

Microheterotrophic utilization of dissolved free amino acids in depth profiles of Southern California Borderland basin waters

Amino acids
Microbial activity
Respiration
Vertical profile
Southern California borderland
Acides aminés
Activité microbienne
Respiration
Profil vertical
Southern California borderland

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ABSTRACT

Microheterotrophic utilization of dissolved free amino acids (DFAA) was examined during the spring season in depth profiles of two California Borderland basins, and the effects of added DFAA concentrations on incorporation and respiration rates were studied. Utilization rates were most rapid in the euphotic zone ($0.4-4.0 \text{ nmol l}^{-1} \text{ h}^{-1}$), and decreased considerably in mid-depth basin waters. Elevated utilization rates were generally observed in the deepest basin waters studied, where bacterial numbers also increased over mid-depth values. Euphotic-zone microheterotrophs, which were adapted to relatively high ambient DFAA levels, were insensitive to DFAA additions as high as 15 nmol l^{-1} , but mid-water populations associated with low ambient DFAA concentrations displayed elevated respiration rates, and elevated incorporation rates to a lesser degree, at DFAA additions greater than approximately 2 nmol l^{-1} . Water column microheterotrophic DFAA utilization was estimated to account for 2-11% of euphotic zone primary carbon production in the basins studied.

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RÉSUMÉ

L'utilisation microhétérotrophique des acides aminés libres dans deux bassins de la bordure continentale de Californie

L'utilisation microhétérotrophique des acides aminés libres dissous (DFAA) a été étudiée au printemps à partir de profils verticaux obtenus dans deux bassins de la bordure continentale de Californie. Les effets d'ajouts de DFAA sur les vitesses d'incorporation et sur les taux de respiration ont été examinés. L'utilisation est la plus rapide ($0.4-4.0 \text{ nmol l}^{-1} \text{ h}^{-1}$) dans la couche euphotique; elle diminue considérablement à mi-profondeur du bassin. L'utilisation est généralement élevée dans le fond du bassin où les bactéries sont plus abondantes que dans les eaux de profondeur moyenne. Les organismes microhétérotrophes de la couche euphotique, qui sont adaptés à des niveaux élevés de DFAA, sont insensibles à des ajouts de DFAA jusqu'à 15 nmol l^{-1} . Les populations de la profondeur moyenne, adaptées à des faibles concentrations ambiantes de DFAA, présentent une respiration et, à un moindre degré, une incorporation en élévation après addition de DFAA jusqu'à 2 nmol l^{-1} ou plus. Dans les bassins examinés, l'utilisation microhétérotrophe de DFAA représente 2 à 11% de la production primaire de carbone de la zone euphotique.

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INTRODUCTION

Although considerable research effort has been directed toward the understanding of microbial utilization of dissolved organic carbon (DOC) in marine euphotic

zone waters (Azam *et al.*, 1983; Azam, Ammerman, 1984; Ferguson, Sunda, 1984; Carlucci *et al.*, 1984 and references therein), much less information exists about the same processes in waters below the euphotic zone (Jannasch, Wirsen, 1982; Karl, 1982; Liebezeit *et al.*,

1980). Profiles of dissolved carbohydrates (Liebezeit *et al.*, 1980) and dissolved free and combined amino acids (DFAA and DCAA: Lee, Bada, 1977; Dawson, Gocke, 1978; Liebezeit *et al.*, 1980; Mopper, Lindroth, 1982) have been presented, and transformation rates either measured or inferred (Dawson, Gocke, 1978; Mopper, Lindroth, 1982). More frequently, only heterotrophic uptake has been reported, but without knowledge of ambient substrate levels. Such measurements generate limited useful information because the *in situ* specific activity of the substrate cannot be determined.

Alternate approaches, employed in the absence of ambient substrate determinations, include: 1) assuming that ambient concentrations are constant throughout a depth profile or during a diel study, so that relative uptake values can be directly compared (*cf.* Li, 1984); 2) calculating turnover rates only and not attempting to determine utilization rates (Ferguson, Sunda, 1984); 3) estimating *in situ* substrate concentrations by plotting turnover times against added concentrations (Wright, Hobbie, 1966; Dietz *et al.*, 1977); or 4) saturating the sample with non-radioactive substrate in addition to the radiotracer, so that ambient levels are comparably negligible (*cf.* Jannasch, Wirsen, 1982). The dangers inherent in an assumption of constant concentration of any growth substrate throughout a depth profile or even within a diel study on a single depth are amply demonstrated in the literature, where several-fold changes in concentration are reported over relatively narrow depth ranges (Dawson, Gocke, 1978; Liebezeit *et al.*, 1980; Mopper, Lindroth, 1982; Poulet *et al.*, 1984) and seasonal or diel cycles (Meyer-Reil *et al.*, 1979; Burney *et al.*, 1982; Mopper, Lindroth, 1982; Laane, 1983; Carlucci *et al.*, 1984). Turnover rate measurements based on single concentration additions are useful, but provide no information on the absolute amount of substrate undergoing transformation. Estimates of *in situ* concentrations derived from plots of turnover time vs added substrate have been criticized because only upper limits to *in situ* concentrations can be determined (Laws, 1983). Saturation with substrate is not advisable, if the purpose of the study is to measure *in situ* rates (Bell, 1984; Ferguson, Sunda, 1984), as changes in population utilization can occur quickly with high added concentrations of substrate.

Although the production of $^{14}\text{CO}_2$ from ^{14}C -labeled organic substrates has been routinely used as an estimate of microbial community respiration (*cf.* Dawson, Gocke, 1978; Jannasch, Wirsen, 1982) the production of $^3\text{H}_2\text{O}$ from ^3H -labeled organic substrates has also been employed as a comparable estimate of respiration (Dietz, Albright, 1978; Carlucci *et al.*, 1984, 1985). While some $^3\text{H}_2\text{O}$ production may be the result of metabolic processes other than respiration of ^3H -organics, Kuparinen and Tamminen (1982) found, in a comparison of $^{14}\text{CO}_2$ and $^3\text{H}_2\text{O}$ production from ^{14}C - and ^3H -labeled glucose, that the ^3H -label resulted in a small (10%) but consistently larger estimate of respiration compared to the ^{14}C -label. Since organic compounds can be labeled to a much higher specific activity with ^3H compared to ^{14}C , the use of the ^3H -label is preferable when the addition of very low levels of tracer is desired.

The study of dissolved free amino acid and dissolved free carbohydrate production, utilization and turnover rate has been vigorously pursued in recent years. A major limitation to progress has been the potential artifact created by the possible existence of several discrete pools of varying availability, of any "dissolved" substrate of interest (Gocke *et al.*, 1981). Consequently, measured ambient pools (and substrate specific activity) could differ significantly from "available" ambient pools (and substrate specific activity), and calculated utilization or turnover rates might then be considerably overestimated (Dawson, Gocke, 1978; Gocke *et al.*, 1981).

Early measurements of DFAA were conducted using techniques which required desalting, concentration, and acidification prior to ligand exchange separation (*cf.* Lee, Bada, 1977; Dawson, Gocke, 1978). Such procedures could induce desorption of dissolved amino acids associated with colloids and possibly hydrolyze peptides (Dawson, Gocke, 1978), so that the final measured "free" dissolved amino acids might not be representative of *in situ* DFAA. However, the advent of high pressure liquid chromatographic (HPLC) techniques for amino acid analysis has much reduced this problem, since samples may be processed with minimal preliminary chemical or pH manipulations (Mopper, Lindroth, 1982). Evidence has been presented recently that DFAA as measured by HPLC are indeed dissolved and free, and the levels thus determined are therefore accurate measures of available *in situ* concentrations in natural samples (Jorgensen, Sondergaard, 1984).

The present communication describes studies of water column microheterotrophic DFAA utilization conducted in the California Borderland basins region in the spring seasons of 1981, 1982 and 1983. The two basins studied, the San Pedro Basin (912 m maximum depth) and the Santa Monica Basin (938 m maximum depth), are interconnected, and mid-depth waters for both basins are provided by a sill (737 m depth) on the southeastern wall of the San Pedro Basin (Jackson, 1982). Utilization rates were measured using individual DFAAs and a commercially-prepared mixture, and the effects of increasing concentration of substrate on water column microbial DFAA utilization were studied.

MATERIALS AND METHODS

The study areas were investigated in conjunction with other members of the Food Chain Research Group (FCRG; Scripps Institution of Oceanography) in an ongoing program on plankton dynamics, referred to as the Southern California Bight Study (SCBS). Stations were occupied in the Santa Monica and San Pedro Basins, and above the San Pedro Basin southeast sill, in the spring seasons of 1981, 1982 and 1983. Pertinent information for station locations is presented in Table 1. All glassware was baked before use and all other supplies were acid-cleaned. Plastic gloves were worn during sample handling to eliminate potential sources of contamination. A 5-1 Niskin bottle was sequentially cleaned with non-detergent soap, tap water, 6% HCl, glass-distilled water, and absolute

Table 1
Information pertinent to water column incubations.

| Cruise Location Date | Station location | Tracer (label position) | Concentration added, nmol l ⁻¹ | Water depths incubated, m |
|--|-------------------------|---|---|---|
| SCBS-18 Santa Monica Basin May 1981 | 33°45.5'N 118°47.6'W | leucine (4,5- ³ H) | 2.42 | 5, 14, 30, 50, 100, 250 500, 753, 842 |
| | | glutamic acid (2,3- ³ H) | 7.06 | as above |
| | | amino acid mixture (NET 250) | 5.39 | as above |
| SCBS-20 San Pedro Basin southeast sill March 1982 | 33°22.9'N 118°10.5'W | glutamic acid (3,4- ³ H) | 1.51 | 10, 25, 150 |
| | | | 3.02 | 250, 500, 600, 700 |
| Santa Monica Basin March 1982 | 33°44.9'N 118°49.8'W | glutamic acid (3,4- ³ H) | 1.51 | 10, 50, 150, 250, 500, 600, 700, 800, 835, 885, 895 |
| SCBS-22 San Pedro Basin May 1983 | 33°34.6'N 118°31.6'W | glutamic acid (3,4- ³ H) | 0.76 | 10, 50, 100, 300, 500, 700, 800, 850, 895 |
| | | | 0.15-15.1 | 50, 500 |
| | | (2,3- ³ H) | 1.51 | 10, 100, 300, 500, 700, 800 |
| | | | 4.96 | 10, 100, 300, 500, 700, 800 |

methanol before use. For microheterotrophic utilization of amino acids, water samples were collected from selected depths, dispensed into glass holding receptacles and stored on ice in the dark until used, usually less than 1 h and more typically, 5-10 min. Incubation and processing followed experimental designs described previously (Carlucci *et al.*, 1984; 1985). Briefly, sample aliquots were dispensed into scintillation vials or pyrex tubes. SCBS-18 incubations were performed in duplicate; all others were performed in triplicate. An additional replicate from each depth was mixed with formalin (2%, final concentration) to serve as a control. All samples were then supplemented with selected ³H-amino acids (New England Nuclear, Boston, MA) as indicated in Table 1, and incubated in the dark at *in situ* temperatures for 1-4 h, depending on the depth of sample origin and expected activity.

Following incubation, samples were processed as described by Carlucci *et al.* (1984; 1985), to determine microheterotrophic utilization of DFAA. Utilization is here defined as the sum of incorporation in the particulate phase plus respiration. Incorporation of the tracer amino acid into the particulate phase was measured by filtering sample aliquots through 0.3 µm millipore filters, rinsing the filter edges carefully with filtered seawater,

and counting the radioactivity on the filter by routine liquid scintillation counting (LSC) techniques. We have found previously that 0.3 µm and 0.22 µm millipore filters were not significantly different with respect to bacterial numbers retained. Filter rate was more rapid under low vacuum for the 0.3 µm filters, so that microbial populations were probably subjected to less stress. Respiration of the tracer was estimated by measuring the production of ³H₂O (see Carlucci *et al.*, 1984 and references therein). The ambient concentrations of dissolved free amino acids (DFAA) were determined by HPLC (Jones *et al.*, 1981; Henrichs, Williams, 1985; Carlucci *et al.*, 1984) on samples which had been filtered immediately upon recovery and frozen until analysis in the shore-based laboratory (Mopper, Lindroth, 1982). Bacterial cell numbers were determined by acridine orange epifluorescence microscopy (Hobbie *et al.*, 1977; Carlucci *et al.*, 1984).

RESULTS AND DISCUSSION

We examined the effect of increasing concentration on microbial utilization of glutamic acid (Tab. 2), using euphotic zone waters (50 m depth) of relatively high ambient total dissolved free amino acid (TFAA) concen-

Table 2

Effect of glutamic acid concentration on incorporation, respiration and percent respiration in waters from 50 and 500 m depth. CV, coefficient of variation for 3 replicates of 4 concentrations; $r > .73$ significant at $\rho = .01$; $r =$ Spearman rank correlation coefficient for amino acid concentration versus incorporation, respiration, or percent respiration.

| Depth, m | Glutamic acid nmol l ⁻¹ | | Incorporation nmol l ⁻¹ h ⁻¹ ($\times 10^{-3}$) | Respiration nmol l ⁻¹ h ⁻¹ ($\times 10^{-3}$) | Percent respiration |
|----------|---------------------------------------|-------|---|---|-------------------------|
| | Ambient | Added | | | |
| 50 | 4.3 | 0.15 | 395.2 | 83.7 | 17.5 |
| | | 1.51 | 299.3 | 45.1 | 13.1 |
| | | 7.56 | 322.5 | 62.4 | 16.2 |
| | | 15.12 | 330.3 | 70.2 | 17.5 |
| | | | | CV = 17.0 $r = -.41$ | CV = 14.2 $r = -.06$ |
| 500 | 0.4 | 0.15 | 7.0 | 1.3 | 15.7 |
| | | 1.51 | 7.4 | 1.2 | 14.0 |
| | | 7.56 | 10.0 | 3.8 | 27.5 |
| | | 15.12 | 10.6 | 4.2 | 28.4 |
| | | | | CV = 18.0 $r = .74$ | CV = 37.0 $r = .78$ |

Table 3

Comparison of ³H-glutamic acid utilization with tritium labels on (2,3-) and (3,4-) carbon atoms. CV, coefficient of variation, $n = 3$.

| Depth, m | Ambient Glutamic Acid, nmol l ⁻¹ h ⁻¹ | Incorporation nmol l ⁻¹ h ⁻¹ ($\times 10^{-3}$) | | Respiration nmol l ⁻¹ h ⁻¹ ($\times 10^{-3}$) | | Percent Respiration | |
|----------|--|---|----------------|---|---------------|------------------------|----------------|
| | | (2,3-) | (3,4-) | (2,3-) | (3,4-) | (2,3-) | (3,4-) |
| | | [CV] | [CV] | [CV] | [CV] | [CV] | [CV] |
| 10 | 4.1 | 136.6 [3.1] | 175.0 [2.8] | 30.6 [14.0] | 22.2 [6.8] | 18.3 [14.2] | 11.3 [8.4] |
| 100 | 0.7 | 44.4 [4.9] | 44.1 [6.6] | 7.5 [13.9] | 4.3 [12.6] | 14.5 [15.9] | 8.9 [15.7] |
| 300 | 0.6 | 10.1 [9.9] | 15.9 [10.9] | 3.1 [12.6] | 2.5 [7.7] | 23.5 [16.5] | 13.6 [10.3] |
| 500 | 0.4 | 7.1 [19.3] | 7.5 [15.0] | 2.7 [16.4] | 1.2 [22.9] | 27.6 [14.0] | 13.8 [31.5] |
| 700 | 1.2 | 3.6 [15.5] | 6.6 [3.8] | 1.7 [44.4] | 1.2 [13.2] | 32.1 [18.7] | 15.4 [14.1] |
| 850 | 0.4 | 5.6 [41.4] | 5.7 [30.1] | 0.9 [59.7] | 1.3 [30.3] | 13.8 [39.6] | 18.6 [15.2] |

tration (TFAA = 23.2 nmol l⁻¹) and deeper waters (500 m depth) with lower ambient TFAA concentration (TFAA = 4.9 nmol l⁻¹). Results in Table 2 are presented as means of 3 replicates for each concentration employed, and coefficients of variation (standard deviation divided by mean) are for all data from each depth ($n = 12$). It is apparent that in microbial populations normally exposed to relatively high ambient TFAA concentrations (50 m), there is no significant correlation (level of significance $\rho > .10$; Spearman rank difference test, Conover, 1971) between additions of 0.15-15.12 nmol l⁻¹ and incorporation, respiration or percent respiration of a selected DFAA.

However, in populations normally exposed to lower ambient DFAA concentrations (500 m), increasing additions of glutamic acid were significantly correlated ($\rho = .01$, Conover, 1971) with increases in all three parameters (Tab. 2). When data were subjected to a Mann-Whitney U test (Tate, Clelland, 1957) the results obtained at 500 m for 0.15 and 1.51 nmol l⁻¹ additions were not significantly different from each other, but were different from the results at 7.56 and 15.12 nmol l⁻¹ additions. Results for all concentrations examined in 50 m waters did not differ significantly. Consequently, we have concluded that DFAA substrate additions in waters from below the euphotic zone must be kept below 2 nmol l⁻¹, and preferably below 1 nmol l⁻¹ to simulate "natural" rates.

It should be emphasized here that our results in Table 2 pertain only to additions where the selected DFAA (in this case, glutamic acid) is also present in natural samples. Glutamic acid was measurable at all depths in all profiles presented subsequently. However, several DFAA were often absent below the euphotic zone. Problems associated with such distributions will be discussed subsequently.

We also examined the effect of isotopic label position on patterns of glutamic acid incorporation and respiration in microbial populations from several depths in the San Pedro Basin (Tab. 3). Although calculated utilization rates were similar for the two labels at all depths examined, the percent of utilization which is represented by respiration was 6-16% greater using the 2,3-³H label, except for the deepest sample studied (850 m), where the difference in percent respiration of the two labels was not significant (Mann-Whitney U test). Incorporation and respiration of the two labels were highly correlated (incorporation (I): $r = .996$, $I_{3,4} = 1.27I_{2,3} - 1.43$ and Respiration (R): $r = .997$, $R_{3,4} = 0.72R_{2,3} - 0.16$; $\rho = .01$ for both), although incorporation via the 2,3-³H label consistently underestimated incorporation via the 3,4-³H label; the reverse was observed for respiration.

As shown previously (Tab. 2), glutamic acid additions below approximately 2 nmol l⁻¹ to 500-m waters appa-

rently did not stimulate utilization, whereas addition of 7 nmol l^{-1} and greater appeared to stimulate respiration rates particularly, and incorporation rates to a lesser extent. We have no information on the addition range between $2\text{--}7 \text{ nmol l}^{-1}$, and must therefore regard additions greater than 2 nmol l^{-1} with considerable caution. Since the $2,3\text{-}^3\text{H}$ labeled glutamic acid is commercially prepared at a much lower specific activity than the $3,4\text{-}^3\text{H}$ labeled glutamic acid (18.1 vs. $44.1 \text{ Ci mmol}^{-1}$, respectively, in this case), a higher concentration addition was necessary to achieve similar isotope additions (4.96 vs. 1.51 nmol l^{-1} , respectively). We have apparently exceeded the maximum concentration threshold in the deeper waters with the $2,3\text{-}^3\text{H}$ label, and this is in part responsible for the greater percent respiration measured there. Some portion of the consistent difference in utilization of the two labels may also be due to alternate metabolic pathways employed in the catabolism of hydrogen protons associated with the 2- and 4-position carbon atoms of glutamic acid.

Bacterial cell numbers were generally higher for the Santa Monica Basin and the San Pedro Basin sill studied in 1982 (SCBS-20) than for either basin in 1981 or 1983 (SCBS-18 and SCBS-22; see Fig. 1a). A substantial increase in cell numbers in the bottom 100 m observed in the Santa Monica Basin in 1982 was not apparent in 1981. Only a slight increase in bottom water cell numbers was observed in the San Pedro Basin in 1983 (Fig. 1a). TFAA concentrations decreased substantially below the euphotic zone in all profiles (Fig. 1b). A secondary increase was observed in bottom waters of both basins, and a large increase over mid-water concentrations was measured in the 700 m sample in San Pedro Basin. The 700-m spike may be due to input of dissolved organic matter at sill depth, as increases were also measured in other dissolved and particulate constituents (data not presented). Distributions of bacterial numbers and TFAA concentrations were well-correlated ($r = 0.87$, $n = 34$, $p = .01$, linear least squares regression).

Based on the results summarized in Tables 1 and 2, we present our DFAA utilization profiles in two groups. The first group ("low" substrate additions) includes glutamic acid utilization profiles for the Santa Monica Basin (1982; SCBS-20) and the San Pedro Basin (1983; SCBS-22), and for the San Pedro southeast sill (1982; SCBS-20). Since additions of 3 nmol l^{-1} were employed for 250-700 m waters for the sill profile, it is, by our classification, intermediate to our "low" and "high" additions. The second group ("high" substrate additions) consists of all Santa Monica Basin data from SCBS-18 (1981). Glutamic acid additions of 7.1 nmol l^{-1} were used throughout the SCBS-18 profile. Leucine was added at 2.4 nmol l^{-1} , but ambient leucine levels were below detection ($<0.1 \text{ nmol l}^{-1}$) at greater than 30 m depth, reappearing only in the deepest sample. The commercial amino acid mixture employed contained 15 amino acids, of which at least seven were below detection limits for most of the water column.

Utilization rates are presented on a log scale because a several orders of magnitude decrease in rates was measured in the depth profiles (Fig. 2). Noting the difference in the rate axes for the two data sets, it is

evident that all populations utilized DFAA more slowly in the 1982 and 1983 profiles (Fig. 2a) than in the 1981 profiles (Fig. 2b). Mid-water utilization rates in the Santa Monica Basin in 1982 were considerably lower than rates measured at the same time at the San Pedro sill or in the San Pedro Basin in 1983 (Fig. 2a).

While the differences between the Santa Monica Basin in 1982 and the San Pedro Basin in 1983 (Fig. 2a), and the Santa Monica Basin in 1981 (Fig. 2b) could be regarded as real differences based on utilization profiles, the percent respiration for the two data sets suggests that the 1981 data are influenced by substrate concentration effects (Fig. 3). All determinations between the surface and 30 m depth range from 10-38% respiration, and are generally highest in the shallowest waters. Below 30 m depth, the two data sets

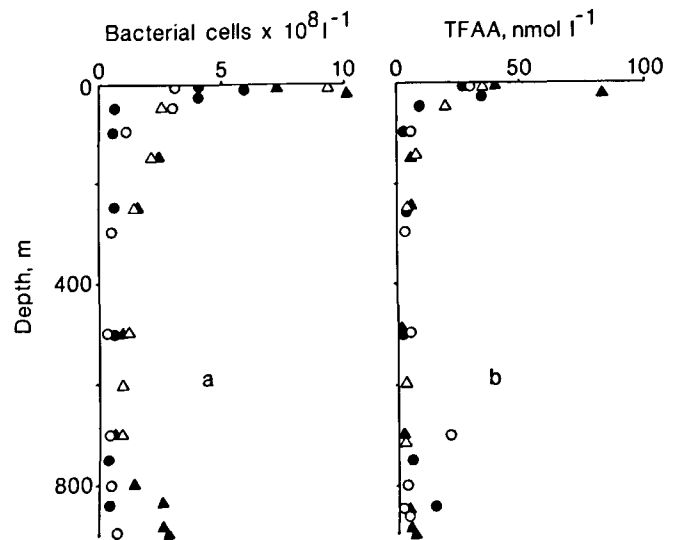


Figure 1
Bacterial numbers as determined by epifluorescence microscopy: a) SCBS-18 (1981), Santa Monica Basin (●); SCBS-20 (1982), San Pedro sill (△), Santa Monica Basin (▲); SCBS-22 (1983), San Pedro Basin (○). b) Total dissolved free amino acids (TFAA), symbols as in Figure 1a.

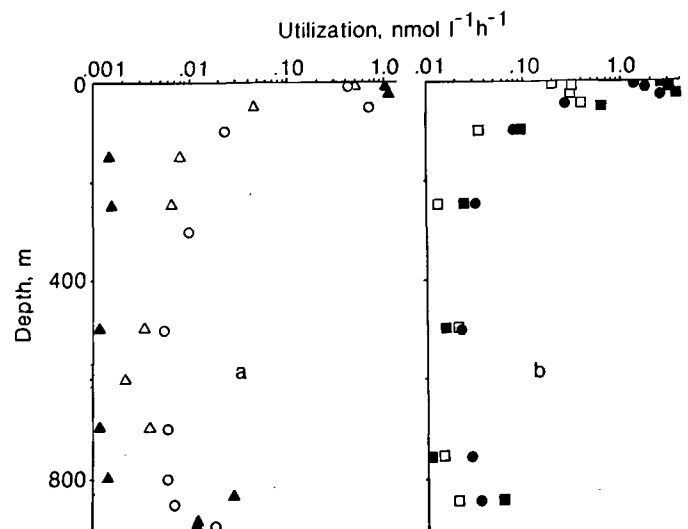


Figure 2
Dissolved free amino acid (DFAA) utilization: a) SCBS-20 (1982), San Pedro sill (△); Santa Monica Basin (▲); SCBS-22 (1983), San Pedro Basin (○), all data for glutamic acid. b) SCBS-18 (1981), Santa Monica Basin, glutamic acid (●); leucine (□); amino acid mixture (■).

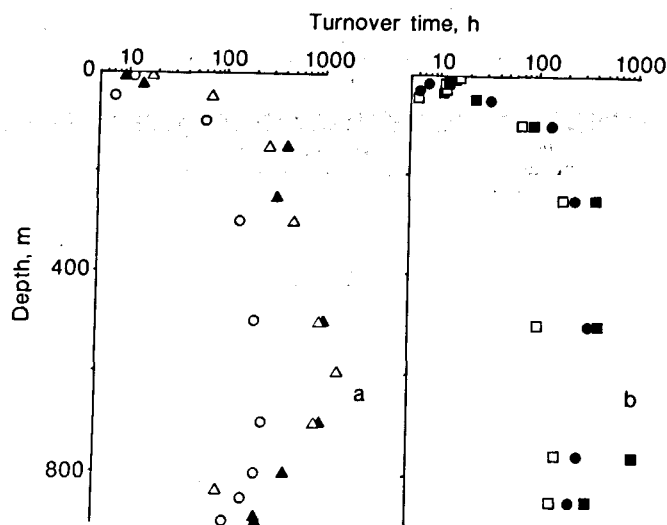


Figure 3
Percent of utilized amino acid which is respired. All symbols as in Figure 2.

diverge (Fig. 3 *a* and *b*). Percent respiration decreased below 100 m depth, and remained almost exclusively below 20% except for the deepest samples, in the "low" substrate addition profiles (Fig. 3 *a*). Percent respiration for the "high" substrate addition profiles was lowest using glutamic acid (which was measurable throughout the profile), but was generally above 40% in all deep waters. However, the percent respiration of leucine increased to as high as 87% in deep waters, where ambient levels of leucine were below detection. The amino acid mixture gave percent respirations as high as 93% at depths where many of the amino acids in the mixture were undetectable in ambient waters (Fig. 3 *b*). While some portion of the differences in the two data sets may be attributed to natural variability, we conclude that such extreme differences as we measured must also be in part the result of stimulation of populations with substrate additions far exceeding *in situ* concentrations, since we have observed that similar additions (Tab. 2) can stimulate respiration considerably.

Calculated utilization per bacterial cell is also presented on a log scale (Fig. 4). It is apparent here that the San Pedro sill waters are more similar to the San Pedro Basin waters studied a year later than to those studied at the same time in Santa Monica Basin. Again, the difference in scale of the rate axes should be noted. It is interesting that the calculated rate per cell is at least an order of magnitude higher in the euphotic zone than in deeper waters. Bacterial doubling times are reported to decrease by four to six-fold over the depth range we examined (Carlucci, Williams, 1978), so we can expect that carbon utilization rates should decrease similarly. An additional factor to consider is that in the "low" addition profiles (Fig. 2 *a*, 3 *a*, 4 *a*), percent respiration decreased considerably at depths below the euphotic zone (Fig. 3 *a*). Consequently, bacterial cells in the mid-waters utilize the lower levels of DOC more efficiently than do their euphotic zone counterparts accustomed to relatively richer waters. This is probably due to high substrate affinities in the mid-water populations. The calculated utilization per cell in the euphotic zone may

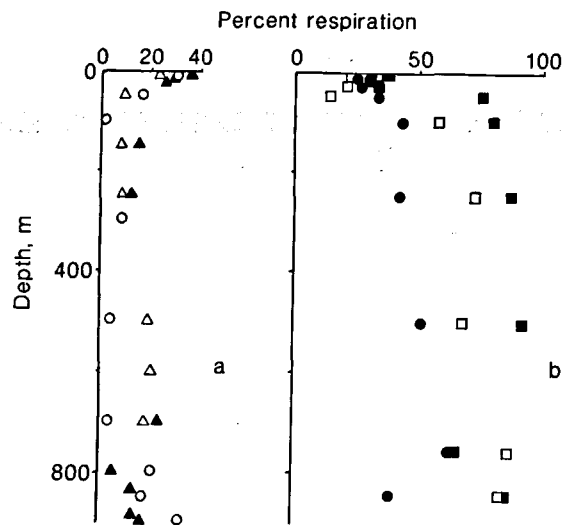


Figure 4
DFAA utilization per bacterial cell. All symbols as in Figure 2.

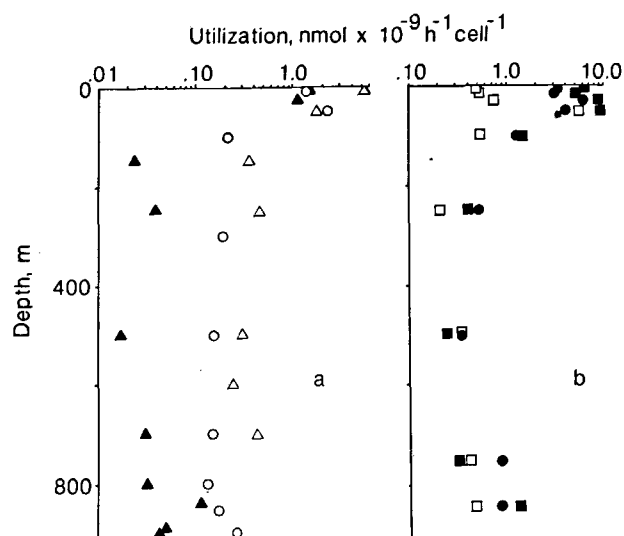


Figure 5
Turnover time. All symbols as in Figure 2.

also be biased to some unknown degree if non-bacterial (microalgal, for instance) utilization of DFAA occurred. Other investigators indicate however, that a large majority of DFAA utilization occurs in the smallest size fraction, which is considered to be dominated by bacterial-activities (*cf.* Palumbo *et al.*, 1983).

Turnover times (*T*) for all substrates used are presented in Figure 5. *T*'s were shortest in subsurface euphotic zone waters. Longest *T*'s were calculated for the mid-water depths, with *T*'s in the deepest waters generally shorter than at mid-depth. Turnover times cannot be as readily grouped as utilization data, because there are no distinctive differences in results among the various treatments. Since *T* is calculated for total (ambient + added) substrate, the cases where added levels greatly exceeded ambient levels cannot be easily interpreted, as calculated *T* may not be indicative of *in situ* processes. Our turnover time estimates are within the ranges given for euphotic zone DFAA pools (Williams *et al.*, 1976; Ferguson, Sunda, 1984; Carlucci *et al.*, 1984) and for euphotic zone and mid-water dissolved carbohydrate pools (Liebezeit *et al.*, 1980).

Table 4
Euphotic zone particulate organic carbon and nitrogen, chlorophyll *a* and of primary carbon production, for Santa Monica and San Pedro Basins measured concurrently with amino acid utilization studies. Numbers in parentheses are respective euphotic zone depths (m).

| Cruise Location Date | Particulate organic carbon, mg m ⁻² | Particulate organic nitrogen, mg m ⁻² | Chlorophyll <i>a</i> mg m ⁻² | Primary carbon production mg m ⁻² day ⁻¹ |
|--|--|--|--|--|
| SCBS-18 Santa Monica Basin May 1981 | 4 400 | 680 | 30 | 1 200 (45) |
| SCBS-20 Santa Monica Basin March 1982 | 4 600 | 810 | 39 | 1 100 (36) |
| SCBS-22 San Pedro Basin May 1983 | 2 700 | 410 | 16 | 570 (51) |

A summary of euphotic zone particulate carbon and nitrogen, chlorophyll *a* and primary carbon production for the three basin studies is presented in Table 4 (unpublished data of R. W. Eppley and other FCRG members). Similar information was not collected for the San Pedro Basin sill station. Parameters for the Santa Monica Basin varied by less than 20% between the 1981 and 1982 studies. The 1983 San Pedro euphotic zone was found to be considerably lower in particulate carbon and nitrogen and chlorophyll *a*, and primary carbon production was approximately half that measured previously in the Santa Monica Basin. However, the 1983 San Pedro study occurred during a period of warm water and low productivity ("el Niño" event [R. W. Eppley, pers. comm.]) so the observed differences in the two closely-associated basins is reasonable.

Phytoplankton are considered to be a major source of labile DOC in the euphotic zone, in part by the process of exudation (*cf.* Hammer, Brockmann, 1983) and by zooplankton grazing (Eppley *et al.*, 1981). Published estimates of the percentage of photosynthetically-fixed carbon transferred to the labile DOC pool range from 10-50% (*cf.* Azam *et al.*, 1984; Carlucci *et al.*, 1984 and references therein). We have estimated the relative importance of TFAA utilization in DOC cycling, using the information presented in Table 5. Depth-weighted mean turnover times were calculated for each profile; for SCBS-18, the turnover times determined for all three substrates were averaged. TFAA concentrations were integrated over the depth of each profile, and converted to carbon as described previously (Carlucci *et al.*, 1984). The amount of carbon utilized as TFAA in each entire water column profile was then compared to the amount of carbon fixed in the euphotic zone of the profile. Since the mean turnover times in Table 5 are depth-weighted, the SCBS-18 data in particular may be influenced by the inclusion of the mid-water values where DFAA additions were sometimes high relative to ambient levels, and may have increased calculated *T*. Consequently, reported mean *T* in Table 5 may be regarded as maximum estimates, and carbon utilization (*i. e.*, mg TFAA-C m⁻² divided by *T*) are conservative estimates. Therefore, the percent utilization (Tab. 5) values are also conservative. Our calculations indicate that TFAA utilization represents approximately 2-11% of primary production in the areas stu-

died, with the higher percentage measured during periods of lower relative primary production.

CONCLUSIONS

1) Microheterotrophic utilization rates of DFAA were most rapid in euphotic zone waters, and decreased considerably in mid-depth waters in profiles from two Southern California Borderland basins. In the deepest waters, utilization rates increased over mid-depth values. Similar distributions were observed for bacterial numbers and TFAA concentrations.

2) Microheterotrophic utilization rates in deeper waters were greatly affected by increasing levels of tracer addition, whereas the same levels had a negligible effect on euphotic zone utilization rates. Hence, in any study of DFAA utilization in deep marine waters, it is of considerable importance to employ suitably low levels of substrate addition, and to stringently monitor all sampling and analyses to avoid inadvertent contamination by hands, reagents or apparatus.

3) Microheterotrophic utilization of DFAA integrated for the full profiles was estimated to account for 2-11% of total carbon fixed by primary production in the euphotic zone. Since DFAA represent only a small fraction of DOC, it is apparent that microheterotrophic processes can be major mediators in the transformation of dissolved organic carbon and nitrogen.

Table 5

Calculated water column microbial carbon utilization based on TFAA metabolism, and percentage it represents of primary production. *T*, depth weighted mean; TFAA, depth-integrated total; C utilization as TFAA; $\mu\text{mol TFAA} \times (4.4 \mu\text{mol C } \mu\text{mol TFAA}^{-1}) \times (12 \mu\text{g C } \mu\text{mol C}^{-1}) \div 1000 \mu\text{g mg}^{-1}$; Percent of primary production represented by TFAA utilization.

| Cruise Location Date | Turnover time, h | TFAA $\mu\text{mol m}^{-2}$ | Carbon utilization mg m ⁻² day ⁻¹ | Percent utilization |
|--|---------------------|--------------------------------|---|------------------------|
| SCBS-18 Santa Monica Basin May 1981 | 260 | 4 900 | 24 | 2.0 |
| SCBS-20 Santa Monica Basin March 1982 | 610 | 9 500 | 20 | 1.8 |
| SCBS-22 San Pedro Basin May 1983 | 160 | 7 900 | 63 | 11.1 |

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