Interactions of bacterivorous grazers and heterotrophic bacteria with dissolved organic matter

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ABSTRACT: Ciliated Protozoa (*Euplotes* sp. and *Uronema* sp.) and natural microzooplankton assemblages (10 to 200 μm) – collected from Los Angeles Harbor, a coastal lagoon, and San Pedro Channel – were fed bacteria previously labeled with $^{14}\text{C}(\text{U})$ -glucose. Dissolved organic carbon-14 (DO^{14}C) released during grazing was collected and subsequently fractionated by AMICON ultrafiltration into 5 nominal molecular weight (NMW) classes: $>10^5$, 10^4 to 10^5 , 10^3 to 10^4 , 5×10^2 to 10^3 , and $<5 \times 10^2$ daltons. Release of labeled organic compounds with low ($<5 \times 10^2$ daltons) and intermediate NMW (10^3 to 10^4 daltons) was most rapid in experimental and control samples and was enhanced in the presence of grazers. Unlabeled bacterioplankton assemblages were incubated 24 h with either fractionated or unfractionated DO^{14}C which was released during grazing experiments. Bacterioplankton utilized $196\% \text{ d}^{-1} \pm 100$ more of the DO^{14}C released in presence of cultured ciliates or microzooplankton than DO^{14}C released in their absence. Low ($<5 \times 10^2$ daltons) and intermediate NMW (10^3 to 10^4 daltons) labeled fractions were incorporated and respired more rapidly by bacteria than the other 3 NMW labeled fractions. Fractionation of DO^{14}C after bacterial incubation revealed a decrease in all NMW classes and a shift toward a dominance of low NMW labeled fractions in the DO^{14}C released in the presence of grazers, but not in the controls. Bacterivorous ciliates and microzooplankton contribute quantitatively and qualitatively to the dissolved organic matter pool in nearshore planktonic systems. Dissolved organic matter released in the presence of bacterivores is a readily available carbon source for bacterioplankton and may thereby influence bacterioplankton metabolism and growth activity.

INTRODUCTION

The microheterotrophically-mediated flow of carbon in marine systems is a topic of keen interest to marine microbiologists. Microheterotrophs, particularly bacteria, have an essential role in regulating the dynamics of carbon flux in aquatic systems (Pomeroy 1974). Recent information – reviewed in Azam et al. (1983), Ducklow (1983), and Sieburth (1984) – suggests that bacterivory (ingestion of bacteria) by microzooplankton (20 to 200 μm in diameter, primarily ciliated Protozoa and Micrometazoa) and nanozooplankton (2 to 20 μm in diameter), primarily heterotrophic mastigophoran and ciliated protozoans, may regulate standing stocks, species composition, and metabolic activity of bac-

terioplankton. By regulating bacterial activity, bacterivores thereby indirectly impact dissolved carbon flux dynamics. This study examines the hypothesis that bacterivorous microzooplankton may also directly influence dissolved carbon flux by releasing, or stimulating release, of dissolved organic matter (DOM). Ciliated protozoans and microzooplankton (operationally defined here as phagotrophic organisms 10 to 200 μm in diameter) were the focus of this study because they can be cultured and collected relatively easily, they are abundant in nearshore planktonic systems, and their trophic role is not well understood.

The ocean represents a large reservoir of DOM. According to Bada & Lee (1977), oceanic waters contain 0.5 to 1.5 mg l^{-1} dissolved organic carbon. The chemical composition of this DOM pool, however, is poorly known. Bada & Lee report that the vast majority of the DOM pool is composed of complex, refractory materials and a small, but significant portion of this

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material is composed of biologically active organic compounds. Of the biologically active dissolved compounds, amino acids, peptides, proteins, mono- and polysaccharides, fatty acids, organic acids, and nucleotides have been detected in seawater (reviewed in Williams 1975). The sources of these compounds are not well known.

In marine systems, planktonic protozoans represent a diverse and ubiquitous component of the water column, contributing a biomass of 1 to 1600 $\mu\text{g C l}^{-1}$ (Taylor 1982). Many of the reported species probably feed on bacteria to varying degrees. Studies of Johannes (1965) and Fenchel & Harrison (1976) demonstrated that bacterivorous protozoan grazers accelerate remineralization of phosphorus and decomposition of detritus. As a result, bacterivory may enhance bacterial metabolic activity by excreting inorganic and organic nutrients and reducing resource competition between members of the bacterioplankton assemblage (Fenchel & Harrison 1976).

To our knowledge, this is the first study to investigate the qualitative spectrum of DOM released during grazing by ciliates and microzooplankton. As a first approximation, we examined the molecular size/weight distribution of DOM released during incubations with bacterivorous protozoans feeding on labeled bacteria. Further microheterotrophic (bacterial) interactions with DOM released during grazing were also examined. Dissolved organic carbon-14 (DO^{14}C), released in the presence of bacterivores fed bacteria previously labeled with ^{14}C (U)-glucose, was separated by ultrafiltration into 5 molecular-weight fractions and compared with DO^{14}C released in the presence of bacteria alone to assess the gross characteristics of 'grazer-enhanced' DOM. Suspensions of unlabeled bacterioplankton were incubated with fractionated and unfractionated DO^{14}C to examine the effects of microheterotrophic utilization on DO^{14}C pool composition.

MATERIALS AND METHODS

Grazing experiments. Grazing experiments using ^{14}C -labeled bacterioplankton were employed to examine DO^{14}C released in the presence and absence of microbial grazers. Details of labeling and grazing protocols appear in Taylor & Sullivan (1984). Dilute inocula of bacterioplankton (ca. 10^4 bact. l^{-1}) were incubated in 0.1 l of low organic labeling medium (f/2 made with precombusted Rila sea salts, 1/10 f/2 vitamin concentration, NH_4Cl substituted for NaNO_3 ; Guillard & Ryther 1962) which contained 100 to 250 $\mu\text{Ci l}^{-1}$ of D-[U- ^{14}C]-glucose (4.86 nCi [nmole] $^{-1}$, New England Nuclear, NEC-042A). Incubations were terminated when cells reached stationary phase and

instantaneous ^{14}C -respiration rates were minimized. Labeled suspensions were then incubated for 4 h with unlabeled glucose (1.0 μM , final conc.) to replace labile ^{14}C -compounds with labile ^{12}C -compounds. Labeled cells were separated from spent medium by centrifugation (10 K rpm for 20 min at 5°C) and washed twice with chilled filter-sterilized seawater (FSSW) and were then concentrated by centrifugation. The concentrate was passed through a 1.0 μm Nuclepore membrane to eliminate large and aggregated cells. This labeling procedure yielded a morphologically diverse bacterial assemblage which retained a high proportion of the isotope in macromolecules (hourly net loss = 1 to 2% of incorporated label and virtually 100% of incorporated label resided in macromolecules, Taylor & Sullivan 1984). Small, labile ^{14}C -molecules in bacterial intracellular pools were minimized by labeling with the pulse-chase technique but not totally eliminated due to endogenous turnover of macromolecules.

Ciliated protozoan isolates (*Uronema* sp. and *Euplotes* sp.) were cultured in organically enriched f/2 medium (boiled extract of rice in seawater, 3 g l^{-1}) and were prepared for experiments at mid-exponential growth phase. Field samples were collected from surface waters by dip bucket or flasks from the following sites: (1) a tidal delta in a Southern California marsh (Mugu Lagoon, 13 Nov 80) on the incoming tide (courtesy of S. Kottmeier); (2) Los Angeles Harbor, 1 km inside breakwater (24 Mar 82); (3) San Pedro Channel, 19 km offshore along transect from Los Angeles Harbor to Big Fisherman's Cove, Santa Catalina Island, CA. (13 Jan 82). To enhance the grazing assay signal, microzooplankton in field samples (2) and (3) was gently concentrated through a 10.0 μm Nitex mesh tube by gravity-driven reverse flow (Capriulo & Carpenter 1980) 19.4 and 40 times ambient concentration, respectively. Microzooplankton in field sample (1) was not concentrated.

To remove grazers from control samples, one half of the grazer suspension was passed through a Nuclepore membrane (5.0 μm for *Uronema* sp.; 10.0 μm for *Euplotes* sp. and microzooplankton assemblages) under low vacuum (< 5 cm Hg). These live controls contained bacterioplankton and in field samples presumably contained some microflagellates although they were not quantified in this study. In order to offset the addition of labeled cells, unlabeled bacteria were removed as follows: (1) one third of the volumes of experimental and control samples were passed through 1.0 μm Nuclepore membranes to remove bacterial fraction; (2) < 1.0 μm fractions were passed through a 0.2 μm Nuclepore membranes to remove bacteria from medium; (3) concentrates of labeled bacteria were added to < 0.2 μm filtrates; (4) labeled sus-

pensions were added back to experimental and control samples to initiate grazing experiments.

Cell concentrations in grazing experiments were: *Uronema* sp. – 2.15×10^6 cells l^{-1} , 10.30×10^9 bact l^{-1} (2 % labeled, $2.30 \mu Ci l^{-1}$); *Euplotes* sp. – 1.65×10^5 cells l^{-1} , 7.25×10^9 bact l^{-1} (10 % labeled, $3.26 \mu Ci l^{-1}$); Mugu Lagoon – 319 ng ATP l^{-1} in $>10.0 \mu m$ fraction (56 % of total ATP, protozoan numbers not determined), 1.48×10^9 bact l^{-1} (30 % labeled, $0.40 \mu Ci l^{-1}$); Los Angeles Harbor – 4.81×10^3 ciliates l^{-1} (biovolume = $2.19 \times 10^6 \mu m^3 l^{-1}$), 1.50×10^9 bact l^{-1} (33.3 % labeled, $0.49 \mu Ci l^{-1}$); San Pedro Channel – 8.48×10^3 ciliates l^{-1} (biovolume = $15.84 \times 10^6 \mu m^3 l^{-1}$), 4.74×10^9 bact l^{-1} (30.2 % labeled, $2.94 \mu Ci l^{-1}$).

Grazing experiments were conducted for 6 h at $18^\circ C$ in darkness. Rates of ^{14}C -incorporation into grazer size fraction, ^{14}C -respiration, and ^{14}C -exudation were measured as described in Taylor & Sullivan (1984). Briefly, ^{14}C -incorporation was determined from radioactivity of rinsed Nuclepore membrane retentates ($5.0 \mu m$ for *Uronema* sp.; $10.0 \mu m$ for *Euplotes* sp. and microzooplankton) from experimental and control samples taken at 0 and 6 h. ^{14}C -respiration rates were determined as described in Hobbie & Crawford (1969). ^{14}C -exudation rates were measured as described in Sharp (1977), except that acidified (0.1 N HCl) samples were shaken vigorously in a fume hood for 40 min instead of sparged. Ingestion and exudation rates were calculated as presented in Taylor & Sullivan (1984).

Bacterial biomass was estimated by epifluorescence microscopy using direct counting and sizing of samples fixed with borate-buffered formalin (2 % final conc.) and stained with acridine orange (Hobbie et al. 1977; Zimmermann 1977). Ciliate and microzooplankton biomasses were estimated by counting and sizing settled cells fixed and stained with Lugol's Iodine (Utermöhl 1931). Carbon biomass was calculated based on estimated biovolume: bacterial carbon content – $87 fg C \mu m^{-3}$ ($fg = 10^{-15} g$), Krempin et al. (1981); protozoan carbon content – $88 fg C \mu m^{-3}$, Heinbokel (1978).

Collection of released labeled DOM. Following grazing experiments, $DO^{14}C$ was collected by passing samples (0.5 to 0.8 l) through a Whatman No. 1 Qualitative prefilter and a $0.2 \mu m$ Nuclepore membrane under 15 cm Hg vacuum at $5^\circ C$. The filtrate was divided into halves and frozen until needed for molecular-size fractionation or bacterial incubation. See Fig. 1 for sample handling protocol.

Characterization of labeled DOM. Procedures for characterization of $DO^{14}C$ were similar to those described for analysis of phytoplankton exudates in Faust & Chrost (1981) and Iturriaga (1981). Prior to ultrafiltration, $DO^{14}C$ samples were thawed, acidified to pH 2.8 with 0.1N HCl, sparged with air for 40 min at

$20^\circ C$ to remove $^{14}CO_2$, and then titrated to pH 7.8 with 0.1N NaOH. The first replicate of each sample (250 to 400 ml) was placed in an AMICON Model 402 Ultrafiltration Cell and ultrafiltered at $0^\circ C$ under maximum prescribed pressures. Prior to ultrafiltration, two 2 ml aliquots were removed and radioassayed for total $DO^{14}C$ activity. Samples were passed sequentially through AMICON DIAFLO XM100, YM10, UM2, UM05 ultrafiltration membranes representing nominal molecular weight (NMW) cut-offs of 10^5 , 10^4 , 10^3 , and 5×10^2 daltons (1 dalton = $1/16$ mass of oxygen atom = $1.65 \times 10^{-24} g$), respectively. Samples were concentrated to approximately 5 ml, filtrate removed, and stored at $0^\circ C$ for subsequent fractionation. The 5 ml concentrate was diluted with 25 ml sterile 3.5 % NaCl in double deionized water and ultrafiltered again to 5 ml to remove labeled molecules smaller than the NMW cut-off of the membrane. By repeating the rinse procedure 3 times, 97.2 % of the labeled molecules

Fig. 1. Schematic of $DO^{14}C$ characterization protocol. Samples were sequentially fractionated

smaller than the NMW cut-off of the membrane were removed from the concentrate (determined arithmetically). The 5 ml concentrates were removed and diluted to 25 ml with sterile 3.5 % NaCl rinse water. Duplicate 2 ml aliquots from the concentrate and filtrate were radioassayed (165 to 8800 dpm aliquot $^{-1}$). The remaining 21 ml of concentrate was stored at $-20^\circ C$ until needed for bacterial incubation. This fractionation procedure yielded NMW distributions of $DO^{14}C$ released in the presence of bacterivorous microzooplankton (microzoo(+) = grazers and bacteria) and in the absence of bacterivorous microzooplankton (microzoo(-) = bacteria only or bacteria and microflagellates).

Bacterial utilization of labeled DOM. The second replicate of the original sample (untreated sample, Fig. 1) and the 5 NMW fractions were incubated with bacteria from the same source as the grazing experiment. The untreated samples were purged of $^{14}CO_2$ as described above. Bacterial suspensions ($< 1.0 \mu m$ fil-

trate) were concentrated 300 to 400 times over a 0.2 μm Nuclepore membrane as described by Hollibaugh et al. (1980) and resuspended in the samples. For the fractionated samples, bacterial suspensions were mixed 1:1 with the 5 fractions. All samples were incubated for 24 h on a New Brunswick Gyrotary Shaker Table at 100 rpm, 18 °C, in the dark. At 0 h, subsamples were fixed in 2 % borate-buffered formalin (v/v, final concentration) as a blank. At 24 h, subsamples were assayed for bacterial incorporation and respiration of DO^{14}C as described by Wright & Hobbie (1966) and Hobbie & Crawford (1969), respectively. Non-aqueous samples were radioassayed in PPO, POPOP, and toluene scintillation fluor and aqueous samples were suspended in Aquasol-2 (New England Nuclear) scintillation fluor. All samples were radioassayed in a Beckman LSC-100 Scintillation Counter and corrected for quench by the external standard method.

Bacterial modification of labeled DOM. After incubation with bacterioplankton (described above), unfractionated DO^{14}C samples were subjected to the same ultrafiltration series as described above. Aliquots of the concentrates and filtrates were radioassayed for experimental and control samples to observe effects of bacterial utilization on NMW distributions. Radioactivities of concentrates and filtrates were used to calculate NMW distributions.

RESULTS

Bactiory and DOM release

Radioassay results indicate that *Uronema* sp. and *Euplotes* sp. ingested 1.58×10^3 and 3.08×10^3 bacteria ciliate $^{-1}\text{h}^{-1}$, respectively (calculated from Table 1). At measured feeding rates, *Uronema* sp. and *Euplotes* sp. released 8.9 and 3.0 % of the ingested ^{14}C -biomass as DO^{14}C , respectively (Table 1, Col 3). Compared to other unpublished grazing-rate data for these species, ingestion rates reported in Table 1 were low, as were specific exudation rates (% carbon exuded / carbon ingested). Bacteria in the ciliate cultures had mean cell volumes of $0.23 \mu\text{m}^3 \pm 0.19$ and contained an estimated 87 fg C μm^{-3} (Krempin et al. 1981) which yielded a cell carbon content of 20 fg C cell $^{-1}$. Based on these calculations, grazing by *Uronema* sp. and *Euplotes* sp. potentially enhanced the DOM pool by 3.07 ± 0.18 and $0.30 \pm 0.07 \mu\text{g C l}^{-1}\text{h}^{-1}$, respectively (Table 1, Col. 4).

Radioassay estimates of bactiory rates for microzooplankton assemblages (10 to 200 μm) in samples collected from surface waters of Mugu Lagoon, Los Angeles Harbor and San Pedro Channel were 7.05, 0.14, and 0.03×10^7 bact l $^{-1}\text{h}^{-1}$, respectively (Table 1).

Table 1. Summary of grazing parameters from DO^{14}C production experiments. Means ($\bar{x}$) and standard deviations (SD) of 3 replicates

Sample	Bacteria ingested $\bar{l}$ ($\times 10^7$ bac l $^{-1}\text{h}^{-1}$)	Bacterial carbon ingested I_c (ngC l $^{-1}\text{h}^{-1}$)	Specific exudation rate e (% of I)	Exuded bacterial carbon E_c (ngC l $^{-1}\text{h}^{-1}$)
<i>Uronema</i> sp. ($G^* = 131$, $N^{**} = 124$)				
$\bar{x}$	172.20	34460	8.9	3067
SD	10.90	2181	0.3	103
<i>Euplotes</i> sp. ($G^* = 741$, $N^{**} = 13$)				
$\bar{x}$	50.82	10170	3.0	305
SD	1.16	232	0.7	71
Mugu Lagoon (STA MID, 13 Nov 80) ($G^* = \text{ND}$, $N^{**} = 12$)				
$\bar{x}$	7.05	552	88.2	487
SD	0.03	2	64.1	354
Los Angeles Harbor (24 Mar 82) ($G^* = 1$, $N^{**} = 12$)				
$\bar{x}$	0.14	11	20.2	2
SD	0.01	1	4.3	0
San Pedro Channel (13 Jan 82) ($G^* = 1$, $N^{**} = 37$)				
$\bar{x}$	0.03	2	26.2	1
SD	0.00	0	20.1	0
G^* microzooplankton biomass, $\mu\text{g C l}^{-1}$				
N^{**} bacterial biomass, $\mu\text{g C l}^{-1}$				
ND not determined				

Microzooplankton in the harbor sample was dominated by the ciliates *Strombidium* sp., *Euplotes* sp., and *Uronema* sp. (biomass = 1.0 $\mu\text{gC l}^{-1}$). In the channel sample, *Strombidium* sp. and 6 species of tintinnids were observed (biomass = 1.1 $\mu\text{gC l}^{-1}$). Microzooplankton identification was not performed on the lagoon sample. Specific exudation rates were variable and higher for natural assemblages than for cultured isolates. In lagoon, harbor, and channel samples, release of DO^{14}C in the presence of microzooplankton was 89, 20 and 26 % of the ingested bacterial carbon 14 , respectively. According to calculations presented in Krempin et al. (1981), mean bacterial cell carbon in these samples was an estimated 8 fg C cell $^{-1}$. Based on radioassay estimates, bactiory by microzooplankton enhanced release of DOM by 487 ± 354 , 2 ± 0 , and 1 ± 0 ng C l $^{-1}\text{h}^{-1}$ in lagoon, harbor, and channel samples, respectively.

Adsorptive losses of labeled DOM

Data from 15 ultrafiltration series were assessed to determine losses of DO^{14}C due to adsorptive properties of the ultrafiltration apparatus and membranes. Radiolabel budgets were calculated by comparing total activity of concentrates, filtrates, and rinses after a run to the activity of the original suspension (% loss = [input - conc. - filtr. - rinses] $\times 100$ input $^{-1}$). Recov-

ered labeled compounds were reduced by 14 to 37 %, $\bar{x} = 26$, at each step in the ultrafiltration series. After running the entire fractionation series, the mean cumulative loss of label for all samples was 44 ± 22 % of the original sample activity. No significant differences (Student's T-test, 95 % confidence limits) in mean adsorption between experimental and control samples were observed for any individual membrane or between the 4 individual DIAFLO membranes. It was not possible to determine if NMW fractions were adsorbed selectively or uniformly at each step.

Characterization of released labeled DOM

Fig. 2 presents histograms of NMW distribution for DO^{14}C released in 5 separate experiments as a result of 6 h incubations in the presence and absence of ciliates and microzooplankton. Within an experiment, free-living bacterial concentrations (labeled and unlabeled) were not detectably different in experimental and control treatments. Fractions A, B, C, D, and E represent the NMW ranges: $< 5 \times 10^2$, 5×10^2 to 10^3 , 10^3 to 10^4 , 10^4 to 10^5 , and $> 10^5$ daltons, respectively.

With the exception of Fig. 2d, ^{14}C -compounds with $< 5 \times 10^2$ (A) and 10^3 to 10^4 (C) NMW were detected in the highest abundance. ^{14}C -compounds with $> 10^5$ (E) NMW represented the third most abundant fraction. Labeled compounds with 5×10^2 to 10^3 (B) and 10^4 to 10^5 (D) NMW were consistently detected in lowest

abundances. To varying degrees, ^{14}C -compounds with $< 5 \times 10^2$ (A) and 10^3 to 10^4 (C) NMW were detected in greater abundance in microzoo (+) treatments than in microzoo (-) treatments in all studies. Grazers enhanced net release of DO^{14}C by 6 to 205 %, $\bar{x} = 73$ %, and release of ^{14}C -compounds with $< 5 \times 10^2$ (A) and 10^3 to 10^4 (C) NMW by 4 to 227 %, $\bar{x} = 99$ % and 9 to 122 %, $\bar{x} = 57$ %, respectively (Fig. 2a to e). These data also reveal that net release of ^{14}C -compounds with $> 10^5$ (E) NMW in microzoo(+) treatments was apparently less than or equal to release of this fraction in microzoo(-) treatments. Release of ^{14}C -compounds with 5×10^2 to 10^3 (B) and 10^4 to 10^5 (D) NMW did not differ discernibly between treatments.

Mean release values, expressed as % of total DO^{14}C , from all 5 experiments are summarized in Fig. 3. Although absolute amounts of material in each fraction varied between treatments, relative proportions of each fraction were similar. The relative proportion of ^{14}C -compounds with $> 10^5$ NMW, however, was 2.5 times greater in the microzoo(-) treatment than in the microzoo(+) treatment (16.5 and 6.6 % of total labeled pool, respectively).

Bacterial utilization of labeled DOM

Bacterial utilization studies were conducted with individual fractions and with unfractionated samples generated in the *Uronema* sp., *Euplotes* sp., and Mugu

Fig. 2. Nominal molecular weight (NMW) distribution of DO^{14}C released in 6 h grazing experiments. Fractions A to E represent $< 5 \times 10^2$, 5×10^2 to 10^3 , 10^3 to 10^4 , 10^4 to 10^5 , $> 10^5$ daltons, respectively. Solid bars: microzooplankton present; open bars: microzooplankton absent. (a) microzooplankton = *Uronema* sp. = 21.50×10^5 cells l^{-1} , bacterial concentration at 0 h, $N_0 = 10.3 \times 10^9$ bact l^{-1} ; (b) microzooplankton = *Euplotes* sp. = 1.65×10^5 cells l^{-1} , $N_0 = 7.25 \times 10^9$ bact l^{-1} ; (c) Mugu Lagoon sample (13 Nov 80), microzooplankton concentration not determined, $N_0 = 1.48 \times 10^9$ bact l^{-1} ; (d) Los Angeles Harbor sample (24 Mar 82), ciliates = 4.81×10^3 cells l^{-1} , $N_0 = 1.50 \times 10^9$ bact l^{-1} , stippled bar: $< 10.0 \mu\text{m}$ fraction; (e) San Pedro Channel sample (13 Jan 82, 15 km offshore), ciliates = 8.48×10^3 cells l^{-1} , $N_0 = 4.74 \times 10^9$ bact l^{-1} . Means and ranges of 2 analytical replicates reported

Fig. 3. Relative abundances of 5 NMW fractions released in (a) Microzoo treatment(+); (b) microzoo(-) treatment during 6 h grazing experiments. Mean activities ($\times 10^3$ dpm l⁻¹) of each fraction from 5 experiments presented as % of total activity

Lagoon grazing experiments. Fig. 4a to c present uptake (total bar length), incorporation (shaded portion), and respiration (open portion) data for these experiments. ¹⁴C-compounds released in both treatments with $< 5 \times 10^2$ (A) and 10^3 to 10^4 (C) NMW were most rapidly incorporated and respired by bacteria. With the exception of ¹⁴C-compounds with 10^4 to 10^5 (D) NMW released in the Mugu Lagoon microzoo(+) treatment (Fig. 4c), ¹⁴C-compounds with 5×10^2 to 10^3 (B) and 10^4 to 10^5 (D) NMW were utilized slowly. In the *Euplotes* sp. study, fraction E ($> 10^5$ NMW) was lost (Fig. 4b), but a comparison of Fig. 2b (fraction E release) and 6b (fraction E remaining after bacterial utilization) reveals that ¹⁴C-compounds with $> 10^5$ NMW were probably utilized relatively rapidly.

Rates of incorporation (I), respiration (R), and uptake (U), expressed as % of total DO¹⁴C d⁻¹ in incubations with bacteria appear in Table 2. All fractions were utilized by bacterial assemblages in these incubations. For the 3 studies, mean uptake rates of all microzoo(+) and microzoo(-) fractions were $51\% \text{ d}^{-1} \pm 15$ and $41\% \text{ d}^{-1} \pm 18$, respectively. To assess whether bac-

terioplankton preferentially select ¹⁴C-compounds in the 5 fractions, mean bacterial uptake rates for the 3 experiments were expressed as proportions of the total DO¹⁴C released in microzoo(+) and (-) treatments. ¹⁴C-compounds with $< 5 \times 10^2$ NMW were taken up in excess of their relative abundances (from Fig. 3), uptake = 60.9 and 55.4 % of total pool, abundance = 53.0 and 45.1 % of total pool, respectively. Conversely, ¹⁴C-compounds released in both treatments with $> 5 \times 10^5$ NMW were taken up in lower proportions than their relative abundances, uptake = 2.7 and 3.9 % of total pool, abundance = 6.6 and 16.5 % of total pool. Relative uptake of the other 3 fractions did not vary consistently from their relative abundances.

Bacterial rates of ¹⁴C-respiration of fractionated and unfractionated samples, calculated as % of uptake (U) were analyzed to assess qualitative differences between microzoo(+) and microzoo(-) DO¹⁴C (calculated from Table 2, data not presented). Only the Mugu Lagoon study displayed any differences in respiration of microzoo(+) and microzoo(-) fractions. In this data set, bacterial respiration rates of ¹⁴C-compounds released in microzoo(+) treatment with $< 5 \times 10^2$, 5×10^2 to 10^3 , 10^3 to 10^4 , 10^4 to 10^5 , and $> 5 \times 10^5$ NMW and the unfractionated sample were 27400, 36, 36, 18, 19, and 348 % of bacterial respiration rate of comparable microzoo(-) samples. In the *Uronema* sp. study, there was no detectable respiration of ¹⁴C-compounds with 10^4 to 10^5 (D) and $> 5 \times 10^5$ (E) NMW for DO¹⁴C released in both treatments and similarly respiration of these fractions was relatively low in the Mugu Lagoon study.

In conjunction with the DO¹⁴C utilization study, cell yields were measured after 24 h incubations in fractionated and unfractionated DO¹⁴C. Table 3 presents results from the *Uronema* sp. and *Euplotes* sp. studies. In general, cell yields were similar for both treatments. In the *Uronema* sp. study, incubations with ¹⁴C-compounds released in microzoo(-) treatments with 10^3 to

Fig. 4. Rates of bacterial uptake (total bar), incorporation (shaded portion), and respiration (open portion) of DO¹⁴C fractions. Suspensions of bacterioplankton were incubated at 18 °C for 24 h with individual DO¹⁴C fractions. (a) DO¹⁴C produced in *Uronema* sp. grazing experiment, $N_0 = 0.71 \times 10^9$ bact l⁻¹ ± 0.12 ; (b) DO¹⁴C produced in *Euplotes* sp. grazing experiment, $N_0 = 3.18 \times 10^9$ bact l⁻¹ ± 0.50 ; (c) DO¹⁴C produced in Mugu Lagoon grazing experiment, $N_0 = 0.74 \times 10^9$ bact l⁻¹ ± 0.09 . Solid bars: DO¹⁴C produced in presence of microzooplankton; stippled bars: DO¹⁴C produced in absence of microzooplankton. Means and ranges of 2 analytical replicates

Table 2. Bacterial utilization rates of fractionated and unfractionated DO¹⁴C. Means of 2 replicates expressed as % of available ¹⁴C d⁻¹

DO ¹⁴ C sample	NMW <5 × 10 ²			5 × 10 ² –10 ³			10 ³ –10 ⁴			10 ⁴ –10 ⁵			> 10 ⁵			Untreated*		
	I	R	U	I	R	U	I	R	U	I	R	U	I	R	U	I	R	U
<i>Uronema</i> sp.																		
Grazer present	38.8	11.2	50.0	38.5	6.9	45.4	30.7	7.4	38.1	67.8	0.0	67.8	25.0	0.0	25.0	24.6	15.2	39.8
Grazer absent	60.3	17.6	77.9	57.6	8.4	66.0	53.2	13.5	66.7	32.8	0.0	32.8	40.6	0.0	40.6	38.9	12.6	51.5
<i>Euplotes</i> sp.																		
Grazer present	35.2	17.7	52.9	28.0	25.4	53.4	26.2	30.4	56.6	15.4	45.8	61.2	ND**			47.0	19.2	66.2
Grazer absent	34.4	15.2	49.6	9.4	23.2	32.6	21.4	30.3	51.7	22.6	14.0	36.6	ND			54.2	22.8	77.0
Mugu lagoon																		
Grazer present	31.3	38.0	69.3	30.2	6.3	36.5	28.9	4.3	33.2	74.0	4.7	78.7	43.7	1.6	45.3	9.9	9.8	19.7
Grazer absent	16.1	0.4	16.5	9.8	9.3	19.1	23.8	13.0	36.8	13.8	7.1	20.9	19.3	4.4	23.7	11.4	1.9	13.3
I incorporation, R respiration, U uptake (U = I + R), * Untreated, i.e. unfractionated DO ¹⁴ C, ** ND not determined																		

 I incorporation, R respiration, U uptake (U = I + R), * Untreated, i.e. unfractionated DO¹⁴C, ** ND not determined

 Fig. 5. Relative proportions of labeled fractions utilized (incorporated and respired) by bacterioplankton during 24 h incubation (summary of data from Fig. 4). DO¹⁴C produced in (a) microzoo(+); (b) microzoo(-) treatments during 6 h grazing experiments. Mean uptake rates ($\times 10^3$ dpm l⁻¹ d⁻¹) of each fraction from 3 experiments presented as % of total uptake

10⁴ (C) and > 5 × 10⁵ (E) NMW yielded slightly more cells than in these fractions from the microzoo(+) treatment. In the *Euplotes* sp. study, however, incubations with ¹⁴C-compounds with < 5 × 10² (A) and 10³ to 10⁴ (C) NMW released in the microzoo(+) treatment produced 220 to 304 % more cells than incubations with those fractions released in the microzoo(-) treatment.

NMW distribution of labeled DOM after bacterial utilization

Fig. 6 presents histograms of NMW distributions from 3 experiments in which unfractionated DO¹⁴C was incubated for 24h with bacterial assemblages. In Fig. 6c, concentrations of Fractions A to D released in the microzoo(-) treatment were calculated based on bacterial utilization data (Fig. 4c) and the original

NMW distribution (Fig. 2c), because the < 10⁵ NMW fraction was lost before it could be fractionated further. Comparison of Fig. 2a to c with Fig. 6a to c reveals substantial reductions in the 3 major peaks; ¹⁴C-compounds with < 5 × 10² (A), 10³ to 10⁴ (C), and > 10⁵ (E) NMW. Few changes were observed in the abundances of ¹⁴C-compounds with 5 × 10² to 10³ (B) and 10⁴ to 10⁵ (D) NMW, with the exception of Fig. 6c in which the concentration of ¹⁴C-compounds with 10⁴ to 10⁵ (D) NMW released in microzoo(+) treatment was reduced 77 % by subsequent bacterial utilization. All 3 figures demonstrate reductions in concentrations of all NMW classes and suggest a shift in the NMW distribution toward low NMW compounds.

Changes in NMW distribution are summarized in Table 4. Data presented in the last column of this table demonstrate that incubation with bacteria reduced the sums of all fractions in microzoo(+) treatments by 56 to 78 %, $\bar{x}$ = 68, and by 41 to 88 %, $\bar{x}$ = 66 in microzoo(-) treatments. In the *Uronema* sp. and Mugu Lagoon studies, concentrations of DO¹⁴C released in microzoo(+) treatments were reduced 2.2 and 2.8 times more rapidly by bacteria than in the microzoo(-) treatments, respectively. In the *Euplotes* sp. study, DO¹⁴C concentrations in the microzoo(+) and microzoo(-) treatments were reduced at comparable rates (22.2 and 26.6 × 10⁴ dpm l⁻¹ d⁻¹, respectively). Based on all 3 studies, concentrations of ¹⁴C-compounds with < 5 × 10² (A) NMW released in microzoo(+) treatments were reduced 1.23 to 8.65 times more rapidly than concentration of the same fraction released in the microzoo(-) treatment. Concentrations of ¹⁴C-compounds with 10³ to 10⁴ (C) NMW released in the microzoo(+) treatment were reduced 2.91, 4.55, and 0.88 times that of the same fractions released in microzoo(-) treatments in the *Uronema* sp., Mugu Lagoon, and *Euplotes* sp. studies, respectively. Concentrations of ¹⁴C-compounds released in the

Table 3. Summary of 24 h cell yields for bacterial assemblages grown in the presence of fractionated and unfractionated DO¹⁴C. Means ($\bar{x}$) and standard deviations (SD) of 10 determinations

Sample		Bacterial yield ($\times 10^6$ cells ml ⁻¹ d ⁻¹)					
		NMW fractions*					Untreated
		A	B	C	D	E	
<i>Uronema</i> sp.							
Microzoo (+)	$\bar{x}$	3.09	1.88	1.66	1.26	1.33	ND
	SD	0.81	0.39	0.21	0.33	0.45	ND
Microzoo (-)	$\bar{x}$	3.57	1.22	3.02	1.29	2.14	ND
	SD	0.75	0.33	0.55	0.24	0.43	ND
<i>Euplotes</i> sp.							
Microzoo (+)	$\bar{x}$	3.04	3.43	9.28	4.08	ND	5.02
	SD	0.95	0.72	1.69	0.80	ND	1.07
Microzoo (-)	$\bar{x}$	0.00	2.35	4.22	4.16	ND	4.43
	SD	0.00	0.64	1.33	1.42	ND	1.32

* NMW fractions: A = $<5 \times 10^2$; B = 5×10^2 to 10^3 ; C = 10^3 to 10^4 ; D = 10^4 to 10^5 ; E = $>10^5$.
Untreated = prior to fractionation
ND not determined

Fig. 6. NMW distribution of DO¹⁴C after 24 h bacterial incubation with unfractionated DO¹⁴C produced in grazing experiments. (a) DO¹⁴C produced in *Uronema* sp. grazing experiment, $N_0 \times 1.32 \times 10^9$ bact l⁻¹ ± 0.24 ; (b) DO¹⁴C produced in *Euplotes* sp. grazing experiment, $N_0 = 2.18 \times 10^9$ bact l⁻¹ ± 0.30 ; (c) DO¹⁴C produced in Mugu Lagoon grazing experiment, $N_0 = 1.21 \times 10^9$ bact l⁻¹ ± 0.22 . Solid bars: DO¹⁴C produced in presence of microzooplankton; open bars: DO¹⁴C produced in absence of microzooplankton. Means and ranges of 2 analytical replicates

Table 4. Summary of changes in DO¹⁴C concentrations after 24 h bacterial incubation. Means of 2 replicates

DO ¹⁴ C sample	NMW		$<5 \times 10^2$		$5 \times 10^2 - 10^3$		$10^3 - 10^4$		$10^4 - 10^5$		$>10^5$		Sum*	
	$\times 10^4$	%	$\times 10^4$	%	$\times 10^4$	%	$\times 10^4$	%	$\times 10^4$	%	$\times 10^4$	%	$\times 10^4$	%
	dpm	of	dpm	of	dpm	of	dpm	of	dpm	of	dpm	of	dpm	of
	l ⁻¹ d ⁻¹	total	l ⁻¹ d ⁻¹	total	l ⁻¹ d ⁻¹	total	l ⁻¹ d ⁻¹	total	l ⁻¹ d ⁻¹	total	l ⁻¹ d ⁻¹	total	l ⁻¹ d ⁻¹	total
<i>Uronema</i> sp.														
Grazer present	-20.91	-35.94	-0.30	-5.56	-40.61	-96.75	+0.45	+60.00	+0.30	+10.00	-61.07	-56.2		
Grazer absent	-13.18	-72.50	-0.52	-48.57	-13.94	-80.70	-0.61	-66.67	+1.21	+61.54	-27.04	-67.9		
<i>Euplotes</i> sp.														
Grazer present	-15.00	-84.74	-0.10	-10.00	-4.50	-78.95	+0.25	+35.71	-2.80	-90.32	-22.15	-78.5		
Grazer absent	-12.15	-85.87	-0.55	-64.17	-5.10	-91.89	-0.45	-56.25	-8.40	-96.56	-26.65	-88.4		
Mugu Lagoon														
Grazer present	-1.73	-67.05	-0.07	-25.96	-0.82	-89.13	-0.46	-76.67	-0.64	-68.08	-3.72	-69.9		
Grazer absent	-0.20	-16.26	-0.07	-19.44	-0.18	-36.00	-0.02	-40.00	-0.87	-76.32	-1.34	-40.7		

* Sum of fluxes in individual NMW classes

microzoo(+) treatment with $>10^5$ NMW, however, were reduced 0.33 and 0.74 times that of the same fraction released in microzoo(-) treatments in the *Uronema* sp. and Mugu Lagoon studies.

In order to examine whether subsequent bacterial utilization altered the proportions of the 5 NMW fractions relative to the total pool, NMW distribution data from the 3 post-bacterial incubations were pooled and the means presented in Fig. 7. Comparison of Fig. 3 (DO^{14}C release) with Fig. 7 illustrates that bacterial utilization has little influence on the relative abundances of ^{14}C -compounds released in the microzoo(-) treatments. The relative abundances of ^{14}C -com-

Fig. 7. Relative abundances of 5 NMW fractions remaining in DO^{14}C after 24 h incubation with bacterioplankton (summary of data from Fig. 6). DO^{14}C produced in (a) microzoo(+); (b) microzoo(-) treatments during 6 h grazing experiments. Mean activities ($\times 10^3 \text{ dpm l}^{-1}$) of each fraction from 3 experiments presented as % of total activity

pounds released in the microzoo(+) treatments, however, were substantially altered subsequent to bacterial utilization. In the microzoo (+) treatments, ^{14}C -compounds with $<5 \times 10^2$ NMW comprised 53.0 and 72.0 % of total pool prior to and after bacterial utilization, respectively, and ^{14}C -compounds with 5×10^2 to 10^3 NMW shifted from 5.1 to 11.4 % of total pool. In the same treatment, ^{14}C -compounds with 10^3 to 10^4 NMW shifted from 33.1 to 5.1 % of total pool subsequent to bacterial utilization. The relative proportions of the remaining two fractions (10^4 to 10^5 and $\geq 10^5$ NMW) were only slightly altered.

DISCUSSION

Quantitative importance of bacterivore exudation

Unpublished data from field experiments performed by Taylor suggest that carbon release resulting from grazing activities of bacterivorous microzooplankton was quantitatively significant, contributing 6.5 to 88.2 %

($x = 38.9 \pm 23.5$) of ingested bacterial carbon to the DOM pool at his study sites. His estimates of bacterivore DOM input varied from $0.13 \text{ ng C l}^{-1}\text{h}^{-1}$ in nearshore waters (San Pedro Channel) to $1351.57 \text{ ng C l}^{-1}\text{h}^{-1}$ in eutrophic waters (Mugu Lagoon). At 3 sampling sites in Mugu Lagoon, DOM release enhanced by bacterivorous microzooplankton potentially contributed 2 to 6 % to total DOM daily (assuming ambient DOM concentration = 0.5 to 1.5 mg C l^{-1} , Bada & Lee 1977). At 3 relatively oligotrophic sites in San Pedro Channel, however, DOM release enhanced by bacterivorous microzooplankton appeared to be quantitatively less, 0.0002 to 0.0006 % of total DOM d^{-1} . The relative importance of bacterivore-mediated DOM flux to these systems is unknown because he measured net fluxes only, and estimates of recycling and instantaneous flux rates are required. Measurement of instantaneous fluxes and recycling, although experimentally unpracticable at present, may yield interpretations different from those based on net fluxes and ambient concentrations.

The dynamics of bacterial recycling is likely to vary between systems and types of dissolved organic compounds. Hence the role of products released by bacterivores in bacterial metabolism cannot be adequately assessed by gross quantitative measurements. Direct measurement of grazer carbon exudation, although highly desirable, is only possible with axenically grown or physically separated grazers as reported in Soldo et al. (1978). Such treatment, however, does not permit investigation of phagotrophic organisms under relevant ecological and physiological conditions. We believe that conditions employed in this study approximate natural conditions more closely than previous studies with axenically cultured ciliates.

Limitations of methodology

Ruby & Neilson (1977) and Dunstall & Nalewajko (1975) reported that a significant portion of nutrient uptake by luminous marine bacteria was released as organic exudates. In suspensions with mixed bacterial species, exudates produced by some species may be incorporated by other species. Use of live labeled bacteria as a food source as reported above, potentially confounded grazer release of DO^{14}C due to bacterial label exudation and short-term recycling. In the present study, the problem of bacterial label release was minimized by: (1) using bacteria whose macromolecules were labeled almost exclusively at the beginning of the experiment (Taylor & Sullivan 1984); (2) comparing experimental treatments with live bacterial treatments. The observed release of DO^{14}C in the bacterial treatments after 6 h incubations may be

attributed to one or more of the following: (1) endogenous turnover of macromolecules; (2) exoenzyme and mucopolysaccharide production; (3) microflagellate grazing; (4) lysis; (5) parasitism. The exact source(s) of DO^{14}C cannot be determined from these experiments. As proposed in Fenchel & Harrison (1976) inorganic nutrient regeneration in microcosms may be enhanced by protozoan grazers as a result of indirect grazing effects on bacterial exudation rather than direct grazer exudation. Cognizant of possible grazer-induced alterations in bacterial activity, we consider DO^{14}C produced in experimental treatments to be 'grazer-enhanced' rather than grazer-produced DO^{14}C . Additionally, correction for short-term bacterial recycling was experimentally not possible. Therefore, DO^{14}C produced during grazing should be considered to be net DO^{14}C released.

Interpretation of our molecular-size fractionation data may be influenced by artifacts introduced by filtration, acidification of filtrate, and adsorption of DO^{14}C to DIAFLO membranes. We believe that filtration effects, i.e. lysis or extraction of soluble pools, were minimal because our filtration technique was relatively mild (15 cm Hg vacuum and 5 °C) and protozoan cells were presumably captured on the prefilter which had an undetectable pressure differential across it.

Acidification of the filtrates to drive off $^{14}\text{CO}_2$ is a protocol previously used in related studies which examined phytoplankton exudates (Faust & Chrost 1981, Iturriaga 1981, Chrost & Faust 1983). Acidification, however, may alter actual NMW distributions by changing size or state of some classes of compounds, e.g. proteins, nucleic acids and polysaccharides, and would thereby change their ultrafiltration characteristics. For example, indeterminate amounts of protein in solution will undergo dissociation of multimeric components or denaturation or agglutination upon acidification (G. Somero, pers. comm.). Some portion of the denatured protein may also undergo renaturation upon titration with 0.1 N NaOH to original pH. The acidification treatment employed in this study is believed to be sufficiently mild to minimize hydrolysis of polysaccharides. Burney & Sieburth (1977) found that complete hydrolysis of soluble polysaccharides in seawater required digestion at pH 1.1 and 100 °C for 20 h. Therefore, soluble polysaccharides, if present, in our samples probably remained in their native state. Acidification artifacts could potentially bias our results by enhancing high NMW fractions due to agglutination and by enhancing low NMW fractions due to denaturation and dissociation. We believe that release of proteins and nucleic acids by protozoan grazers is negligible because numerous studies have reported the presence of nucleases, proteases, peptidases, and

low pH (<3.0) in the food vacuoles of many taxa of phagotrophic protozoa (reviewed in Muller, 1967). However, we cannot rule out the possibility that the artifactual effects outlined above may have acted on bacterially-produced exudates.

Selective adsorption of some labeled compounds to the DIAFLO membranes may also bias apparent NMW distributions by altering amounts of some labeled products dissolved in the retentates and filtrates. Quantitative adsorption was not statistically different between treatments or between membranes. However, our data do not allow qualitative assessment of this problem. Despite its limitations, we believe ultrafiltration is the best tool available to examine molecular-weight distributions of DOM in grazing experiments and natural waters.

Qualitative importance of bacterivore exudation

Net rates of DO^{14}C production and grazer impact on NMW distribution varied widely between experiments and were positively correlated with bacterivory rates. As a result of bacterivory, DOM pools were apparently enriched in organic compounds with $<5 \times 10^2$ and 10^3 to 10^4 NMW. The other 3 NMW fractions did not vary consistently with respect to grazer enhancement in our study.

Maurer (1976) reported the following steady-state NMW distribution for natural DOM at a station in the Gulf of Mexico: $>10^3$ daltons, 10 to 15 % of total DOM; $>10^4$ daltons, <10 % of total DOM; and $>10^5$ daltons <5 % of total DOM. He proposed that DOM released from herbivorous grazing and phytoplankton exudation may be mostly proteins and carbohydrates in the 10^3 to 10^4 NMW range. Findings in the present studies partially support Maurer's proposal if applied to bacterivorous grazers. But for reasons stated above, release of proteins by bacterivorous protozoans is probably negligible. Bacterivory appears to enhance production of compounds with 10^3 to 10^4 NMW, but the $<5 \times 10^2$ NMW fraction appears to be more important. Release of large macromolecules ($>10^5$ NMW) also appears to be important, although not demonstrably 'grazer-enhanced'. High steady-state concentrations of large macromolecules dissolved in seawater have also been reported by Khailov & Finenko (1970).

The chemical nature of compounds released by bacterivores and bacteria is largely unknown. In field studies of oceanic DOM composition, Degens et al. (1964), Maurer (1976), Sieburth et al. (1976), Ogura (1977), and Burney et al. (1982) report significant (μg to mg^{-1}) concentrations of dissolved mono- and polysaccharides, proteins, amino acids, and unidentified macromolecules. Heterotrophic microflagellates (Pro-

tozoa-Mastigophora) in culture and at a coastal station in the Southern California Bight have been shown to enhance the production of dissolved free amino acids (Andersson et al. in press). In axenic laboratory cultures, Soldo et al. (1978) demonstrated that *Parauronema acutum* (Protozoa-Ciliophora) released guanine and hypoxanthine to the medium. Data cited above suggest the possibility that bacterivory directly or indirectly enhances levels of dissolved mono- and polysaccharides, purines, amino acids, and proteins. The substantial release of $>10^5$ NMW compounds reported for both treatments in the present study may be due to bacterial lysis or bacterial release of extracellular enzymes and mucopolysaccharides (Stanier et al. 1976, Geesey 1982).

Bacterial utilization of DOM

Rates of bacterial utilization are apparently rapid for the 3 abundant NMW fractions. Comparison of Fig. 3 and 5 suggests that NMW fractions are utilized by bacterioplankton at rates proportional to their concentration. Similar trends have been reported for phytoplankton exudates in Iturriaga (1981). Mean utilization rates of unfractionated samples were $41.9\% \pm 23.3$ and $47.3\% \pm 32.1$ of the total d^{-1} for $DO^{14}C$ produced in microzoo(+) and microzoo(-) treatments, respectively. In terms of total $DO^{14}C$ release and utilization, in 24 h bacteria utilized $196\% \pm 100$ more $DO^{14}C$ released in microzoo(+) treatments than in microzoo(-) treatments. In the *Uronema* sp. and Mugu Lagoon studies, $DO^{14}C$ released in microzoo(+) treatments was 2.74 and 1.61 times greater than that released in the microzoo(-) treatment, respectively. In the same studies, $DO^{14}C$ released in the microzoo(+) was utilized (uptake) by bacteria 2.26 and 2.78 times faster than that released in the microzoo(-) treatments. In the *Euplotes* sp. study, however, microzoo(+) release of $DO^{14}C$ was 93.6% of microzoo(-) $DO^{14}C$ release. $DO^{14}C$ utilized by bacteria was 83.1% as great in the microzoo(+) as in the microzoo(-) treatment. Inconsistencies introduced by the *Euplotes* sp. study may be a function of observed low bacterivory and exudation rates relative to the sensitivity of the radioassay methods and possible interference by recycling.

Bacterial respiration, expressed as % of uptake, was considered to be an approximate index of differences in nutrient quality. This index did not demonstrably vary between microzoo(+) and microzoo(-) fractions in laboratory culture studies, but varied substantially in the Mugu Lagoon study. Laboratory culture studies indicated that bacterial incorporation and respiration of each microzoo(+) fraction differed in total amount from microzoo(-) fractions, but not in relative proportions. As reported in Iturriaga (1981) for phytoplankton

exudates, macromolecular fractions (10^4 to 10^5 and $>10^5$ NMW) in this study also were more slowly respired by bacteria than lower NMW fractions.

Bacterial bioassay of nutrient quality of the 5 $DO^{14}C$ fractions yielded inconsistent results. This analysis suggested that labeled compounds in the $< 5 \times 10^2$ and 10^3 to 10^4 NMW fractions yielded higher bacterial growth than the other three fractions. In the *Euplotes* sp. study, bacterial growth in microzoo(+) fractions was substantially higher than in microzoo(-) fractions for the $< 5 \times 10^2$ and 10^3 to 10^4 daltons fractions. Net release of these $DO^{14}C$ fractions, however, was only slightly enhanced in the presence of ciliates. The observed enhancement of bacterial growth suggests that either a readily utilizable substrate or some bacterial growth promoting factor was released in the *Euplotes* sp. incubation. At present, too few data exist to test this hypothesis.

Bacterial modification of DOM

$DO^{14}C$ utilization and fractionation results (post-bacterial incubation) suggest that most of the $DO^{14}C$ produced during grazing is recycled within 24 h by bacteria and the residual $DO^{14}C$ is predominantly in the lower NMW fractions. As a result of bacterial incubation, overall concentrations of dissolved organic ^{14}C were reduced (45.3 to 67.9% in 24 h) and ^{14}C -compounds with 10^3 to 10^4 NMW were reduced to the greatest extent (43.2 to 75.9% in 24 h). Data for bacterial utilization (Fig. 5) indicated that ^{14}C -compounds with $< 5 \times 10^2$ NMW were utilized in excess of their relative abundance in both treatments. However, after bacterial utilization (Fig. 7a), relative abundances of ^{14}C -compounds from microzoo(+) treatments with $< 5 \times 10^2$ and 5×10^2 to 10^3 NMW were enhanced 19 and 6.3% above their relative abundances prior to 24 h incubations with bacteria (Fig. 3a). In contrast, the NMW distribution of ^{14}C -compounds from the microzoo(-) treatments was only slightly altered by subsequent bacterial interaction (Fig. 3b and 7b). These observations suggest that in at least 1 treatment bacteria released low NMW ^{14}C -compounds as a result of taking up the spectrum of ^{14}C -compounds offered.

Quantitative estimation of recycling rates of these products in nature is not possible due to the experimental manipulation employed. These results, however, strongly suggest that the bulk of DOM produced as a result of bacterial-bacterivore interactions is available for recycling by bacterioplankton. We suggest that bacterial activity may be responsible for altering the NMW distribution of DOM released as a result of bacterivorous grazing by the following mechanisms: direct uptake, production of DOM, and modification of DOM by exoenzymes.

CONCLUSIONS

Our bacterivore-bacterial-DOM interaction studies suggest that bacterivore grazing activity alters the DOM pool quantitatively and qualitatively. The presence of bacterivores appears to enhance the DOM pool by contributing organic compounds with $< 5 \times 10^2$ and 10^3 to 10^4 NMW. Varying amounts of bacterially utilizable substrates are made available by bacterivore activity. The reported findings support earlier proposals by Johannes (1965, 1968) and Fenchel & Harrison (1976). These authors correlated phosphate regeneration, detrital decomposition, and bacterial growth rates with protozoan grazing activity. As suggested by Fenchel & Harrison (1976), bacterivorous ciliates and microzooplankton may stimulate bacterial growth by reducing growth-inhibitory bacterial competition through grazing and by supplying dissolved organic matter through excretion and through stimulation of bacterial exudation. Although ciliated protozoans were the focus of the present study, there are no *a priori* reasons to suspect that bacterivorous zooflagellate-bacteria-DOM interactions are qualitatively different, although they may be quantitatively different. We suggest that bacterivore grazing activity and attendant release processes enhance production of biologically available DOM and thereby stimulate bacterial heterotrophic activity and growth. Further resolution of bacteria-bacterivore-DOM interactions awaits creative experimentation with analytical techniques which are highly specific, sensitive, and less subject to artifacts.

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