing. The relationship of PA concentration vs. time in these samples was usually well described by a linear model ($r > 0.83$; $P < 0.01$) over several days in otherwise untreated samples (Table 2). Likewise, unfortified samples with *in situ* PA concentrations above background levels (>0.14 μM PA) showed significant linear decreases with time (Table 1). Thus on seven out of nine sampling dates, the PA uptake patterns were *not* those expected for strictly carbon-limited bacterial populations.

Samples collected from both 3- and 30-M depths on 31 July 1984 had higher initial natural concentrations of PA and also exhibited more rapid removal rates at both depths than samples collected on other dates (Tables 1 and 2). Initial *in situ* PA concentrations were about 0.5μM as compared to more usual concentrations of 0.1 to 0.2 μM. Although uptake kinetics were only examined for 3 days in this experiment, PA levels were reduced at constant rates (between 4 to 9 nmole PA $L^{-1}h^{-1}$ for the different bottles) in both the controls and in bottles with amino acid additions (Fig. 3). This compares to removal rates of <2 nmole PA $L^{-1}h^{-1}$ for samples collected earlier in the season (Table 1).

As was true for July/August 1984 samples, the natural concentration of PA in water collected 31 July 1985 was relatively high (Tables 1 and 2). However, in contrast to results from most other lake water experiments, the PA uptake patterns (nonautoclaved sample of Fig. 2) resembled those expected in substrate-limited samples. Uptake rates were moderate the first day (1.4 to 2.1 nmole $L^{-1}h^{-1}$) but then increased to a rate of ca. 12 nmole $L^{-1}h^{-1}$ on days 7–8 in the sample receiving 1.26 μM amino acids. Similar patterns were observed in the bottles receiving 0.64 μM amino acids and in the control bottles receiving no amino acids, but the concentrations in these treatments were reduced to background levels sooner (at about 7 and 4 days, respectively) because of their lower initial amino acid concentrations (Fig. 2).

Water, sampled on 6 June 1984, with concentrated zooplankton and added amino acids, exhibited slow but significant PA removal rates over the first 8 days (Fig. 5; Table 2), but then showed greatly increased removal rates at both amino acid treatment levels during the last 2 days (Fig. 5). In contrast, lake water without added zooplankton

*TABLE 2. Conditions of incubation and amino acid flux in samples of unfiltered Lake Michigan water fortified with amino acid substrates. Flux was calculated both as the mean change in primary amine concentration during the first 20–24 h of incubation and as the slope of primary amine concentration vs. time over several days when the relationship was linear. Carbon flux was calculated from primary amine flux using a factor of 84.7 (see text).*

| Sampling date | Sample depth (m) | Initial PA conc. (μM) | Incubation volume (mL) | Incubation conditions | Conc. of added amino acids (μM) | Amino acid flux (nmole PA/L/h) Day 1 | Slope (r) | F (ngC/L/h) Slope |
|---|---|---|---|---|---|---|---|---|
| 29/2/84 | 3 | 0.38 | 70 | Dark, 4°C | 0.59 | +0.50 | nonlinear decrease | |
| " | " | " | " | " | 1.18 | -1.10 | " | |
| 27/3/84 | 3 | 0.14 | " | " | 1.18 | -1.23 | -1.05 (-0.968) | -80 |
| " | " | " | " | Light, 4°C | 0.59 | -1.72 | -1.21 (-0.922) | -102 |
| " | " | " | " | " | 1.18 | -3.65 | -1.59 (-0.995) | -135 |
| 6/6/84 | " | 0.10 | 580 | " | 0.36 | 0 | -0.03 (-0.217) | -3 |
| " | " | " | " | ", 10 × Zooplankton | 0.36 | -0.14 | -0.63 (-0.909) | -54 |
| " | " | " | " | " " | 0.71 | -0.13 | -0.83 (-0.867) | -70 |
| 20/6/84 | " | 0.18 | 70 | Light, 10°C | 0.18 | -0.08 | -0.12 (-0.346) | -10 |
| " | " | " | " | " | 0.30 | -0.61 | -0.28 (-0.926) | -23 |
| " | 30 | 0.13 | " | " | 0.12 | -0.53 | -0.30 (-0.986) | -25 |
| " | " | " | " | " | 0.30 | -0.64 | -0.45 (-0.831) | -38 |
| " | " | " | 580 | " | 0.14 | -1.32 | -0.35 (-0.989) | -30 |
| " | " | " | " | " | 0.36 | -1.13 | -0.55 (-0.985) | -46 |
| 5/7/84 | 3 | 0.11 | 650 | Light, 16°C | 0.13 | -0.73 | -0.62 (-0.947) | -52 |
| " | " | " | " | " | 0.32 | -1.68 | -1.54 (-0.949) | -130 |
| " | 30 | 0.13 | " | Light, 8°C | 0.13 | -1.56 | -0.53 (-0.975) | -45 |
| " | " | " | " | " | 0.32 | -0.35 | -0.82 (-0.974) | -70 |
| " | " | " | " | " | 0.51 | -0.47 | -1.00 (-0.975) | -85 |
| 31/7/84 | 3 | 0.49 | " | Light, 21°C | 0.32 | -6.54 | -5.93 (-0.997) | -502 |
| " | " | " | " | " | 0.64 | -8.93 | -7.18 (-0.995) | -608 |
| " | 30 | 0.62 | " | Light, 12°C | 0.32 | -6.05 | -5.61 (-0.995) | -475 |
| " | " | " | " | " | 0.64 | -6.40 | -5.74 (-0.997) | -486 |
| 22/8/84 | 3 | 0.27 | " | Light, 23°C | 0.32 | -2.54 | -2.34 (-0.991) | -198 |
| " | " | " | " | " | 0.64 | -0.04 | -2.54 (-0.983) | -215 |
| " | 30 | 0.35 | " | Light, 8°C | 0.32 | -0.95 | -1.54 (-0.972) | -130 |
| " | " | " | " | " | 0.64 | -1.62 | -1.74 (-0.988) | -147 |
| 5/6/85 | 3 | 0.10 | " | Light, 4°C | 0.64 | -1.26 | -0.93 (-0.989) | -79 |
| " | " | " | " | " | 0.95 | -0.52 | -1.10 (-0.934) | -93 |
| " | " | " | " | " | 1.27 | -2.65 | -1.19 (-0.936) | -101 |
| 31/7/85 | " | 0.33 | " | Light, 18°C | 0.64 | -1.82 | nonlinear decrease | |
| " | " | " | " | " | 1.27 | -2.08 | " | |

did not exhibit detectable PA uptake in this experiment. Natural bacterial uptake activity was below our detection limits in this unmodified sample.

The short-term containment experiments with radiolabeled amino acids indicated some changes in uptake rate with time of incubation. In the presence of the high concentration (1.2 μM) of the unlabeled mixture, total amino acid carbon flux estimates increased moderately from 28 ng C $L^{-1}h^{-1}$ at 4 h to 53 and 48 ng C $L^{-1}h^{-1}$ at 20 and 40 h. However, in the low (5.9 nM) addition tracer experiment, uptake rates after 20 and 40 h were almost 4-fold faster than those observed at 4 h (data not shown); actual fluxes were not calculated for this tracer experiment because the concentration of available substrate was not known.

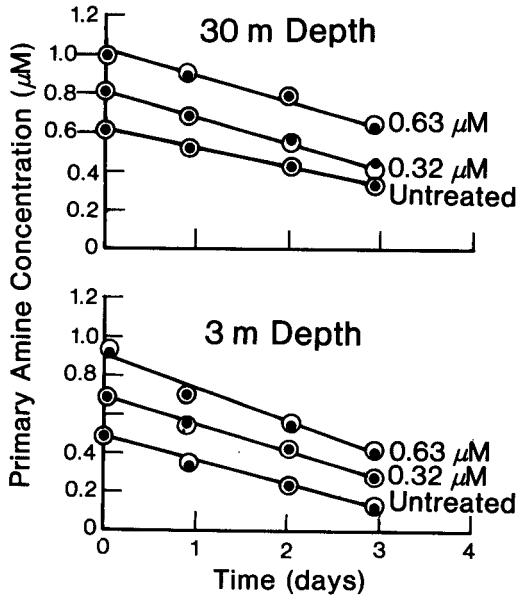


*FIG. 3. Primary amine concentration vs. time of incubation for Lake Michigan water sampled at two depths and fortified with 0, 0.32, and 0.63 μM of an amino acid mixture.*

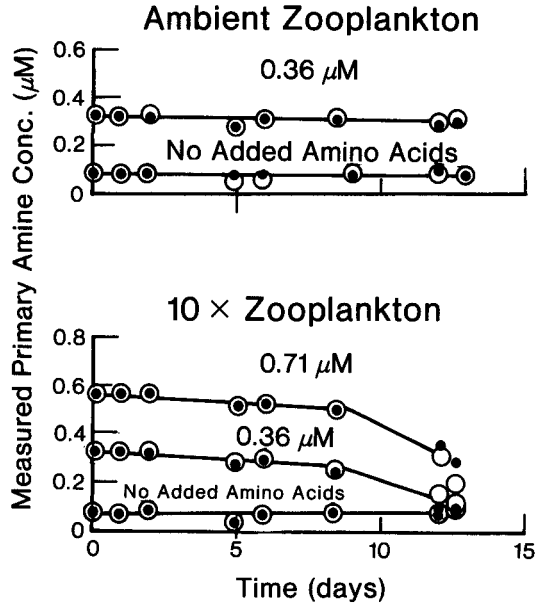


*FIG. 5. Effects of a 10-fold increase in zooplankton levels on PA removal patterns in Lake Michigan water fortified with amino acids.*

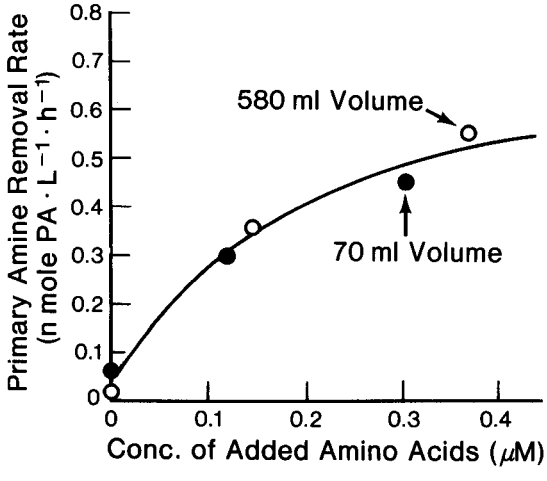


*FIG. 4. Dependence of PA removal rates (determined as the slope of PA concentration over time) on concentration of added amino acids, and on incubation volume, for a Lake Michigan sample collected from a depth of 30 m on 20 June 1984.*

## DISCUSSION

### Methodology Considerations

The current approach of adding amino acids to an untreated water sample and then monitoring concentration decreases complements other methods used to examine the flux of labile organic compounds, and provides insights about the status and potential activity of microbes in the water (Williams and Gray 1970). Unlabeled, rather than isotopically-labeled, substrates were used so that removal rates could be conveniently followed over periods exceeding bacterial generation times without isotope dilution/exchange problems. An amino acid mixture was selected as the substrate because amino acids are important labile compounds used by planktonic bacteria (Williams and Gray 1970), Crawford *et al.* 1974) that can be conveniently measured at near-ambient levels. Discrete-injection automatic chemical analysis of PA offered the precision needed to evaluate small changes against a relatively high background signal.

The background levels of >0.07 μM PA that we always observed in lake water suggests that chemical measurements may increasingly overestimate PA concentrations actually available to bacteria as measured PA concentrations approach this level in Lake Michigan water. Similarly, in other lakes and in the Baltic Sea, dissolved free amino acids measured chemically were higher than substrate levels apparently available to bacteria (Burnison and Morita 1974,. Dawson and Gocke 1978); however this was not the case in Danish lakes (Jorgensen

and Sondergaard 1984). The reasons for elevated background levels of PA in lake water as compared to NOPN are not completely clear. It was not due to background fluorescence in the absence of OPA, but may have been caused by "bound PA" (Gardner and Stephens 1978) that responded to the OPA reagent but were not available to bacteria. An alternative explanation is that natural lake bacteria, normally accustomed to limited substrate inputs, may "turn off" their uptake mechanisms at very low levels of substrate because the uptake of subthreshold levels of labile substrates is energetically too costly. In contrast, cultured microbes accustomed to sufficient substrate may not be constrained by this threshold (J.E. Hobbie, Marine Biological Laboratory, Woods Hole, MA, personal communication). To examine this possibility, we mixed *Daphnia*-culture microbes with both NOPN and lake water (sampled 6 December 1985) and observed their effect on background PA levels. The laboratory microbes again reduced the PA levels to $<0.02$ $\mu$M in NOPN but not in lake water; although the laboratory bacteria thrived in the lake water, PA response in the latter never decreased to $<0.07$ $\mu$M. We therefore concluded that the "background PA" were more likely due to chemical interference than to a high threshold for uptake by the lake bacteria.

Possible "containment" effects must be considered in interpreting substrate-enrichment experiments of several days duration. Microbial population changes caused by containment effects tend to be selective for culturable species (Ferguson *et al.* 1984). In agreement with previous substrate-addition experiments (Williams and Gray 1970, Hollibaugh 1979), our experiments indicated that containment effects on PA uptake rates were not great after the substrate addition experiments were initiated. Bacterial counts, when examined, remained in the range of $3 \times 10^5$ to $11 \times 10^5$ cells $mL^{-1}$ within and among experiments. A comparison of the effects of incubation volume on PA removal rates on portions of the same sample suggested the absence of significant size-related bottle effects on uptake rates (Fig. 4). A significant linear relationship ($r = 0.88$) was observed between "first day" PA uptake rates and rates obtained from the slopes of PA concentration vs. time over several days (data from Tables 1 and 2). This indicated that continued containment in the bottles did not, in general, greatly affect PA uptake rates by microbes during the course of our incubations. However, for treatments having relatively low long-term removal rates, the first-day rates tended to be higher than these long-term estimates (Tables 1 and 2). This trend was not evident in the treatments showing fast removal rates.

In the above experiments, we did not evaluate compositional changes in the bacterial communities nor changes in uptake activity during the interval between sample collection and initiation of uptake experiments, when the samples were stored in insulated carboys for up to 24 h. Bacterial abundance did not significantly change during a 26-h containment experiment, but if dramatic changes in uptake activity occurred during this period, our flux (F) values would be biased. Even if samples were fortified with amino acids immediately after sampling, the 24-h interval needed to provide measurable changes could cause some error in estimating *in situ* flux rates by this method. Short-term containment effects were examined using radiolabeled amino acids. Although the uptake rate of labeled compounds was significantly lower at 4 h (28 ng C $L^{-1}h^{-1}$) than at 20 or 40 h (53 and 48 ng C $L^{-1}h^{-1}$) of incubation, changes were small relative to the ranges in F that we observed on different days (Tables 1 and 2). The changes were more pronounced in the tracer experiment suggesting possible decreases in natural substrate concentrations with increased incubation time. These results are consistent with the observation, mentioned above, that 1-day rates tended to be higher than the long-term rates when PA levels and removal rates were low (zero amino acid additions) but were similar when PA levels were high (0.6 to 1.4 $\mu$M) (Tables 1 and 2). Thus containment effects on amino acid uptake rates appeared to be minimized by addition of near-saturation levels of amino acid substrate.

The examination of substrate-limitation vs. grazer control as the first-order process controlling bacteria populations should not be affected as much by containment effects as would the absolute flux rates. This is because the limitation factor should not change unless the activities of the bacterial grazers change greatly with time as a result of containment. If this happens, a constant substrate removal rate with time, as we usually observed, would not be expected.

Contamination with bacteria not native to the samples could occur during sample collection or during the subsequent amino acid addition or subsampling steps. For example, in preliminary experiments (not reported here) we found that selective prefiltrations, to remove different-sized organisms, resulted in more variable and unpredictable

results than we observed with unfiltered samples, possibly because of bacterial or amino acid contamination during filtration or treatment. In the present study we achieved good precision by minimizing pretreatments of the samples. With only a few exceptions, results from duplicate test bottles were remarkably similar. Although care was taken to maintain needle cleanliness during amino acid additions and PA sampling, we did not use sterile techniques. This practice potentially could have introduced non-native microbes (as apparently occurred in some of the autoclaved lake samples, Fig. 2). However, the constant removal rates over time that we observed for the majority of samples during several days of incubations and the good agreement between most duplicate samples suggests that the experimental system was relatively "robust" and insensitive to bottle effects or bacterial contamination during the sampling routine for non-autoclaved samples.

Splitting water among containers must also be considered as a potential source of error when using small containers for substrate enrichment experiments. This potential subsampling problem could be important, for example, if "meso" zooplankton have a major role in the cycling of free amino acids in the water, but would probably not be a major factor if components of the microbial food web (bacteria, small algae, protozoans) are the principle components controlling amino acid cycling. The close agreement among replicate samples, the similarity in uptake rates with the different-sized containers, and the low-level effects of zooplankton on amino acid levels or uptake rates, over the first several days of incubation, suggest that subsampling was not a major problem in these experiments.

### Seasonal Flux of Amino Acids Added to Lake Michigan Samples

Although, as mentioned above, our uptake data may have been affected by containment effects between sample collection and incubation, we did observe differences in PA levels and uptake rates in samples collected on the different dates. PA removal rates were not examined on enough dates to confidently differentiate actual seasonal changes from day-to-day variations but, as would be predicted from temperature differences, higher rates were observed in the summer than in the spring. Both ambient PA levels and PA removal rates were higher during this period, particularly in the 31 July 1984 sample that showed far higher removal rates at all levels of PA additions than the other samples. This response may have reflected an "event" rather than a seasonal trend; the sample taken during the same period in 1985 did not exhibit extraordinary high initial PA removal rates. A peak in thymidine uptake rate was also observed on 31 July 1984 (Scavia *et al.* 1986). The high PA removal rates in these samples, relative to those observed on other dates, suggests the presence of a relatively active bacterial population.

Results from adding different levels of amino acids indicated that our substrate-addition approach, with chemical measurement of uptake rate, may offer a reasonable estimate of $V_{max}$ for amino acids. This estimate is conservative, of course, because it does not consider the uptake of amino acids being supplied by natural processes to the bacteria. The small differences in uptake rates among portions of samples treated with relatively high but different levels of amino acids (e.g., 0.6 vs. 1.2$\mu$M; Table 2) indicated that the substrate uptake sites were approaching saturation at approximately 1 $\mu$M. By this approach, $V_{max}$ varied from $<1$ nmole PA $L^{-1}h^{-1}$ in the spring samples up to 8–9 nmole PA $L^{-1}h^{-1}$ in the 31 July 1984 sample (Table 2). The spring values compare favorably with a $V_{max}$ of about 0.4 nmole amino acids $L^{-1}h^{-1}$ obtained, using tracer kinetics and a radioactive amino acid suite, on Lake Michigan samples collected in June 1983 and October 1985 (Scavia and Laird, unpublished data). Except for the relatively high values for $V_{max}$ observed for 31 July 1984, our $V_{max}$ values (Table 1) agree well with those observed in the Gulf of Mexico (0.003 to 1.7 nmole amino acids $L^{-1}h^{-1}$; Ferguson and Sunda 1984).

Amino acid carbon uptake rates in samples fortified with amino acids ranged from 10 ng C $L^{-1}h^{-1}$ in some June samples to ca. 600 ng C $L^{-1}h^{-1}$ in the 31 July 1984 sample fortified with 0.64 $\mu$M amino acids (Table 2). These rates resemble amino acid assimilation rates in Lake Almind, Denmark (200 to 750 ng C $L^{-1}h^{-1}$; Jorgensen 1984) and natural fluxes for dissolved organic solutes in estuarine and ocean waters (ca. 4 to 200 ng $L^{-1}h^{-1}$; Wright 1984) but were lower than amino acid $V_{max}$ values observed in the eutrophic Upper Klamath Lake, Oregon (600–6,000 ng C $L^{-1}h^{-1}$; Burnison and Morita 1974). Our rates are also lower than uptake rates estimated from concentration differences in sequential sampling from given stations (1,000 to 10,000 ng C $L^{-1}h^{-1}$ as summarized by Wright 1984).

Primary production rates, determined by $^{14}C$

uptake, in Lake Michigan water sampled from the same sites in 1984 ranged from ca. 1,000 to 4,000 ng C $L^{-1}h^{-1}$ (G.L. Fahnenstiel and D. Scavia, unpublished data). Although the comparison may not be valid, our values of amino acid carbon flux, F, cover the approximate range that would be expected for bacteria production if 1 to 20% of the primary production (= 10 to 800 ng C $L^{-1}h^{-1}$) is released as dissolved labile material (Laird *et al.* 1986) and subsequently used by the heterotrophs. However, on 31 July 1984 our F values (335 to 608 ng C $L^{-1}h^{-1}$) constituted a much higher fraction of primary production (960 ng C $L^{-1}h^{-1}$; Scavia *et al.* 1986) than was true on other sampling dates. Similar high results were observed for bacterial production rates as measured by thymidine uptake (Scavia *et al.* 1986). This implies that bacteria production was not directly coupled with primary production on this sampling date.

Our F values were generally lower than those calculated from bacterial growth rates of $0.3 \times 10^8$ to $2 \times 10^8$ cells $L^{-1}h^{-1}$ over the same season (Scavia *et al.* 1986). If a typical bacteria cell is assumed to have $9.0 \times 10^{-9}$ μg C ($1.21 \times 10^{-7}$ μg C $um^{-3}$, Watson *et al.* 1977; 0.074 $\mu m^3$ $cell^{-1}$, Scavia *et al.* 1986) and if bacteria are assumed to have growth efficiencies of about 50% (Cole *et al.* 1982, 1984), this represents a bacterial carbon production rate of 500 to 3,360 ng C $L^{-1}h^{-1}$, as compared to our calculated values of 1 to 600 ng C $L^{-1}h^{-1}$ (Tables 1 and 2). Possible reasons for this apparent discrepancy are: (1) the bacteria could be growing primarily on substrates other than amino acids (Jorgensen 1984) and not be physiologically equipped to adapt to added amino acid substrates as their sole carbon source (if this is true, amino acid uptake rates cannot be extrapolated to total carbon flux, but would simply reflect potential amino acid uptake rates), (2) our estimate of net uptake of added amino acids could underestimate total uptake of labile organic compounds if natural input rates for these compounds were high relative to our amino acid additions, and (3) the conversion factors used to convert thymidine incorporation to carbon flux may be in error. Problems associated with these various conversion factors have been examined in detail (Scavia *et al.* 1986).

### Bacterial Community Response: Substrate Limitation Vs. Grazer Control

In a conceptual model, Wright (1984) proposed that one of three states of idealized steady-state bacterioplankton communities could be feasible based on the effects of substrate limitation, grazing, and dormancy on bacteria populations. These were: (1) active, grazer-controlled community; (2) active, substrate-limited community; or (3) dormant, substrate-limited community. In this model, dormancy sets the lower limit on the number of bacteria in the system, whereas grazers set the upper limit on the bacterial numbers. The second state is probably only transient in nature because new consumers of bacteria would be expected to develop in response to this available food supply. Although bacterial numbers can remain within a rather narrow range among these different communities, a large range of heterotrophic activity is thought to exist among them. Dormancy favors the presence of inactive cells whereas grazing favors active cells (Wright 1984, Wright and Coffin 1984b).

In our experiments, PA removal rates increased with increased concentrations of added amino acids until uptake rates reached a plateau (Table 1; Fig. 4). The agreement of this pattern with that expected for Michaelis-Menton uptake kinetics (e.g., Wright and Hobbie 1966) suggests that bacterial growth rates were substrate-limited in our samples. However bacterial abundance (and corresponding total-population uptake activity) are potentially controlled not only by growth rates but also by grazing pressure on the bacteria.

Our kinetic examination of PA-uptake patterns provides insight about these factors that control abundances of active bacteria. For example, uptake results with microbes from the aged *Daphnia* culture resembled those expected from a substrate-limited community without grazers. Substrate removal rates increased rapidly with time of incubation and were proportional to the amount of substrate added (Fig. 1b). In agreement with results reported for estuarine waters (Vaccaro 1969; Hollibaugh 1979), similar non-linear patterns were observed after substrate additions for the Lake Michigan samples collected on 27 February 1984 (data not shown) and 31 July 1985 (Fig. 2). In contrast, other lake samples we examined did not exhibit the pattern expected of populations controlled in size only by substrate limitation. The rates were constant over time, within the limitations of the chemical measurements, i.e., the linear relationships between PA and time were highly significant (Tables 1 and 2). The linear relationship of PA concentration vs. time implies either that amino acids did not provide the nutrition needed for substrate-limited bacterial growth or that uptake activity of the total bacterial population

activity was controlled by factors other than substrate supply, e.g., grazer control of population size (Wright and Coffin 1984a).

Bacterial abundance is relatively invarient with season in Lake Michigan (Scavia *et al.* 1986), but almost no information is available on bacterial grazers in the Great Lakes. However, recent evidence on micro food webs in Lakes Superior (Fahnenstiel *et al.* 1986) and Ontario (Caron *et al.* 1985) and in marine systems (Davis and Sieburth 1982, Sherr and Sherr 1984, Fenchel 1982, Sieburth 1984, Fuhrman and McManus 1984, Wright and Coffin 1984a) suggests the importance of microzooplankton (mainly flagellates) in controlling microbe populations. Although the process has not been studied in the Great Lakes, these protozoans may in turn be consumed by relatively large zooplankton and thereby contribute to the transfer of carbon up the food chain. Some "large" zooplankton (e.g., cladocerans) can filter bacteria but they probably do not consume enough to significantly affect bacterial populations (Porter 1984, Pedros-Alio and Brock 1982). In agreement with this thesis, increasing the zooplankton levels tenfold above natural levels in one experiment had only a minimal immediate effect on amino acid concentrations or removal rates during the first few days after substrate addition, but appeared to substantially enhance amino acid removal rates several days after the experiment was begun (Fig. 5). This is consistent with the micro grazer-control scenario, because removal of the bacterial grazers by zooplankton over a period of time could feasibly lower the grazing pressure on bacteria and switch the primary factor controlling bacterial population from grazers to substrate limitation. Additional substrate addition studies, with experimental manipulations to remove or evaluate predator effects (e.g., Wright and Coffin 1984a), are needed to further elucidate the importance of predators relative to other factors in controlling bacterial abundances in lakes.

In conclusion, amino acid substrate addition experiments offer insights about potential rates of carbon flux from the dissolved pool to bacteria in lake water. Containment appears to have little effect on PA uptake rates when Lake Michigan samples are supplemented with amino acids at concentrations high enough to saturate bacterial uptake sites. Conservative chemical estimates of $V_{max}$ for amino acids in Lake Michigan samples ranged from ca. 1 nmole PA $L^{-1}h^{-1}$ in the spring up to >7 nmole PA $L^{-1}h^{-1}$ on one sampling date (31 July 1984). Primary amine uptake rates were higher in samples with elevated (>0.14 $\mu$M PA $L^{-1}$) ambient PA levels than in samples containing only background levels of PA. Measured flux rates for added amino acids were similar in magnitude to potential rates of carbon release from phytoplankton, if 1 to 20% of photosynthesized carbon is assumed to be excreted as labile compounds. The hyperbolic shape of the curve of PA removal rate vs. concentration of added amino acids agreed with Michaelis-Menton kinetics and suggested that bacterial growth rates in Lake Michigan were substrate dependent. However, kinetic studies of PA concentration decreases with time over several days suggested that bacterial abundance, as reflected by PA uptake activity, was usually not controlled by carbon (at least amino acid) limitation alone in Lake Michigan. The linear uptake patterns over time that were often observed were instead consistent with patterns expected for grazer-controlled populations or for populations limited by nutritional factors not provided by amino acids.

## ACKNOWLEDGMENTS

We are grateful to G.L. Fahnenstiel, J.E. Hobbie, S.J. Tarapchak, H.A. Vanderploeg, and two unidentified referees for their suggestions and constructive criticisms of the manuscript.

## REFERENCES

Burney, C. M., Johnson, K. M., and Sieburth, J. McN. 1981. Diel flux of dissolved carbohydrates in salt marsh and a simulated ecosystem. *Mar. Biol.* 63:175–187.

Burnison, B. K., and Morita, R. Y. 1974. Heterotrophic potential for amino acid uptake in a naturally eutrophic lake. *Appl. Microbiol.* 27:488–495.

Caron, D. A., Pick, F. R., and Lean, D. R. S. 1985. Chroococcoid cyanobacteria in Lake Ontario: Vertical and seasonal distribution during 1982. *J. Phycol.* 21: 171–175.

Cole, J. J., Likens, G. E., and Strayer, D. L. 1982. Photosynthetically produced dissolved organic carbon: an important source for planktonic bacteria. *Limnol. Oceanogr.* 27:1080–1090.

———, Likens, G. E., and Hobbie, J. E. 1984. Decomposition of planktonic algae in an oligotrophic lake. *Oikos.* 42:257–266.

Crawford, C. C., Hobbie, J. E., and Webb, K. L. 1974. The utilization of dissolved free amino acids by estuarine microorganisms. *Ecology* 55:551–563.

Davis, P. G., and Sieburth, J. McN. 1982. Differentiation of the photosynthetic and heterotrophic popula-

tions of nanoplankton by epifluorescence microscopy. *Ann. Inst. Oceanogr.* (Paris) 58(Suppl): 249–260.

Dawson, R., and Gocke, R. 1978. Heterotrophic activity in comparison to the free amino acid concentrations in Baltic Sea water samples. *Oceanol. Acta* 1:45–54.

Ducklow, H. W., and Hill, S. M. 1985. The growth of heterotrophic bacteria in the surface waters of warm core rings. *Limnol. Oceanogr.* 30:239–259.

Fahnenstiel, G. L., Sicko-Goad, L., Scavia, D., and Stoermer, E. F. 1986. Importance of picoplankton in Lake Superior. *Can. J. Fish. Aquat. Sci.* 43:235–240.

Fenchel, T. 1982. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. *Mar. Ecol. Progr. Ser.* 8:225–231.

Ferguson, R. L., and Sunda, W. G. 1984. Utilization of amino acids by planktonic marine bacteria: Importance of clean technique and low substrate additions. *Limnol. Oceanogr.* 29:258–274.

_____, Buckley, E. N., and Palumbo, A. V. 1984. Response of marine bacterioplankton to differential filtration and confinement. *Appl. Environ. Microbiol.* 47:49–55.

Fuhrman, J. A., and Azam, F. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar. Biol.* 6:109–120.

_____, and McManus, G. B. 1984. Do bacteria-sized marine eukaryotes consume significant bacterial production? *Science* 224:1257–1260.

Gardner, W. S. 1978. Microfluorometric method to measure ammonium in natural waters. *Limnol. Oceanogr.* 23:1069–1072.

_____, and Miller, W. H., III. 1980. Reverse-phase liquid chromatographic analysis of amino acids after reaction with o-phthalaldehyde. *Anal. Biochem.* 101:61–65.

_____, and Miller, W. H. III. 1981. Intracellular composition and net release rates of free amino acids in *Daphnia magna*. *Can. J. Fish. Aquat. Sci.* 38:157–162.

_____, and Stephens, J. A. 1978. Stability and composition of terrestrially derived dissolved organic nitrogen in continental shelf waters. *Mar. Chem.* 6:335–342.

_____, and Vanderploeg, H. A. 1982. Micro sample-filtering device for liquid chromatography or flow-injection analysis. *Anal. Chem.* 54:2129–2930.

Hagstrom, A., Larsson, U., Horstedt, P., and Normark, S. 1979. Frequency off dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. *Appl. Environ. Microbiol.* 27:805–812.

Hobbie, J. E., Daley, R. J., and Jasper, S. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33:1225–1228.

Hollibaugh, J. T. 1979. Metabolic adaption in natural bacterial populations supplemented with selected amino acids. *Estuar. Coast. Mar. Sci.* 9L215–230.

Ittekkot, V., Borckman, U., Michaelis, W., and E. T. Degens, W. 1981. Dissolved free and combined carbohydrates during a phytoplankton bloom in the northern North Sea. *Mar. Ecol. Prog. Ser.* 4:299–305.

Jorgensen, N. O. G. 1984. Occurrence and heterotrophic turnover of dissolved free amino acids in the thermally stratified Lake Almind. *Verh. Internat. Verein. Limnol.* 22:785–789.

_____, and Sondergaard, M. 1984. Are dissolved free amino acids free: *Microb. Ecol.* 10:301–316.

Laird, G. A., Scavia, D., and Fahnentiel, G. L. 1986. Algal organic carbon excretion in Lake Michigan. *J. Great Lakes Res.* 12:136–141.

Lee, C., and Bada, J. L. 1975. Amino acids in equatorial Pacific Ocean water. *Earth Planet. Sci. Lett.* 25:61–68.

Lehman, J. T. 1980. Release and cycling of nutrient between planktonic algae and herbivores. *Limnol. Oceanogr.* 25:620–632.

Mopper, K., and Lindroth, P. 1982. Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis. *Limnol. Oceanogr.* 27:336–347.

Moriarty, D. J. W. 1984. Measurements of bacterial growth rates in some marine ecosystems using the incorporation of tritiated thymidine into DNA. In *Heterotrophic Activity in the Sea*, J. E. Hobbie and P. J.leB. Williams (eds.), pp. 217–232. New York: Plenum Press.

Pedros-Alio, C., and Brock, T. D. 1982. Assessing biomass and production of bacteria in eutrophic Lake Mendota, Wisconsin. *Appl. Environ. Microbiol.* 44:203–218.

Porter, K. G. 1984. Natural bacteria as food resources for zooplankton. In *Current Perspectives in Microbial Ecology*, M. J. Klug and C. A. Reddy (eds.), pp. 340–345. Washington, D.C.: American Society for Microbiology.

Scavia, D., Laird, G., and Fahnenstiel, G. L. 1986. Production of planktonic bacteria in Lake Michigan. *Limnol. Oceanogr.* 31:612–626.

Sherr, B. F., and Sherr, E. B. 1984. Role of heterotrophic protoza in carbon and energy flow in aquatic ecosystems. In *Current Perspectives in Microbial Ecology*, M. Klug and C. Reddy (eds.), pp. 412–423. Washington, D.C.: American Society for Microbiology.

Sieburth, J. McN. 1984. Protozoan activity in pelagic marine waters. In *Heterotrophic Activity in the Sea*, J. E. Hobbie and P. J.leB. Williams (eds.), pp. 405–444. New York: Plenum Press.

Vaccaro, R. F. 1969. The response of natural microbial populations in sea water to organic enrichment. *Limnol. Oceanogr.* 14:726–735.

Watson, S. W., Novitsky, T. J., Quinby, H. L., and Valois, F. W. 1977. Determination of bacterial number and biomass in the marine environment. *Appl. Environ. Microbiol.* 33:940–954.

Williams, P. J.LeB., and Gray, R. W. 1970. Heterotrophic utilization of dissolved organic compounds in the sea. II. Observation on the responses of heterotrophic marine populations to abrupt increases in amino acid concentration. *J. Mar. Biol. Assoc. US* 50:871–881.

Wright, R. T. 1984. Dynamics of pools of dissolved organic carbon. In *Heterotrophic Activity in the Sea*, J. E. Hobbie and P. J.leB. Williams (eds.), pp. 12–154. New York: Plenum Press.

_____, and Burnison, B. K. 1979. Heterotrophic activity measured with radiolabelled organic substrates. In *Native Aquatic Bacteria: Enumeration, Activity and Ecology*, J. W. Costerton and R. R. Colwell (eds.), pp. 140–155. Philadelphia: American Society for Testing and Materials, STP 695.

_____, and Coffin, R. B. 1984a. Measuring microzooplankton grazing on planktonic marine bacteria by its impact on bacterial production. *Microb. Ecol.* 10:137–149.

_____, and Coffin, R. B. 1984b. Factors affecting bacterioplankton density and productivity in salt marsh estuaries. In *Current Perspectives in Microbial Ecology*, M. J. Klug and C. A. Reddy (eds.), pp. 485–494. Washington D.C.: Amer. Soc. for Microbiol.

_____, and Hobbie, J. E. 1966. The use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology* 47:447–464.