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Close coupling between release and uptake of dissolved free amino acids in seawater studied by an isotope dilution approach*

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ABSTRACT: Dissolved free amino acids (DFAAs) play a major role in the flux of organic carbon and nitrogen in marine biotic systems. In this study, DFAA release and uptake rates in samples from Long Island Sound and the Atlantic continental shelf were measured simultaneously using a tritium isotope dilution approach in conjunction with high pressure liquid chromatography (HPLC). Numerous measurements over a 20 mo period showed that release and uptake rates were usually similar, and net changes in DFAA concentration were much slower than (typically less than 30 % of) the gross uptake or release rates; this indicates close coupling between these 2 processes. DFAA turnover was rapid, with summer turnover times typically 0.5 h or less, and concentrations of individual DFAAs were usually a few nanomolar. Comparisons of total uptake rates with estimates of bacterial heterotrophic production confirm that DFAAs represent a significant source (>10 %) of carbon and nitrogen for bacterial growth. DFAA release mediated by copepods, either through 'sloppy feeding' or by excretion, can be of comparable magnitude to direct release by microplankton.

INTRODUCTION

In recent years it has been found that as much as 60 % of total primary production in planktonic marine ecosystems cycles through bacterioplankton, primarily via dissolved organic matter (DOM; Hagström et al. 1979, Fuhrman & Azam 1980, 1982, Williams 1981, 1984, Azam et al. 1983, Ducklow 1983). Uptake of DOM has been studied for many years (e.g. Parsons & Strickland 1962, Wright & Hobbie 1965, Williams 1970, Azam & Holm-Hansen 1973, Crawford et al. 1974, Azam & Hodson 1977, 1981). However, it is probably the rate of release of DOM that controls the amount available to bacteria, so understanding the release processes is an important goal. Previous studies of particular release mechanisms have given primary attention to release by photosythesizing algae (see review by Sharp 1977), zooplankton (e.g. Webb & Johannes 1967), and heterotrophic microflagellates (Andersson et al. 1985). However at this time we have little knowledge about how individual release mechanisms contribute to total release and how release and uptake processes are coupled. These are the problems addressed by this study.

Uptake processes are generally easier to measure than release. Studies of uptake are relatively simple with the use of radioisotopes because the tracer is taken up by organisms that can be easily separated from the medium by filtration. However, release is more difficult to measure because the released material enters a pool from which material is constantly being removed by uptake. Therefore released material will not simply accumulate unless uptake is stopped by a mechanism that does not affect release. Radioisotopic studies of release by phytoplankton in natural marine environments have so far been limited to those in which the phytoplankton have been incubated with ^{14}C-bicarbonate and accumulation of dissolved organic ^{14}C has been followed. This basic protocol has been modified in some studies where subsequent bacterial uptake has been followed by size fractionation (e.g. Larsson & Hagström 1982) or a mathematical model has been used to estimate the gross release rate (e.g. Lancelot 1979). However, these sorts of studies can only measure release of organic

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matter fixed during the incubation period, and such material is only a small portion of the total carbon potentially available for release.

The problem of measuring release in the presence of rapid uptake is not unique to dissolved free amino acid (DFAAs) and has already been largely solved for dissolved amonium; an isotope dilution procedure can be used (e.g. Alexander 1970, Blackburn 1979, Glibert et al. 1982). In this sort of experiment, an isotopic tracer is added to the dissolved pool at the beginning of the experiment, and the method takes advantage of the fact that both labelled and unlabelled molecules are taken up, but in a short experiment only unlabelled molecules are released. This asymmetry allows calculation of both release and uptake rates over the course of the experiment from measurements of concentrations and isotope ratios. With ammonium, the work is done with the stable isotope ^{15}N and the experimental data are concentrations (measured by wet chemistry) and isotope ratios (by mass spectrometry); most of the optimization of calculations has been worked out for these sorts of data (Glibert et al. 1982, Garside & Glibert 1984). The data provided by an experiment with a radioisotope require slightly different calculations, such as those described by Harrison (1983) for work with radioactive phosphate.

In this study, isotope dilution experiments in conjunction with high performance liquid chromatography (HPLC) analyses were used to measure simultaneously the rates of release and uptake of DFAAs in seawater. The results demonstrate close coupling between the rates, and also give indications of important DFAA sources in seawater.

MATERIALS AND METHODS

Water samples were collected in acid-washed plastic bottles from a pebble beach at Crane Neck on Long Island Sound (40° 55.3′ N, 73° 09.3′ W) and transported (~ 20 min) in an insulated container to the laboratory, where they were kept in a seawater-temperature incubator lighted at 100 μE m^{-2} s^{-1} (~10% surface sunlight). Subsamples (usually 100 to 500 ml) were dispensed into rinsed sterile polyethylene Whirlpak bags. Tritiated amino acids (New England Nuclear or ICN) were added at 0.1 to 1 nM final concentration (concentrations of stock solutions were first tested by HPLC as described below) and replicate 10 ml subsamples were immediately filtered through pre-rinsed Millipore mixed-ester filters (25 mm diam., 0.45 μm nominal pore size; type HA) in an acid-washed stainless steel and teflon filter unit (Hoefer Scientific). The filtrates were collected in acid-washed vials and removed, and the filters then were rinsed 5 times with 0.22 μm-filtered seawater, and removed to scintillation vials. The seawater sample in the Whirlpak bag was kept in the incubator, and the filtration procedure was repeated at intervals ranging from 10 to 30 min, with total incubation times rarely exceeding 2 h.

The concentrations of DFAAs in the filtrates were measured by a modification of the pre-column *o*-phthaldialdehyde derivatization technique of Mopper & Lindroth (1982). One ml subsamples were derivatized with 10 μl of a reagent consisting of 12.5 mg *o*-phthaldialdehyde, 250 μl of methanol, 12.5 μl of 2-mercaptoethanol, and 250 μl 1 M sodium borate buffer (pH 13). After exactly 1 min, 5 μl of 10% (vol/vol) acetic acid was added to stop the reaction, and 250 μl was injected into the HPLC (Spectra Physics model 8700, Kratos model FS-970 fluorescence detector set at 340 nm excitation and >410 nm emission, Spectra Physics model 4270 integrator). The chromatographic column had dimensions of 4.6 × 100 mm and was commercially packed with Microsorb 3 μm C18 bonded silica (Rainin Instruments, Woburn, Massachusetts, USA). Mobile phases were (A) 50 mM sodium acetate, pH 5.8, with 2% tetrahydrofuran, and (B) methanol (Jones et al. 1981). The elution gradient was changed over the course of the study to optimize separations (elution profiles varied somewhat from column to column and with column age), but was essentially from 25 to 80% B over 15 to 20 min.; changes usually involved holding the %B for a minute or so at some intermediate value in order to separate fused peaks. Reagent blanks were filtered HPLC-grade water. Such water was also used as a 'dummy sample' to test for contamination in the containment, pipetting, and filtration steps; these tests sometimes discovered contamination in the filter unit despite previous cleaning, and prompted further cleaning before experimental samples were filtered. All reagents were HPLC grade. Further details of the HPLC methodology are presented by Fuhrman & Bell (1985).

Radioactivity was measured by liquid scintillation spectroscopy. The filters were dissolved in 1 ml ethyl acetate, and then 5 ml of NEN Formula 963 scintillation fluid was added. Radioactivity in filtrates was measured by adding 0.5 ml to 5 ml of scintillation fluid. Respiration of the tritium label formed tritiated water which was measured as loss from filtrates upon freeze-drying; freeze-dried duplicate 0.5 ml filtrates were rehydrated with 0.5 ml distilled water and radioactivity was assayed as for filtrates. Counts were corrected for quench by internal and external standards.

Release and uptake rates were calculated from the following equations (Glibert et al. 1982, Harrison 1983):

$$\text{Release rate} = \frac{\ln(R_o/R_t)}{\ln(C_o/C_t) \cdot t} \cdot (C_o - C_t) \qquad (1)$$

$$\text{Uptake rate} = \frac{D_o - D_t}{\bar{R} \cdot t} \quad (2)$$

$$\bar{R} = \frac{R_o}{k \cdot t} \cdot (1 - e^{-kt}) \quad (3)$$

$$k = \frac{\ln(R_o/R_t)}{t} \quad (4)$$

where R_t = specific activity (radioactivity mole^{-1}) of the dissolved amino acid pool at time t; C_t = concentration (moles l^{-1} of the dissolved amino acid at time t; and D_t = radioactivity in the dissolved amino acid at time t (dpm l^{-1}). Note that R=D/C. The C term was measured by HPLC as described above, and the D term was most commonly determined as radioactivity (dpm) in the freeze-dried filtrate, but we also measured it as radioactivity in the material eluting from the HPLC at the time of the fluorescent peak corresponding to the added labelled amino acid (collected with a fraction collector and assayed for radioactivity like the filtrates). The second method allows for the simultaneous uptake measurement of more than one amino acid in the same sample, but has the problem that the conversion to the fluorescent derivatized product is not 100 % efficient, sometimes causing variable loss of radioactivity in these peaks. In our experiments these 2 methods yielded rate measurements within 20 % of each other, suggesting they are both appropriate. An alternative way of measuring the term D_o–D_t, which is the total radioactivity taken up, was by dividing the radioactivity on the filter by the fraction incorporated (filter dpm/[filter dpm + ^3H_2O dpm]), with the fraction averaged over the entire experiment. This method improves precision over the other 2 methods at low activities because under these conditions the latter depend on accurately determining differences between large numbers (each with considerable variability). The 'fraction incorporated' was averaged over the experiment to minimize the influence of errors in individual measurements. The coefficient of variation of individual measurements (between time points of the same sample) was 16.5 %.

In experiments with copepods, these animals were collected from Long Island Sound with a plankton net, and swimming individuals of the species *Acartia tonsa* were picked out by pipette and manipulated with acid-washed nitex netting. Water free of adult copepods was prepared by filtration through clean 202 µm-mesh nitex, and water free of all microorganisms was prepared by filtration through rinsed 0.22 µm pore-size Millipore filters (47 mm diam.). Experiments had 3 treatments: (i) 202 µm nitex mesh-filtered water containing microplankton but no adult copepods; (ii) 202 µm-filtered water plus adult or late copepodite copepods (about a 10 to 100-fold concentration above natural abundance); and (iii) water filtered through a rinsed 0.22 µm Millipore filter (to remove all plankton) plus rinsed adult or late copepodite copepods. Isotope dilution experiments with these samples were as described above.

Most experiments used labelled alanine (> 50 Ci mmol^{-1}), but glutamic acid, leucine, serine, and glycine (highest specific activity available, typically > 20 Ci mmol^{-1}) were also used. Alanine was chosen for most experiments because it is one of the more concentrated of the DFAAs in seawater (cf. Mopper & Lindroth 1982, Fuhrman & Bell 1985) and the fluorescent and radioactive peaks eluting from the HPLC were minimally interfered with by other amino acids.

Because of the ubiquity of potential DFAA contaminants (Fuhrman & Bell 1985), the most critical parameter in the isotope dilution experiments was the DFAA concentration measurement; a few contaminated samples can make results uninterpretable (e.g. negative regeneration rates). Thus it was sometimes necessary to exclude occasional random high DFAA concentration measurements, typically one in a set of triplicates and comprising up to 20 % of all the samples. Error in concentration measurement was the factor limiting the precision of absolute uptake and release rates, and it is probably unreasonable to expect a particular individual rate measurement to be much better than within a factor of 1.5 to 2 of the true rate.

Thymidine incorporation into cold-TCA-insoluble material was measured as described by Fuhrman & Azam (1982) on samples collected from Crane Neck and incubated in the laboratory under simulated *in situ* temperature and light conditions.

RESULTS

Over the course of a typical isotope dilution experiment, the total amount of radioactivity (filter + filtrate) in an incubation declined slightly (probably due to adsorption onto the container walls), and the radioactivity accumulated in the organisms (filter) or in tritiated water produced by respiration (Fig. 1A). At the same time, the amount of soluble non-volatile radioactivity (in the lyophilized samples or collected from the HPLC effluent) declined exponentially, as shown by a linear semi-log plot (Fig. 1B). This is the behavior expected from a tracer added to a pool that is turning over but still in near-steady state. The observation that the HPLC eluate is always lower than the lyophilized filtrate is apparently due to low molar efficiency of HPLC derivatization rather than to radioactive contaminants in the lyophilized filtrate; both yield the same rate measurement. Note that in the experiment depicted in Fig. 1 the alanine concentration declined by less than a factor of 2 over 50 min, but the rate of

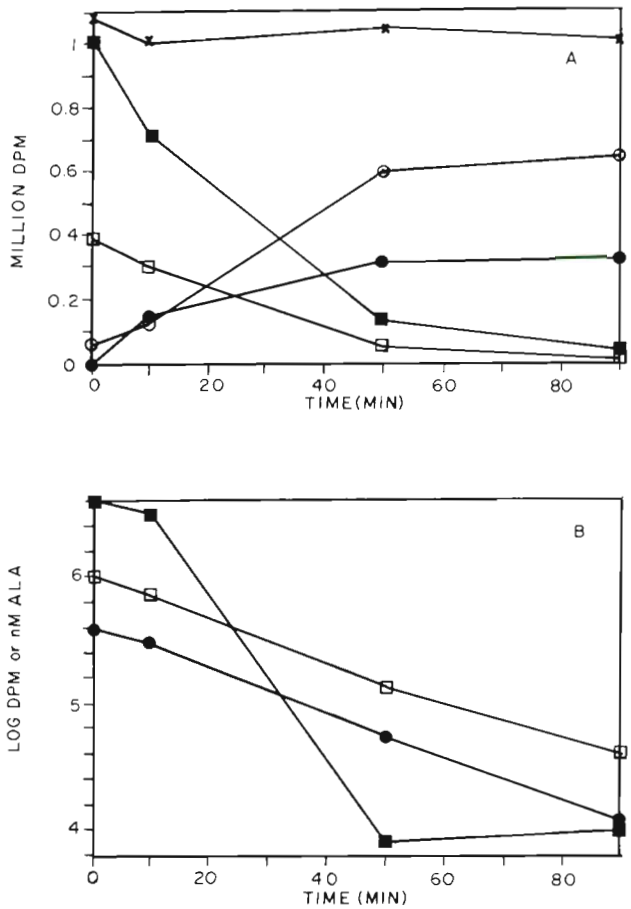

Fig. 1. Isotope dilution results from 16 Jun 1983. (A) Time course of distribution of radioactivity within the sample, normalized to 10 ml volume. (X) Total from an unfiltered sample; (■) lyophilized filtrate; (□) eluted from the HPLC during the fluorescent derivatized alanine peak; (○) tritiated water, measured by difference between filtrate and lyophilized filtrate; (●) collected on the filter. (B) Time course of alanine concentration and $\log_{10}$ of radioactivity. (■) Alanine concentration; (□) lyophilized filtrate; (●) eluted from HPLC during the fluorescent derivatized alanine peak

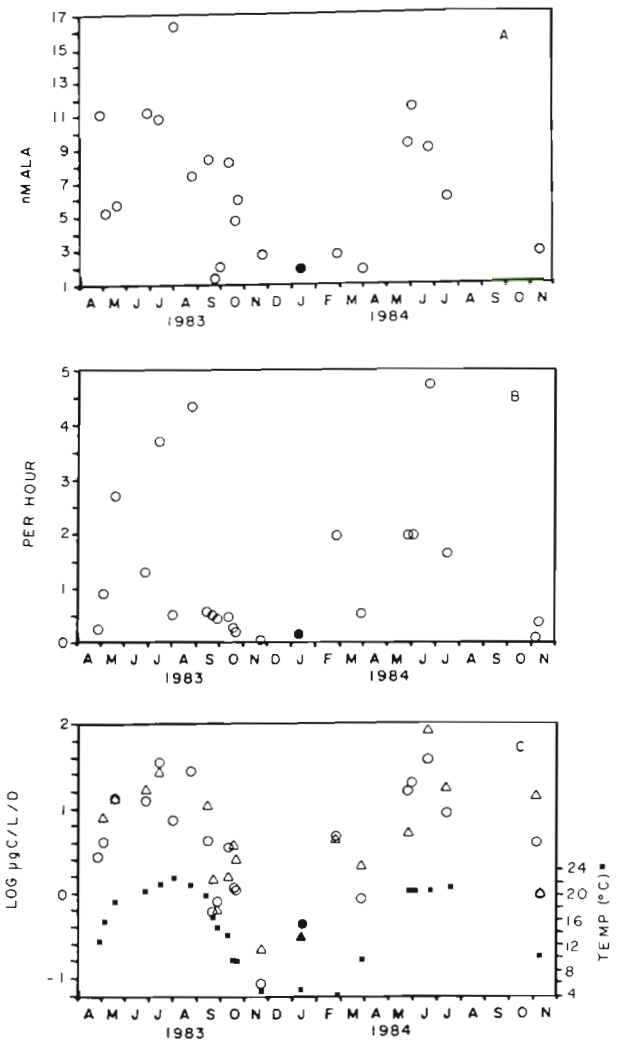

Fig. 2. Alanine experimental data from Apr 1983 to Dec 1984. (A) Alanine concentrations. (B) Turnover rates assuming steady state (note that steady state was not always observed). (C) Release (△) and uptake (○) rates (note log scale) and (▪) water temperature. Open circles and triangles are from Crane Neck, Long Island Sound, and filled circles and triangles are from Atlantic continental shelf off Montauk Point, New York

decline was much less than that of the radioactive tracer, which dropped by about a factor of 7. This clearly demonstrates isotope dilution. The decline in concentration showed that the uptake rate was faster than the release rate, but the fact that the tracer declined so much faster indicated that the release rate was still substantial (14.8 nM h^{-1} compared to 15.5 nM h^{-1} uptake). Not all experiments showed declining concentrations; many had no significant change and some showed increases over time, indicating release > uptake, but in all experiments the concentration of dissolved tracer declined.

Several experiments similar to those depicted in Fig. 1 were performed on samples from Long Island Sound from May 1983 through the end of 1984. Alanine concentrations ranged from 1 to 16 nM (Fig. 2A), and turnover rates, calculated as if the system were near steady state, ranged from 5 h^{-1} in summer to < 0.5 h^{-1} in winter (Fig. 2B). Measurements of uptake and release calculated as flux through the dissolved pool (µg C l^{-1} d^{-1}) showed that within the precision of the measurement, these processes were closely coupled to each other and were strongly correlated to temperature (Fig. 2C). Overall, there was no significant difference between release and uptake rates ($p > 0.2$, t-test). Averaged over all the experiments, the release and uptake rates were, respectively, 12.06 ± 4.03 and 9.36 ± 2.38 µg C l^{-1} d^{-1} (mean ± SE), so there was a small (29 %) excess of release over uptake, although this may indicate an artifact of sample capture and containment (e.g. injury to delicate plankton or contamination).

Table 1. Uptake of amino acids other than alanine

Amino acid	Date	Uptake rate ($\mu gC\ l^{-1}\ d^{-1}$)	Ala. uptake[a] ($\mu gC\ l^{-1}\ d^{-1}$)
Glutamic acid	13 May 1983	9.9	2.7
	31 Jul 1984	4.8	8.7
Glycine	30 Nov 1983	1.2	1.1
	31 Jul 1984	1.4	8.7
Leucine	30 Nov 1983	0.9	1.1
	6 Jul 1984	2.1	28.2
	19 Jul 1984	2.4	36.7
	31 Jul 1984	1.2	8.7

[a] If not measured on same date as other rate, then from closest date measured (all within 2 wk)

Compared on a seasonal basis, alanine was generally taken up at a similar or higher rate than leucine, or glycine, but sometimes less than glutamic acid (Table 1). The average percentage incorporation:

(100 × label incorporated/[incorporated + respired])

for alanine was 51 ± 2.7 % (mean ± SE, n=20). This applies specifically to the #3 carbon (methyl group) that was tritiated.

Other amino acids were found to affect the uptake of alanine, possibly by competition. With a sample from 16 November 1984, separate additions of 30 nM glutamic acid, glycine, serine, or leucine reduced the alanine uptake rate to 38, 35, 45, and 34 %, respectively, of the control (no addition) rate. Because the alanine concentration was 2.4 ± 0.75 nM (mean ± SE, n=4), a reduction to 40 % of control rates is equivalent to addition of 3.6 nM of an equal competitor, if the result is due to competition. However, because this effect was from 30 nM additions, it appears that these amino acids were individually at most about ⅛ as effective as a 'perfect' competitor. An alternative explanation for the reduction is that the bacteria slowed their alanine assimilation due to the other amino acid better meeting current needs, especially when available at higher concentrations.

The alanine uptake rate ($\mu g\ C\ l^{-1}\ d^{-1}$) was significantly correlated to the incorporation rate of tritiated thymidine into cold-TCA-insoluble material ($r = 0.60$, $p < 0.05$, n=21). The latter can be used as a measure of heterotrophic bacterial production (Fuhrman & Azam 1980, 1982), and it is thus possible to make an estimate of the alanine contribution to the total carbon and nitrogen demand of the bacteria. Using a factor of 2×10^{18} to convert moles thymidine incorporated into cells produced (Fuhrman & Azam 1982) and a C and N content of 20 and 5 fg $cell^{-1}$, respectively (Lee & Fuhrman unpubl.), the alanine incorporation rate (excluding respiration) as a fraction of total bacterial secondary C production was 8.3 ± 1.6 % (mean ± SE, n=21). Regarding nitrogen, if it is assumed that all the amino N taken up is retained (i.e. no release of nitrogenous substances), then the alanine uptake represented an average of 24.8 ± 4.8 % of the calculated bacterial N demand. Because most of the experiments reported here were not designed to quantify all the DFAAs, we cannot report the exact mol% of alanine, but in those experiments where an estimate was possible, the mol% appeared to fall in the 10 to 30 % range, similar to that reported previously (Carlucci et al. 1984, Fuhrman & Bell 1985). To put the results into context of primary productivity, the chlorophyll concentration in this region ranges roughly from 3 to 15 $\mu g\ l^{-1}$ and primary productivity ranges roughly from 30 to 600 $\mu g\ C\ l^{-1}\ d^{-1}$, with highest rates April to October (S. G. Horrigan pers. comm.).

Table 2. Copepod *Acartia tonsa* involvement in DFAA uptake and release

Treatment	Uptake rate[a] (nmol $l^{-1}\ h^{-1}$)	Release rate[a] (nmol $l^{-1}\ h^{-1}$)	Extrapolated release[b]
26 Oct 1983, [^{3}H] alanine tracer			
< 202 µm	0.9	1.3	–
< 202 µm + 150 ind l^{-1}	4.2	30	0.3–3
< 0.22 µm + 150 ind l^{-1}	< 0.1	< 0.1[c]	< 0.1
29 Nov 1984, mixture of [^{3}H] ala + glu + gly + ser			
< 202 µm	7.6	9.9	–
< 202 µm + 125 ind l^{-1}	8.7	17.7[d]	0.2–1.8
< 0.22 µm + 125 ind l^{-1}	< 0.1	6.2[e]	0.1–0.6

[a] All rates are only for DFAAs traced in that experiment
[b] Rates extrapolated to approx. natural copepod abundance by dividing measured rates by 10 to 100
[c] Increase in glycine noted (ca 9 nmol $^{-1}\ h^{-1}$)
[d] Mostly glycine + alanine; also noted increase in glutamine, histidine, and taurine
[e] Mostly glycine + alanine, also noted increase in arginine

Copepods released DFAAs both in the presence and absence of other, smaller, plankton, but the release rate was higher when there were microplankton available for copepod feeding (Table 2). When the release rates were extrapolated to approximate natural copepod abundance (dividing rates by a factor between 10 and 100), it was found that the DFAA release from copepods in microplankton-free water (< 0.22 µm filtered) was much lower than release from microplankton alone (202 µm filtered water). However, the same extrapolation shows that in the first experiment, the copepods incubated in the presence of other plankton released alanine at a rate comparable to that from the < 202 µm plankton alone (Table 2).

DISCUSSION

For isotope dilution studies of particular DOM molecules in seawater, DFAAs have advantages over other classes of molecules: (1) individual DFAAs can be measured in unconcentrated seawater by HPLC (Mopper & Lindroth 1982, Fuhrman & Bell 1985); (2) as the building blocks of proteins, DFAAs are one of the largest single classes of monomers used by all organisms, making them important sources of carbon and nitrogen for bacteria; (3) they are available with high specific activity tritium labels, allowing addition of true trace levels (sub-nanomolar); (4) they include a diverse variety of functional groups, including aliphatic, aromatic, acidic, basic, and thiol moieties; thus they can represent a wide array of DOM molecules.

The alanine uptake rate calculated above for Long Island Sound was found to represent roughly 8 % of the C requirement and 25 % of the N requirement for bacterial growth (assuming 50 % C and 100 % N growth efficiencies). These numbers seem high, given that alanine is only one substrate out of many. However, it should be realized that alanine is one of the more concentrated of the DFAAs and has a particularly high flux rate. Still, our calculation may be an overestimate of the alanine contribution to growth because the thymidine-based production rate using the conservative conversion factor may be an underestimate by perhaps a factor of 2 (Ducklow & Hill 1985). The contribution to N demand may be overestimated if there is some N release by bacteria. However, even including these possible errors, the data indicate that DFAAs represent a significant source of nutrition for marine bacteria.

The copepod experiments were designed to see if processes related to zooplankton feeding have an effect on DFAA release. The results showed that the combination of copepods + food had a much higher release rate than either one alone. This suggests that the processes of 'sloppy feeding', ingestion, and egestion cause release of DFAAs into seawater. The release from the copepods without food was low on a per-individual basis, suggesting that the metabolic processes of non-feeding animals need not release much DFAAs, although in both experiments glycine was released to a small extent. When the estimated release rates from copepods + food were extrapolated to the natural abundance of the copepods, the copepod-mediated release was comparable to that of the microplankton alone in the first experiment, but much lower in the second experiment (Table 2). This suggests that zooplankton-mediated DFAA release can be as important as direct release from the microplankton, as has been suggested by the indirect measurements of Eppley et al. (1981), although the microplankton alone may sometimes be much more important.

It was a general phenomenon in virtually all of our experiments that the net changes in DFAA concentrations were very slow (30 % or less) compared to the gross release and uptake rates. In other words, the bacterial DFAA utilization rates closely corresponded to the rates at which the DFAAs were released, so the DFAAs neither accumulated nor disappeared appreciably compared to the changes one would expect from release or uptake alone. Similar results were found when DFAA release and uptake were measured at much shorter intervals over diel cycles (unpubl.). This suggests good regulatory mechanisms whereby the bacteria use the DFAAs as rapidly as they become available. At this time, we do not know the exact mechanisms by which the bacteria respond to changes in release, but 2 related factors are probably involved: (a) uptake kinetics (function of concentration) causing increased uptake as concentrations increase from increased release rates, and (b) bacterial uptake and assimilation controlled by C and N demand for growth, with growth adapted to the available substrate supply rate.

A simple conceptual model of such a closely-regulated system suggests that there is an 'equilibrium concentration' that would be maintained by the balance between release and uptake rates. Because the uptake rate ordinarily increases with increasing concentration (until saturation) and we have shown that uptake approximately balances release, one would expect that this equilibrium concentration would increase as the release rate increases. Described mathematically, at equilibrium

$$E = U \quad (5)$$

where E and U = release and uptake rates, respectively. Assuming a simplified first-order relation between uptake and concentration,

$$U = k_u C \quad (6)$$

where C = concentration (in this case an equilibrium concentration because E = U), and k_u = the first-order rate constant. Combination of the equations yields

$$E = k_u C \text{ or } C = E/k_u \quad (7)$$

Examination of our data indicates that in accordance with the expectations of this model the concentrations generally covaried with release (Fig. 3), and the correlation between these 2 parameters was statistically significant ($r=0.48$ and significantly different from zero, $p<0.05$). The correlation was not very strong probably due to short-term fluctuations (possibly including experimental contamination), and other variables that are not included in the model, such as saturation uptake kinetics. Evidence indicates that saturation of uptake rate is uncommon at typical natural

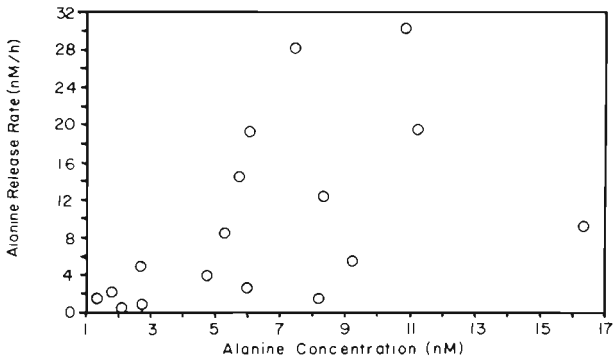

Fig. 3. Alanine release rates plotted against concentrations

DFAA concentrations (Jørgensen & Søndergaard 1984, Fuhrman & Ferguson 1986). Overall, the basic correlation between release rate and concentration observed in our study is consistent with the hypothesis that an increased DFAA supply (release) rate produces a higher equilibrium DFAA concentration and results in higher uptake, via the mechanism of sub-saturation uptake kinetics.

Close coupling between release and uptake is not surprising considering the extremely fast DFAA turnover rates that we observed. With rapid turnover, if there is no large 'buffer' concentration of substrate, the uptake rate is limited by the rate at which substrate becomes available. Measurements of DFAA concentrations in most pelagic marine systems show that DFAA concentrations are low (Mopper & Lindroth 1982, Carlucci et al. 1984, Ferguson & Sunda 1984, Fuhrman & Bell 1985, Fuhrman & Ferguson 1986), suggesting the lack of a buffer. A dimensional analysis can be used to put upper limits on substrate concentrations and show that low concentrations (<50 nM total) of all rapidly cycling organic subtances should be typical: for rich coastal waters, if we take 100 µg C l^{-1} d^{-1} as a typical primary production rate (Parsons et al. 1977), a conservative turnover time of 2.4 h for labile organics, and a liberal estimate of 50% conversion from particulate to dissolved primary production, then the expected total concentration of the labile organic matter is 5 µg C l^{-1}, or 50 nM with a C molecular weight of 100. In offshore waters, DFAA turnover rates are slower, but so is primary production, resulting in even lower concentrations (Ferguson & Sunda 1984). Situations that can lead to much higher concentrations may include (a) higher primary productivity, (b) selective inhibition of bacterial activity such as by near-freezing temperatures (Pomeroy & Deibel 1986), and (c) a sudden burst of DOM release, such as from a declining bloom, or a combination of these factors.

In conclusion, I have shown that in the temperate coastal waters studied, release and uptake of DFAAs are tightly coupled, as evidenced by direct measurements as well as very rapid turnover. Such tight coupling implies good regulatory mechanisms whereby bacterial uptake tracks the release involved with several processes, including direct exudation by microplankton as well as more indirect routes such as those related to zooplankton feeding. The exact nature of the regulatory mechanisms is poorly understood, and deserves further study. Isotope dilution is a very powerful tool for these and similar studies where measurement of rates in highly dynamic systems is required. Further applications in marine studies should greatly increase our understanding of interactions within complex marine ecosystems.

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