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Bacterial utilization of transient plankton-derived dissolved organic carbon and nitrogen inputs in surface ocean waters

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ABSTRACT: The majority of bacterial growth and respiration in the upper ocean is thought to result from coupling between microheterotrophic populations and the reactive soluble components of planktonic primary and secondary production. However, we know little about the potential turnover of these components and the concomitant growth of bacteria under conditions of intermittent or transient inputs of natural dissolved organic matter (DOM) compared to quasi-steady state, low DOM conditions. The present study evaluated the short-term (~3 d) rates and net extents of utilization (as measured losses) of selected constituents of plankton-derived DOM (DOM_{PD}) by indigenous bacterioplankton populations in eastern North Pacific surface waters, and assessed bacterial growth efficiencies (BGE) during temporarily non-limiting DOM conditions. Approximately 28% of the starting dissolved organic carbon (DOC) and 34% of the dissolved organic nitrogen (DON) in incubations supplemented with DOM_{PD} could be characterized as dissolved free and combined amino acids (DFAA and DCAA, respectively) and monosaccharides (MCHO). Up to 31% of the added DOC and 26% of the added DON was utilized in $+DOM_{PD}$ incubations; however, BGE under supplemented conditions (~4 to 5%) was similar to estimates for ambient oligotrophic waters. Of the net DOC consumed, 75% was accounted for by DFAA (which alone was 61% of the total), DCAA, and MCHO, while the remaining non-characterizable 25% may represent an inherent or rapidly formed component of lower reactivity. In contrast to DOC, net DON utilization was supported entirely by DFAA and DCAA, with DFAA alone accounting for the vast majority (up to 99%). Together, DCAA and MCHO accounted for only ~13% of the DOC consumed and ≤~5% of the DON (i.e. as DCAA) utilized. These findings are consistent with bacterial growth in the open ocean being controlled predominantly by inputs of a small fraction of bulk DOM, and further suggest that bacteria may function primarily as remineralizers even during transient periods where labile DOC and DON is relatively available.

KEY WORDS: Plankton-derived DOM · Bacterial growth efficiency · DOC · DON · Dissolved free amino acids · Dissolved combined amino acids · Monosaccharides

INTRODUCTION

Oceanic dissolved organic matter (DOM) is one of the largest active reservoirs of reduced carbon at the earth's surface (Hedges et al. 1997). Seawater DOM consists of a wide array of C-, N-, and P-containing organic compounds (see reviews by Benner 2002, Bronk 2002, Karl & Bjorkman 2002) ranging from highly refractory, aged humic material which is turned over on the order of thousands of years (Williams & Druffel 1987, Bauer et al. 1992, Cherrier et al. 1999, Bauer 2002), to quasi-refractory material having turnover times of months to years (Ogura 1972, 1975, Søndergaard et al. 2000), to highly reactive and recently produced components that cycle on the order of hours to days (see Carlson 2002 for a thorough review of this topic). Surface seawater bulk DOM may therefore be viewed as an assemblage of co-occurring organic components possessing continua in both reactivity and age.

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The relatively small degree of variability in surface ocean DOM concentrations (i.e. bulk dissolved organic carbon and nitrogen [DOC and DON] as well as some component compound groups) both spatially and temporally suggests that DOM production and consumption processes are ultimately coupled. As a result, the 'background' standing stock of bulk DOM may be dominated by constituents of lower biological reactivity remaining after (1) the selective removal of more reactive components by bacterial heterotrophs (Kirchman et al. 1991, Noorman et al. 1995, Carlson & Ducklow 1996, Cherrier et al. 1996, Amon et al. 2001, Carlson et al. 2002), and (2) the bacterially mediated formation of refractory DOM from previously labile DOM (Brophy & Carlson 1989, Tranvik 1993, Heissenberger et al. 1996, Ogawa et al. 2001). For example, using seawater cultures Carlson & Ducklow (1996) found that bacterial growth in surface Sargasso Sea waters was stimulated only when DOC concentrations became elevated above ambient mixed-layer background concentrations (67 to 69 µM C). They also suggested that the 'surplus' DOM, while only ~6 to 7% of total mixed layer DOM, represented the component of bulk DOM that was utilized for bacterial growth and maintenance.

When evaluating the potential turnover of DOM in pelagic systems, consideration of such factors as substrate quality and reactivity (e.g. C:N, molecular makeup and weight, and age; Goldman et al. 1987, Williams 2000) may be especially important due to the oftentimes large energetic constraints placed on indigenous bacterial populations (Cherrier et al. 1996, Morita 1997, del Giorgio & Cole 1998, 2000, Carlson et al. 2002, del Giorgio & Duarte 2002). Both temporal and spatial variability in substrate quality and availability may have an important influence on the physiologic status and phylogenetic makeup of pelagic bacterial communities (Jørgensen 1987, Cherrier et al. 1996, Weiss & Simon 1999, Cottrell & Kirchman 2000, Carlson et al. 2002). Bacterial cycling of reactive DOM constituents is based largely on studies of analytically recognizable compounds such as amino acids (e.g. Williams et al. 1976, Keil & Kirchman 1993, 1999, Rosenstock & Simon 1993, 2001, Middelboe et al. 1995; see Kirchman 2003 for review) or carbohydrates (e.g. see Jørgensen & Jensen 1994, Tranvik & Jørgensen 1995, Hanisch et al. 1996, Rich et al. 1996, Jørgensen et al. 1998, Skoog et al. 1999, Kirchman 2003). However, to assess the contributions of various C- and N-containing constituents, their concurrent utilization along with that of DOM must be followed.

A growing body of evidence suggests that the soluble products of primary and secondary production are key for sustaining the microbial loop in oceanic systems. However, much less is known about the temporal nature of these inputs and the responses of microbial communities to intermittent or transient forms of reactive DOM (see, e.g., Hansell et al. 1995) resulting from such factors as exudation and excretion by primary and secondary producers (Baines & Pace 1991, Kirchman et al. 1991, Nagata 2000, Søndergaard et al. 2000, Carlson 2002 and references therein), grazing activities (Jumars et al. 1989, Nagata & Kirchman 1992, Nagata 2000, Steinberg et al. 2000), and viral lysis of photoautotrophs (Suttle 1994, Fuhrman 1999; see Wommack & Colwell 2000 for a review). The overall objectives of the present study were to evaluate the potential rates and extents of utilization of specific constituents of a transient pulse of fresh, plankton-derived DOM by indigenous bacterial populations in surface ocean waters, and how each of these constituents contributed to overall DOC and DON turnover. Given the carbon, nutrient, and energy constraints under which heterotrophic bacteria often subsist in oligotrophic waters (Carlson & Ducklow 1996, Morita 1997, del Giorgio & Cole 2000, Williams 2000), we hypothesized that (1) N-enriched components would be preferentially utilized relative to less enriched components due to N-limitation in oligotrophic waters, and (2) bacteria would be released from their typical degree of substrate limitation when plankton-derived DOM was available, thus allowing for proportionately greater allocation of C and N toward biomass relative to substrate remineralization.

MATERIALS AND METHODS

Experimental design. Experiments were conducted in July 1993 at a hemi-pelagic site (Stn M, 34° 50′ N, 123° 00′ W; water depth of ~4100 m) in the northeast Pacific, ~220 km west of Point Conception, CA. The southward-flowing California Current influences surface productivity here (Michaelson et al. 1988, Smith et al. 1988), resulting in the highest fluxes of particulate organic carbon (POC) in early to mid-summer and a smaller secondary maximum in late fall (Smith et al. 2001).

Shipboard time-series incubations were conducted to assess how different soluble organic constituents of the bulk plankton may support bacterial growth in quasi-oligotrophic waters. Briefly, seawater was collected from the chlorophyll *a* (chl *a*) maximum at 85 m (Bianchi et al. 1998) using acid-cleaned 12 or 30 l Go-Flo bottles. The seawater was gravity-filtered through a pre-combusted GF/F filter (nominal pore size ~0.7 µm), to remove particles and bacterial grazers. The filtered water was then distributed into 2 l acid-cleaned polycarbonate incubation bottles. Because *in situ* O_2 concentrations could not be maintained with

gravity filtration, unfiltered water for oxygen measurement was dispensed directly into a series of pre-combusted 60 ml BOD bottles (Wheaton). Thus, reported changes in O_2 concentration are potential estimates of overall 'micro-community' (i.e. all microorganisms) respiration, rather than heterotrophic bacterial respiration exclusively (Griffith et al. 1990). However, earlier work indicated that nearly all of the O_2 utilization in these waters was due to the <0.8 µm fraction (Cherrier et al. 1996, Cherrier 1997). Plankton-derived DOM (DOM_{PD}) was obtained from a zooplankton-dominated assemblage from the cod end of a 335 µm Nitex mesh net after an 85 m depth evening net tow. The assemblage was warmed to ~30°C, to induce DOM release, and was then filtered through a pre-combusted (525°C for 4 h) GF/F filter to remove plankton debris and other particulates. The resultant concentrate (DOC = 32 mM, total dissolved nitrogen [TDN] = 7.2 mM) was stored at –20°C in a pre-combusted amber glass bottle until use (within 2 to 3 d).

Incubations were carried out in the dark at *in situ* temperature (13 ± 0.5°C) following supplementation of bottles with DOM_{PD} ('+DOM_{PD}') to give starting DOC and DON concentrations of 230 µM C and 35 µM N, respectively. The rationale for adding elevated DOM_{PD} was (1) to attempt to mimic episodic DOM release reflective of bloom, grazing, or other events, (2) to release bacteria from reactive DOC and DON limitation, and (3) to ensure that starting concentrations of individual DOM_{PD} constituents were great enough to detect changes over short time intervals (hours to days). Experimental controls consisted of non-supplemented incubation bottles having mean ambient seawater DOC concentrations of 66 µM C and ambient DON below detection. A series of BOD bottles either supplemented with DOM_{PD} to the same final concentrations as the 2 l bottles, or maintained at ambient DOC and DON levels, were incubated in parallel with the polycarbonate bottles. Triplicate polycarbonate and BOD bottles for each treatment were monitored at 6 to 12 h intervals over 3 d for dissolved combined amino acids (DCAA), dissolved free amino acids (DFAA), dissolved monosaccharides (MCHO), nitrate plus nitrate, ammonium, oxygen, and heterotrophic protist and bacterial abundances. For purposes of net mass balances, only initial and final concentrations of DOC and DON were measured.

Analyses. ***Dissolved combined and free amino acids:*** Subsamples from all incubations were pre-filtered through 0.2 µm polycarbonate filters (Poretics) under a sterile hood using an all-glass filtration apparatus and collected into glass scintillation vials equipped with acid-washed Teflon-lined closures and stored at –20°C for subsequent analyses. All glassware, filters, and other materials contacting the samples were pre-combusted for 4 h at 550°C. Total dissolved amino acids (TDAA, as DCAA + DFAA) and corresponding blanks were determined following vapor-phase hydrolysis as outlined by Tsugita et al. (1987) as described in Keil & Kirchman (1991a). Both TDAA and DFAA were quantified by reverse-phase HPLC using modifications of the o-phthaldialdehyde (OPA) method outlined by Lindroth & Mopper (1979), with a buffering system and elution gradient modified from Hill et al. (1979) as described by Shultz (1994), and having a detection limit of better than1 pM. Samples were analyzed using a Shimadzu (Shimadzu Instruments) HPLC system with a LPM 600 pump, RF 535 fluorescence detector, and 250 mm Adsorbosphere column (Alltech). DCAA were estimated by subtracting DFAA from TDAA concentrations following blank correction of TDAA and DFAA.

Total amino acid concentrations were determined by summing the concentrations of each individual amino acid calculated using Pierce amino acid mixture H (Pierce Biochemical) as the analytical standard. Procedural blanks for DFAA and DCAA averaged 3 and 18 nM, respectively, while system blanks averaged ≤0.5 nM. Analytical replication was better than ~±1% of the mean, while sample replication averaged ±8% of the mean for both DFAA and DCAA. The elution gradient used during HPLC analysis prohibited the separation of glycine and threonine. α-amino butyric acid (Sigma Chemical) was used as the internal standard. Average C and N contents for amino acids were estimated by summing the respective molar equivalents of C and N in each amino acid, and averaging these values on a relative percentage basis of individual amino acid abundance to obtain conversion factors for amino acids of 5 and 1.5 for C and N, respectively.

Dissolved monosaccharides: Monosaccharides (MCHO) and corresponding blanks were quantified spectrophotometrically as standard glucose equivalents using the modified (Pakulski & Benner 1992) 3-methyl-2-benzothiazolinoe hydrazone (MBTH) method of Johnson & Sieburth (1977) and Johnson et al. (1981). Blank absorbances were subtracted from all sample absorbances to correct for free aldehydes and turbidity, and sample precision averaged ≤±10% for triplicate analyses. A molar conversion factor for MCHO to glucose-C equivalents of 6 was used.

DOC and TDN: DOC and TDN were measured using modifications of the high temperature (680°C) flow-through catalytic oxidation methods outlined by Bauer et al. (1993) and Williams et al. (1993). Standard DOC calibration curves were generated using both glucose and CO_2 gas, and DON calibration curves used EDTA, urea, and NO gas. The resultant CO_2 produced from DOC oxidation was detected using a Beckman model 880 non-dispersive IR detector. Nitric oxide produced

from TDN oxidation was detected using an Antek (Houston TX) chemiluminescence detector. Mean overall standard deviations for DOC and TDN were 3.5 and 0.5 µM, respectively. Nitrate and nitrite were determined on a Thermo-Environmental Instruments model 42 chemiluminescence NO-NO_2-NO_x analyzer, using the method outlined by Garside (1982) and Braman & Hendrix (1989). Ammonium was determined by the standard colorometric techniques of Solorzano (1969). DON was determined as the difference between TDN and the sum of nitrate plus nitrite plus ammonium.

Dissolved oxygen: Oxygen was monitored at each time point by harvesting triplicate 60 ml BOD bottles containing the same bacterial consortia and DOM concentrations as the polycarbonate incubation bottles. A YSI Dissolved Oxygen Meter (model 58) with a self-stirring BOD Bottle Probe (YSI model 5730), and having an analytical precision of ±1.5 µM, was used.

Heterotrophic bacteria and protists: Subsamples for bacterial and protist enumeration were collected at different time points and preserved in the dark in 4% filtered (0.2 µm polycarbonate filters, Poretics) buffered formalin. Cells were stained using 4,6-diamidino-2-phenylindole (DAPI, Sigma Chemical; Porter & Feig 1980) at 0.1 µg ml^{-1} DAPI (Cherrier et al. 1996), and counted by epifluourescence microscopy within 1 mo. This lag in counting may have resulted in slightly lower numbers than immediate counting (Turley 1993). Heterotrophic protist abundances were determined using the primulin (Sigma Chemical) staining method of Caron (1983). Bacterial C and N biomass was estimated from the product of bacterial abundance and constant biomass conversion factors of 19.6 fg C and 5.6 fg N per cell (Lee & Fuhrman 1987). The factor for C is virtually identical to the average for oceanic and coastal bacteria measured by Fukuda et al. (1998), and thus assumed to be reasonable for this hemi-pelagic environment.

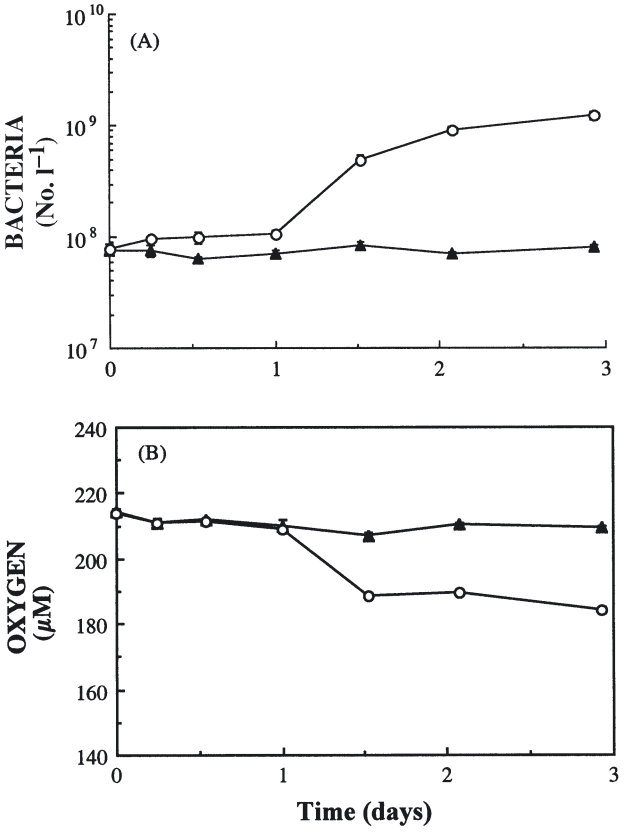

Fig. 1. Changes in (A) bacterial abundances and (B) oxygen over time for non-supplemented ambient seawater (▲) and DOM_{PD}-supplemented (○) incubations. Error bars represent ±1 SD of triplicate samples

RESULTS

Time-series incubations of seawater, either supplemented with DOM_{PD} or maintained at ambient DOM levels, were carried out to evaluate bacterial utilization of DFAA, DCAA, and DCHO as a function of bulk DOC and DON utilization and bacterial growth. No measurable increases in heterotrophic protist abundances were observed in any of the incubations (data not shown). Bacterial abundances and O_2 concentrations in non-supplemented incubations remained relatively constant throughout the incubation period (Fig. 1A,B, Table 1). Bacterial growth in +DOM_{PD} incubations followed a typical batch culture pattern of lag, exponential, and stationary phases with exponential growth occurring between Days 1 and 2 (Fig. 1A). Oxygen utilization in supplemented incubations showed a 1 d lag, followed by rapid consumption between Days 1 and 2, then slower rates of utilization after Day 2 (Fig. 1B).

No utilization of bulk DOC or DON was detected in non-supplemented incubations (Table 1). However, in +DOM_{PD} incubations, net 3 d decreases in DOC and DON averaged 51 ± 9 µM C and 9 ± 2.8 µM N, respectively (Table 1). No changes were observed in nitrate or nitrite for any of the treatments (mean NO_2^- + NO_3^- in both treatments was 11 ± 0.8 µM, data not shown).

Initial concentrations of MCHO, DCAA, and DFAA in +DOM_{PD} incubations were 1.4 ± 0.02, 2.2 ± 0.8, and 6.4 ± 0.6 µM, respectively, compared to those in ambient seawater of 0.30 ± 0.03, 0.55 ± 0.13, and 0.039 ± 0.02 µM, respectively (Fig. 2A–C). No changes in MCHO, DCAA, DFAA, and NH_4^+ concentrations were observed during the course of the experiment in the non-supplemented incubations (Fig. 2A–D, Table 1). In C and N equivalents, the DFAA accounted for 19 ± 0.4% of the added DOC_{PD} (i.e. +DOC_{PD} *minus* ambient

Table 1. Net changes and rates of utilization (–) or production (+) of parameters measured in 3 d seawater incubations with DOM_{PD} added. Errors in parentheses represent ±1 SD for triplicate incubations. nd = not determined

Measured parameter[a]	+DOM_{PD} Net change (µM)[b]	+DOM_{PD} Rate (µM d^{-1})[c]
Bacterial carbon	1.9 (0.20)	0.7 (0.08)
Bacterial nitrogen	0.54 (0.06)	0.2 (0.02)
Oxygen	–30.0 (1.5)	–1.3 (0.6)
DOC	–51.0 (9.0)	nd
DON	–9.0 (2.80)	nd
NH_4^+	9.1 (1.70)	3.5 (0.8)
MCHO	–0.87 (0.34)	–0.26 (0.06)
C equivalents	–5.22 (2.04)	–1.56 (0.36)
DCAA	–0.35 (0.9)	–0.14 (0.2)
C equivalents	–1.75 (4.5)	–0.70 (1.0)
N equivalents	–0.52 (1.4)	–0.21 (0.3)
DFAA	–6.22 (0.63)	–2.5 (0.4)
C equivalents	–31.1 (3.15)	–12.5 (2.0)
N equivalents	–9.33 (0.94)	–3.75 (0.7)
Uncharacterized[d]	–13.0 (5.8)	nd

[a]Net changes and rates of parameters were below detection in all ambient treatments (i.e. not supplemented with DOM_{PD})
[b]Net changes in +DOM_{PD} treatments represent differences in concentrations between initial and final incubation time points
[c]Rates in +DOM_{PD} treatments were determined by the slopes of least-squares linear regressions of parameters against time
[d]Uncharacterized component estimated as DOC – (MCHO + DCAA + DFAA), all in C equivalents; see text for details

treatments) and 27 ± 0.9% of the added DON_{PD}, while DCAA represented 5 ± 2.8 and 7 ± 0.4%, respectively. The MCHO represented only ~4 ± 0.02% of the added DOC_{PD}. Small decreases in MCHO and DCAA were observed in seawater supplemented with DOM_{PD} following the initial 1 d lag period (Fig. 2A & B, respectively). In contrast to all other measured parameters, a slight decrease in DFAA concentration was observed during Day 1 of incubation, followed by rapid utilization into Day 2 (Fig. 2C). Only 2% of the DFAA remained by the end of the 3 d incubation. Concurrent with DFAA loss and bacterial growth was ammonium production (Fig. 2D), which, with the exception of Day 1, was balanced stoichiometrically by DFAA-N remineralization (Fig. 3, Table 1).

Rates of MCHO, DCAA, DFAA, and O_2 utilization (–µM d^{-1}), and NH_4^+ and bacterial C and N production (+µM d^{-1}) were determined by least-squares linear regression of each parameter against incubation time for all data points in triplicate samples (Table 1). Lag periods were not observed consistently in all measured parameters (e.g. DFAA). Thus, for purposes of compar-

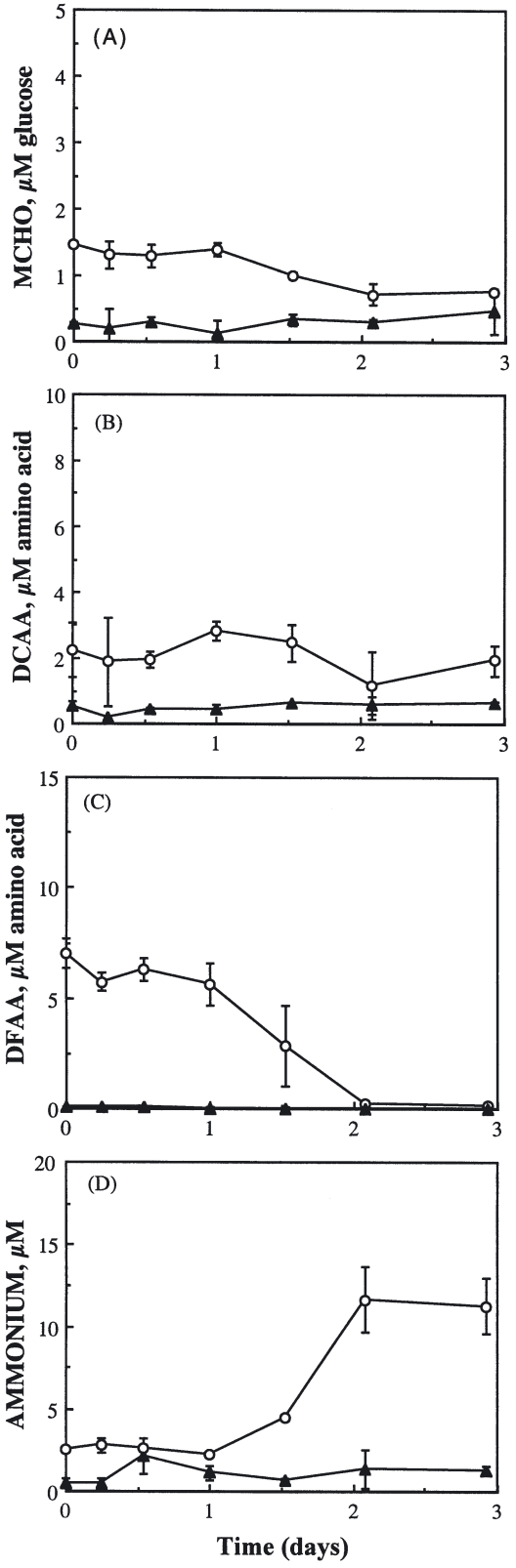

Fig. 2. Changes in (A) MCHO, (B) DCAA, (C) DFAA, and (D) NH_4^+ over time for non-supplemented ambient seawater (▲) and DOM_{PD}-supplemented (○) incubations. Error bars represent ±1 SD of triplicate samples

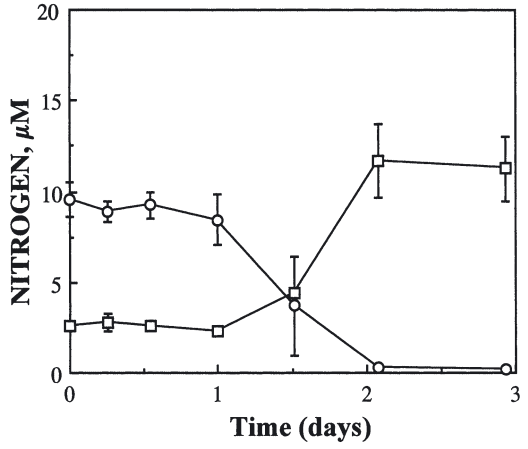

Fig. 3. Total DFAA utilization (○) and ammonium production (□) in N equivalents for DOM_{PD}-supplemented seawater. Error bars represent ±1 SD of triplicate samples

ison and consistency between parameters, the lag period was not taken into account in the regressions. Maximal rate estimates of the various parameters are therefore considered conservative.

In the $+DOM_{PD}$ incubations, utilization rates of MCHO (0.26 ± 0.06 µM glucose d^{-1} or 1.56 ± 0.36 µM C d^{-1}) and DCAA (0.14 ± 0.2 µM amino acid d^{-1} or 0.7 ± 1.0 µM C d^{-1} and 0.21 ± 0.3 µM N d^{-1} equivalent) were 1 order of magnitude lower than DFAA rates (2.5 ± 0.4 µM amino acid d^{-1} or 12.5 ± 2.0 µM C d^{-1} and 3.75 ± 0.7 µM N d^{-1}) (Fig. 2A–C, Table 1). The utilization rate of DFAA (i.e. in N equivalents) was equivalent to NH_4^+ production (i.e. 3.5 ± 0.8 µM N d^{-1}). Bacterial C and N production rates in $+DOM_{PD}$ incubations were 0.7 ± 0.1 µM C d^{-1} and 0.2 ± 0.02 µM N d^{-1}, respectively, with a corresponding O_2 utilization rate of 13.1 ± 2.1 µM O_2 d^{-1} (Table 1).

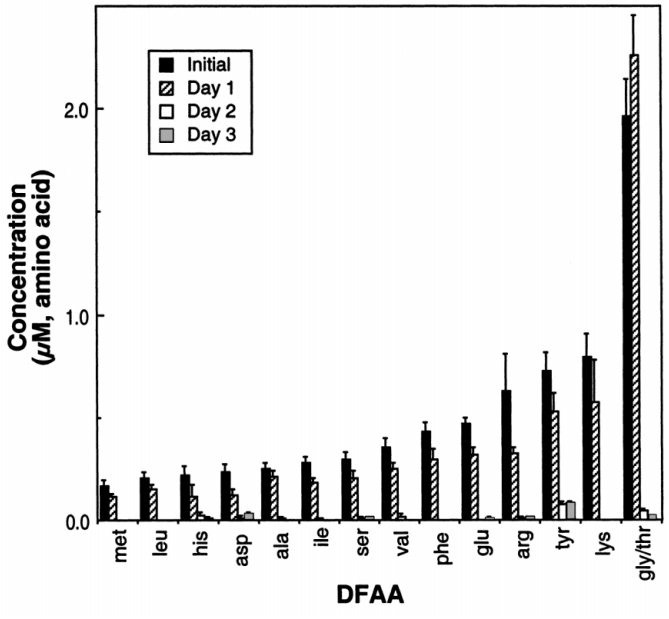

Fig. 4. Concentrations of individual DFAA (in µM amino acid) in DOM_{PD}-supplemented seawater at Time 0, Day 1, Day 2, and Day 3 of the incubation. Error bars represent ±1 SD of triplicate samples

Concentrations of individual DFAA in the $+DOM_{PD}$ treatment for selected time points (i.e. initial, and Days 1, 2, and 3; Fig. 4) were initially as low as 0.17 ± 0.03 µM for methionine to 1.96 ± 0.18 µM for glycine/threonine. No significant changes were observed for any of the individual DFAA during Day 1 of the incubation. However, dramatic decreases for all DFAA occurred between Days 1 and 2 (Fig. 4). After Day 2, no further significant changes in individual DFAA concentrations were noted. In addition, statistically significant differences in individual amino acid uptake rate constants were not observed (data not shown), suggesting non-selective utilization of individual DFAA.

DISCUSSION

Previous investigations of bacterial DOM utilization in temperate eastern North Pacific surface waters (Cherrier et al. 1996) found that bulk DOC_{PD} and DON_{PD} were utilized at relatively low bacterial growth efficiencies (BGE ≈ 4%), suggesting that the majority of seawater DOC and DON is used to meet the energetic needs of the microbial community. The present study expanded upon the earlier findings by assessing how specific DOM constituents of the planktonic community potentially support bacterial production and remineralization in pelagic waters.

Starting $+DOM_{PD}$ incubations contained ~163 µM added DOC (i.e. 229 µM DOC total minus 66 µM DOC ambient) and 35 µM added DON. We were able to successfully characterize 28 ± 3% of this DOC_{PD} as DFAA (19 ± 0.4%), DCAA (5 ± 0.6%), and MCHO (4 ± 0.02%; Figs. 2A–C & 5, Table 1). The remaining 'uncharacterized' DOC_{PD} accounted for 72 ± 3.4% of the carbon, estimated as the difference between the total added DOC (163 µM C) and the sum of MCHO, DCAA, and DFAA carbon. Approximately 34 ± 4.7% of the DON in the added DOM_{PD} could be accounted for by DFAA (27 ± 2.7%) and DCAA (7 ± 3.8%) nitrogen, with the remainder (66 ± 7.2%) being uncharacterized (Fig. 2B,C). Compounds associated with zooplankton ingestion, digestion, death, and initial decay potentially contributing to the uncharacterized fraction in DOM_{PD} may include urea, amino sugars, lipids, nucleic acids, and methlyamines, colloids and, depending on

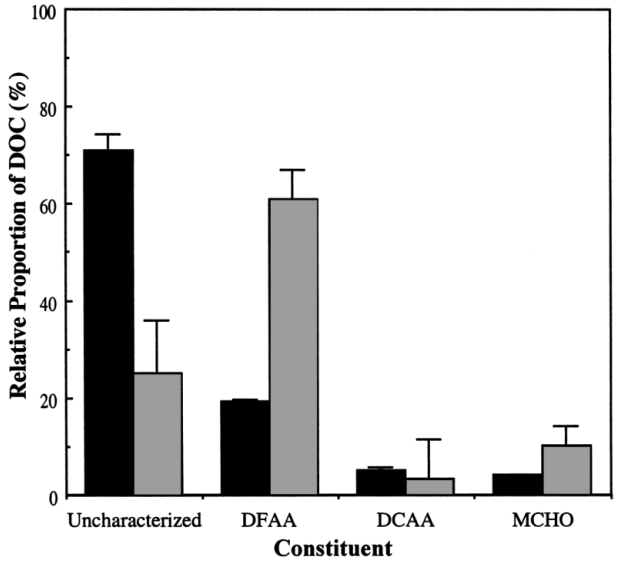

Fig. 5. Relative proportions of carbon constituents in DOM_{PD}-supplemented seawater at Time 0 (black bars) and consumed during the 3 d incubation period (gray bars). Error bars represent ±1 SD of triplicate samples

formation rates, possibly humic-bound organics (Hubberton et al. 1995, Kaiser & Benner 2000, Nagata 2000, Benner 2002, Bronk 2002, Carlson 2002).

Net DOC utilization (51 ± 9 µM C), O_2 consumption (30 ± 1.5 µM O_2), and bacterial C production (1.9 ± 0.2 µM C) in $+DOM_{PD}$ incubations were similar to net changes observed by Cherrier et al. (1996; 48 ± 9 µM DOC, 31 ± 9 µM O_2, and 1.7 ± 0.4 µM bacterial C, respectively) in June 1992 for similarly supplemented samples. However, net DON utilization (9.0 ± 2.8 µM N) in the present study was about 3-fold greater than in June 1992 (2.9 ± 1.8 µM N), suggesting that the degree of C and/or energy limitation may have been greater in July 1993. Such limitation may be due to the relatively lower concentrations of bioavailable substrates (e.g. DFAA and MCHO) in seawater at 85 m at Stn M in July 1993 (0.039 µM DFAA and 0.300 µM MCHO; this study) compared to June 1992 (0.26 µM DFAA and 2.3 µM MCHO; Shultz 1994).

Microheterotrophic utilization of plankton-derived DOM constituents

Approximately 31 ± 5% of the added DOC and 26 ± 8% of the added DON in the DOM_{PD}-supplemented seawater was utilized during the course of the 3 d incubation (Table 1). The degree of labile DOC reactivity (i.e. having turnover times on the order of minutes to days) is within the range reported for other marine systems under conditions of transiently elevated DOM (Ogura 1972, 1975, Kirchman 1990, Amon & Benner 1994, 1996, Søndergaard & Middelboe 1995, Weiss & Simon 1999). Approximately 50% of the MCHO, 14% of the DCAA, 98% of the DFAA (Fig. 2A–C, respectively) and 25% of the uncharacterized DOC (Fig. 5) in the $+DOM_{PD}$ incubations were utilized over the course of 3 d, indicating a wide range in the availabilities of these different constituents in fresh plankton-derived material. It is also interesting to note that despite the differences in bulk DOC concentrations in ambient (66 µM) and $+DOM_{PD}$ (112 µM) incubations by Day 3, the relative proportions of characterized and uncharacterized DOM were similar for both (Fig. 6). This implies that refractory DOM may either be an inherent component of 'fresh' plankton (Williams 2000) or form rapidly upon release to seawater (Brophy & Carlson 1989, Ogawa et al. 2001).

While DFAA, DCAA, and MCHO together represented only ~28% of the added DOC in the $+DOM_{PD}$ incubations, their collective utilization accounted for 75 ± 11.5% of the total DOC consumed (Fig. 5). Rates of DFAA utilization (2.4 ± 0.4 µM amino acid d^{-1} or 12.5 ± 2.0 µM C d^{-1}) in $+DOM_{PD}$ incubations were comparable to those in other studies (e.g. Carlucci et al. 1986, Fuhrman 1987, Middelboe et al. 1995, Rosenstock & Simon 2001). However, the relative contribution of DFAA to overall bacterial carbon demand (BCD, 61 ± 6%; Fig. 5) in $+DOM_{PD}$ incubations is significantly higher than that in other oligotrophic systems (≤20% for the Sargasso Sea; Suttle et al. 1991, Keil & Kirch-

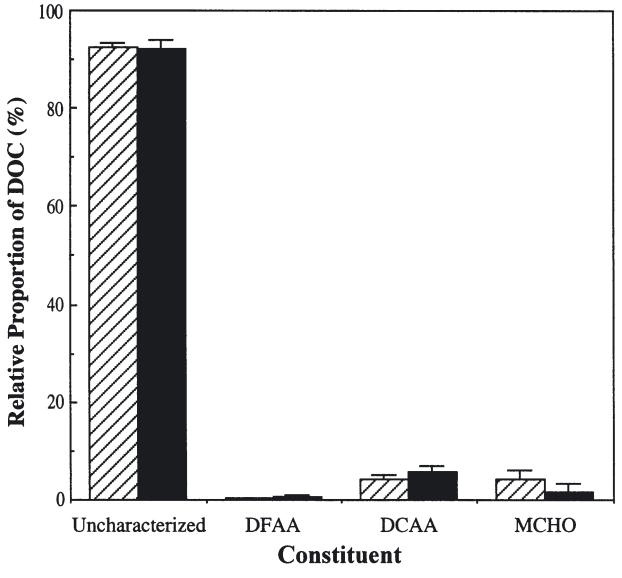

Fig. 6. Relative proportions of uncharacterized and characterized carbon constituents in non-supplemented ambient (cross-hatched bars) and DOM_{PD}-supplemented (black bars) seawater at the conclusion of the 3 d incubation period. Error bars represent ±1 SD of triplicate samples

man 1999), but within the range of diel and seasonal maxima reported in some non-marine systems (i.e. Jørgensen 1987, Tranvik & Jørgensen 1995, Rosenstock & Simon 2001). Thus, N-rich substrates such as DFAA may, when available in adequate concentrations, fulfill as much or more bacterial C demand as non-N compounds (Rich et al. 1996, 1997, Keil & Kirchman 1999, Skoog et al. 1999, Kirchman et al. 2001).

The relative contribution of DCAA to BCD was slightly lower (3 ± 8%; Fig. 5) than what has been found in other marine systems (i.e. ~10 to 25%; Jørgensen et al. 1993, Keil & Kirchman 1993, 1999, Middelboe et al. 1995). The BCD estimate derived for MCHO in the present study (10 ± 4%; Fig. 5) is similar to Gulf of Mexico bacterioplankton (5 to 10%; Skoog et al. 1999), but lower than observed for the equatorial Pacific (15 to 45%; Rich et al. 1996). One reason for these differences may be that our measurements reflect changes in the total MCHO pool, whereas Rich et al. (1996) and Skoog et al. (1999) examined changes in the neutral sugars sub-fraction of the MCHO pool, which is believed to be the most bioavailable fraction of the total MCHO pool (Benner 2002 and references therein). The MBTH method used here measures all monosaccharides (including neutral sugars), and thus net changes in MCHO may be due almost exclusively to neutral sugars, leading to lower than expected BCD estimates.

In contrast to carbon, all of the DON consumed could be accounted for by DFAA (99 ± 10%) and DCAA (6 ± 15%) utilization (Table 1). Thus, DFAA and DCAA met 100% of the bacterial N requirements and accounted for 100% of the DON remineralized to NH_4^+. These observations are similar to those of Fuhrman (1990) and Jørgensen et al. (1993) who reported that DOM, in the form of DFAA, supported greater than 64% of the C demand and 100% of the N demand of coastal bacterial populations. However, the essentially complete fulfillment of N demand by DFAA at Stn M in $+DOM_{PD}$ incubations also contrasts with a number of other open ocean environments where the range was lower under ambient conditions (~4 to 41%; Kirchman 2000). It is possible that when labile substrates such as DFAA are present at low, ambient levels, bacteria may compete more intensely for these substrates, thus forcing their greater dependence on 'less-preferred' forms of DON. In the presence of high, transient DFAA concentrations, however, bacteria may be released from this competition and reliance on other DON forms, and, over the short term at least, rely entirely on the more labile DFAA. The present findings further indicate that the indigenous populations at Stn M are primarily remineralizers of N, similar to what has been found in other aquatic systems (see, e.g., Cotner & Gardner 1993 and Haga et al. 1995), even when labile forms of DON are presumably non-limiting over short timescales.

The remainder of the DOC (25 ± 11%) could not be characterized as 1 of the 3 constituents analyzed in this study (Fig. 5). Because total hydrolyzable carbohydrates (i.e. polysaccharides, PCHO) are often considered to be a bioavailable component of seawater DOM (see, e.g., Williams & Yentch 1976, Ittekkot et al. 1982, Benner et al. 1992, Weiss & Simon 1999, Amon et al. 2001), the consumption of the uncharacterized DOM_{PD} could be attributable, at least in part, to PCHO, which were not analyzed as part of this study. Approximately 69 and 71% of the added DOC and DON, respectively, remained by Day 3 of the incubation. This residual material potentially represents semi-labile or refractory fractions of the bulk DOC and DON pools that cycle on week-month or much longer timescales (Ogura 1972, Carlson & Ducklow 1995, Cherrier et al. 1996, Carlson et al. 2002). Further work is thus needed to elucidate the composition and utilization of this relatively unavailable but geochemically young DOM fraction.

The higher utilization rate for DFAA relative to MCHO in $+DOM_{PD}$ seawater (Table 1) is consistent with observations by Ittekkot et al. (1982) who reported higher *in situ* removal rates of TDAA than total dissolved sugars during a phytoplankton bloom in the North Sea. These findings are also similar to those of Williams & Yentsch (1976) who observed higher turnover rates of amino acids versus carbohydrates from phytoplankton exudates. Our findings contrast, however, with those of Amon et al. (2001) who reported higher utilization rates of total hydrolyzable neutral sugars (THNS) than total hydrolyzable amino acids (THAA) during bacterial growth on algal-derived DOM. They attributed the higher THNS utilization rate to the 'higher overall bioreactivity of neutral sugars relative to amino acids'.

The variable findings from these different studies may arise at least in part from differences in analytical procedures. In the present study, the DFAA, DCAA, and MCHO constituents of DOM_{PD} were monitored, whereas Amon et al. (2001) evaluated THNS (i.e. MCHO + PCHO) and THAA (DFAA + DCAA). A possible advantage of following changes in individual constituents (i.e. MCHO and PCHO; DFAA and DCAA) versus THNS and THAA is that the relative ratio of the low molecular weight to higher molecular weight constituents in each substrate class (i.e. MCHO:PCHO or DFAA:DCAA) may be ascertained. Without this knowledge, it is difficult to determine whether a higher utilization rate associated with one substrate class over another is due to its overall greater bioavailability or whether it is merely a function of the presence of a higher proportion of either LMW or HMW

labile constituents. The variability in findings from different studies may also result from regional and other differences in the microheterotrophic communities. Since the physiologic status and taxonomic composition of bacterial assemblages is known to vary both temporally and spatially (del Giorgio & Cole 2000, Giovannoni & Rappé 2000), it stands to reason that the fate of potential substrates (i.e. growth vs respiration) will also vary. These factors may be especially important in open ocean systems where the energetic constraints on bacterial growth and survival may be quite high (del Giorgio & Cole 2000, Carlson 2002 and references therein).

BGE under DOM-supplemented conditions

BGE was estimated for the $+DOM_{PD}$ incubations using net changes in bacterial C production (as estimated from bacterial abundances) and DOC utilization (Table 1) as $BGE_{DOC} = (\Delta B_{carbon}/\Delta DOC) \times 100$ yielding a mean BGE of 3.7 ± 1.8%. An independent estimate of BGE was also calculated using the sum of the carbon equivalent net utilization for the DFAA, DCAA, and MCHO constituents (i.e. 38 ± 5.8 µM C d^{-1}; Table 1), resulting in a slightly higher value ($BGE_{\Sigma CONSTITS}$) of 5 ± 0.2%. These estimates of BGE, although quite low, are within the range (~3 to 5%) observed previously for this region of the Pacific by Cherrier et al. (1996) using bulk DOC and DON utilization. These values are also within the range of BGE estimates (2 to 9%) summarized by Carlson (2002) for oligotrophic Atlantic and Pacific waters using $\Delta B_{carbon}/\Delta DOC$, and are approximately half of the oceanic median BGE value of 9% as summarized by del Giorgio & Cole (2000).

There are several possible reasons for low BGE in this and other oligotrophic ocean regions, including C- and energy limitation and bulk metabolism of mixed substrates by a taxonomically diverse population. The quality of the available substrates as well as the energetic costs associated with surviving in an oligotrophic environment (Morita 1997, del Giorgio & Cole 2000, del Giorgio & Duarte 2002) suggest that low growth efficiencies could be related to the degree of C- and energy limitation of the indigenous bacterial populations at Stn M. In addition, the estimated BGE represents mean bacterial growth on a mixture of substrates having different degrees of bioavailability. Although the DOM_{PD} contained significant concentrations of DFAA, which theoretically should have resulted in higher growth efficiencies (~9% as observed by Cherrier et al. 1996, for Stn M natural bacterial consortia grown on a pure DFAA mixture), it also contained DCAA, MCHO, as well as a suite of other uncharacterized and perhaps more complex substrates that were utilized or co-metabolized (Carlson et al. 2002) over the course of the incubation. Other factors potentially contributing to the low observed BGE include secondary or indirect factors such as depletion of limiting inorganic nutrients (e.g. N, P, or trace elements). Finally, as suggested by del Giorgio & Cole (2000), low BGE values may be an artifact of only a small subpopulation of bacteria in the incubations actually growing, and a larger component of the population remineralizing the DOM_{PD} but not actively growing (see, e.g., Cottrell & Kirchman 2000, Carlson 2002). As a cautionary note, Turley & Hughes (1992) showed that formalin-preserved seawater samples stored up to 40 d resulted in up to a 39% decrease in bacterial cell numbers. However, even if we adjust bacterial cell numbers and resultant net changes in bacterial C (2.4 µM C) to account for this maximum potential 39% loss, the BGE estimates (i.e. 5% for BGE_{DOC} and 6% for $BGE_{\Sigma CONSTITS}$) are not altered significantly.

Role of individual DFAA in DOC and DON turnover and net bacterial production

Ambient levels of DCAA (0.55 µM) and DFAA (0.039 µM) measured at 85 m at our study site are consistent with earlier findings of these compound classes in open ocean surface waters (Lee & Bada 1977, Mopper & Lindroth 1982, Keil & Kirchman 1991b, 1999). In contrast, in $+DOM_{PD}$ incubations, initial DCAA abundances were proportionately lower than DFAA (DCAA:DFAA = 0.34). Due to the often tight coupling between DFAA production and utilization processes in marine systems (Billen & Fontigny 1987, Fuhrman 1987, Suttle et al. 1991, Keil & Kirchman 1999), DFAA are not expected to accumulate to the high concentrations observed in the DOM_{PD}-supplemented seawater. However, within 2 d, the DFAA in $+DOM_{PD}$ incubations were drawn down to levels (0.20 µM) close to levels (0.260 µM; Shultz 1994) observed at this site in June 1992. Additionally, by Day 2 of incubation, DCAA:DFAA in the $+DOM_{PD}$ incubation had increased to 6.2, and by Day 3 had re-attained ambient values of approximately 14.

At the start of the incubation, glycine and threonine were present in the highest concentrations, followed by lysine, tyrosine, and arginine, respectively (Fig. 4). Except for tyrosine and threonine, these amino acids are important zooplankton osmolytes (Jeffries & Alzara 1970), which likely accounts for their elevated concentrations in the DOM_{PD} compared to the other amino acids. The elution gradient used during HPLC analysis of the DFAAs did not allow for the separation of glycine and threonine. However, given that (1) oceanic concentrations of threonine are typically much lower

than those for glycine (Gardner & Paffenhofer 1982, Ittekkot et al. 1984a,b) and (2) threonine is not stored by zooplankton as an osmolyte (Jeffries & Alzara 1970), the co-eluted glycine/threonine was likely comprised primarily of glycine.

Approximately 12% of the DFAA were consumed during Day 1 of incubation without concomitant measurable changes in O_2 utilization ammonium production (i.e. via deamination), suggesting that DFAA were either (1) being used primarily for biosynthetic purposes or (2) an artifact of sorptive removal, for example to the container walls or colloidal particles. The most significant decrease in all individual DFAA occurred between Days 1 and 2 (Fig. 4), during which ~97% of the remaining DFAA were utilized. In contrast to Day 1 of incubation, the uptake of DFAA between Days 1 and 2 coincided with high rates of respiration and NH_4^+ production, indicating that the community had switched to using these amino acids primarily as energy sources after an initial period of biosynthesis (del Giorgio & Cole 1998, 2000 and references therein).

The only DFAA above ambient levels by the end of the +DOM_{PD} incubation were tyrosine, arginine, glutamate, and aspartate (Fig. 7), suggesting that certain DFAAs may persist longer than others, thus serving either as an extended source of C, N, and energy, or as precursors of quasi-refractory or refractory DOM. Final concentrations of histadine, serine, and glycine in DOM_{PD}-supplemented seawater at Day 3 were approximately equal to their respective ambient concentrations suggesting that at background concentrations, these DFAA are not utilized effectively due to threshold or reactivity limitations (Fuhrman & Ferguson 1986, Keil & Kirchman 1999). In contrast, methionine, leucine, alanine, isoleucine, and lysine were all depleted to below detection by the conclusion of the +DOM_{PD} incubations (Fig. 7), suggesting that these specific DFAA potentially contribute to the highly labile fraction of DOM that turns over on the order of hours to days (Kirchman et al. 1993, Carlson & Ducklow 1995, 1996, Cherrier et al. 1996).

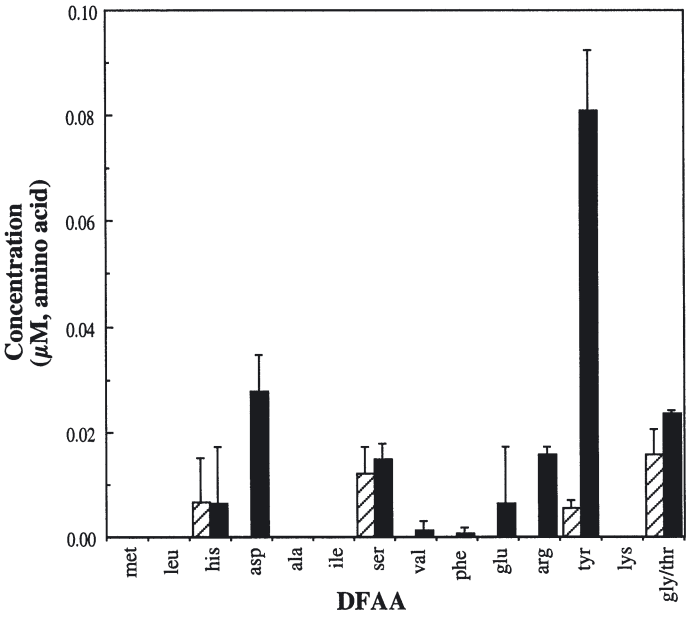

Fig. 7. Concentrations of individual DFAA (in μM amino acid) in non-supplemented ambient (hatched bars) and DOM_{PD}-supplemented (black bars) seawater at the conclusion of the 3 d incubation period. Error bars represent ±1 SD of triplicate samples

These findings collectively suggest that in permanently or temporally energy-limited systems such as Stn M, DFAA and other DOM components from episodic events (e.g. bloom die-offs, grazing activity, etc.) may be an important intermittent, short-term source of C and N for bacterial heterotrophs. Additionally, the rapid depletion to near ambient levels of the plankton-derived DFAA, DCAA, and MCHO, along with their relatively low concentrations in ambient seawater, support the contention that bacterial growth in oceanic systems is supported by a relatively small part of the total DOM (Kirchman et al. 1991, Noorman et al. 1995, Carlson & Ducklow 1996, Cherrier et al. 1996, Keil & Kirchman 1999, Amon et al. 2001, Carlson 2002, Carlson et al. 2002). However, despite the elevated short-term availability of these seemingly reactive constituents in DOM_{PD}-supplemented incubations, BGE estimates were still very low. Thus, even during short, transient periods of greater reactive substrate availability, oceanic bacteria may remineralize the vast majority of DOC and DON.

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