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Measurement of zooplankton grazing using particles labelled in light and dark with [methyl-^{3}H] methylamine hydrochloride

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ABSTRACT: Zooplankton grazing rates were estimated by measuring ingestion of [methyl-^{3}H]methylamine hydrochloride (^{3}H-MeA) labelled particles. Experiments were carried out in laboratory culture and in diverse natural plankton assemblages (Chesapeake Bay and Sargasso Sea). Uptake of MeA by particulate matter was linear in both light and dark conditions over the first hour of incubation and was highest in bacteria-sized particles compared to phytoplankton. In situ grazing rates estimated for zooplankton > 200 µm using particles labelled during daylight with both ^{3}H-MeA and $Na^{14}CO_3$ were similar. The technique was used to measure zooplankton grazing above and below the euphotic zone, day and night in the Chesapeake Bay during May 3 to 5, 1989. Filtration rates showed no diel pattern but were 2- to 10-fold higher in the deep chlorophyll maximum compared with surface waters. From these data, we conclude that the ^{3}H-MeA technique is useful for measuring grazing on natural particles at night and at depth where $Na^{14}CO_3$ incorporation by phytoplankton is not sufficient for in situ grazing experiments.

INTRODUCTION

A primary challenge facing zooplankton ecologists is how to accurately estimate grazing impact of zooplankton on natural phytoplankton stocks. One approach to this problem involves labelling particles with radioactive isotopes (usually $NaH^{14}CO_3$ or $^{32}PO_4$) and then estimating grazing by the rate at which isotope appears in the zooplankton (Sorokin 1966, Haney 1971, 1973, Daro 1978). Using in situ chambers for the incubations, this technique can be used repeatedly at discrete depths to estimate grazing by specific zooplankton species (Haney 1973, Lampert & Taylor 1985) or the entire community (Roman & Rublee 1981).

Zooplankton commonly exhibit diurnal feeding with significant grazing occurring at night (Boyd et al. 1980, Dagg & Grill 1980, Baars & Oosterhuis 1984, Stearns 1986, Roman et al. 1988a, Tiselius 1988) and at depth under low light conditions (Roman et al. 1986, Napp et al. 1988). Therefore, any estimate of phytoplankton loss to grazing must include measurements made throughout the water column, both day and night. Limitations specific to the experimental methods and radiolabelled compounds commonly used in tracer grazing studies affect our ability to make these measurements accurately. Carbon-14 labelled bicarbonate is a suitable tracer of carbon flow, but is not transported by phytoplankton in the dark, restricting its use for in situ labelling to the photic zone during day. Alternatively, phytoplankton may be prelabelled in vivo and offered to zooplankton at depth (Haney 1971, 1973, Lampert & Taylor 1985). This technique produces uniformly labelled cells, but uses cultured or naturally occurring phytoplankton that have been handled extensively (Daro 1978, Roman & Rublee 1981). Phosphorus-32 used to label phosphate is taken up by phytoplankton in both light and dark, but is a high-energy beta emitter which requires special handling and safety precautions that are difficult to implement on ships. Napp & Long (1989) have used the lower energy ^{33}P-phosphate to label natural particulate matter for in situ grazing studies under both light and dark conditions. At present, the commercial production of ^{33}P is limited and it is very difficult to obtain. Given the above constraints, we began looking for a radiolabelled compound which is taken-up by phytoplankton in light and dark, relatively safe to use and readily available.

Radiolabelled methylamine (CH_3NH_2) or MeA, has been used to examine ammonium uptake kinetics in phytoplankton because of its similar molecular size and chemical properties (Wheeler 1980, Wheeler & McCarthy 1982, Balch 1986). Because MeA is an ammonium

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analog, it also should be taken up by bacteria that utilize ammonium directly (Wheeler & Kirchman 1986). The uptake of MeA by phytoplankton is inversely related to ambient ammonium concentrations (Wheeler 1980) and occurs in the light and dark (Balch 1985). Because of this latter property, we considered using MeA as a label of phytoplankton for in situ grazing experiments. Carrier MeA is available in 2 tracer forms; the methyl group being labelled with either ^{14}C or ^{3}H.

In order to test the usefulness of this carrier, we performed a series of laboratory and field experiments. In the laboratory, we tested the uptake of MeA by cultured phytoplankton: (1) in light and dark and (2) in different ammonium concentrations. In the field we: (1) tested the linearity of ^{3}H-MeA uptake by particulate matter during light, dark, and at depth, (2) measured the uptake of ^{3}H-MeA into different size fractions of particles, and (3) measured grazing rates using in situ chambers with both ^{3}H-MeA and ^{14}C-bicarbonate. From this data, we determine the usefulness of MeA in zooplankton grazing studies.

METHODS

Laboratory experiments. We measured the uptake of MeA in light and dark by the diatom *Thalassiosira weissflogii* during log phase growth. Cells were grown in batch cultures with 20 ppt salinity, f/2 media (Guillard 1975) on a 12 h light/dark cycle at 20 °C. At midday in light (106 $\mu E\ m^{-2}\ h^{-1}$) and again at midnight in the dark, 15 beakers were filled with 50 ml of diatom culture (100 $\mu g\ chl\ l^{-1}$) and inoculated with 2.5 μCi ^{14}C-MeA (specific activity: 21 $Ci\ mmol^{-1}$). The contents of each of 3 beakers were poured through 3.0 µm polycarbonate (PC) Nuclepore filters after 0 (control), 10, 20, 40, 60, and 120 min (light) and 0 (control), 10, 20, 40, 60, and 90 min (dark). The isotope activity retained on the filters was determined in all experiments by liquid scintillation counting.

To determine the range of ammonium concentrations that will effectively suppress MeA uptake, we also measured the affect of ammonium concentration on the uptake of MeA by *Thalassiosira weissflogii* in the laboratory. Cells grown as above were transferred to new 20 ppt salinity, filter-sterilized f/10 culture media (Guillard 1975) 1 d prior to the experiment to control the amount of available nitrate (176 µM) and ammonium (< 0.1 µM). Three flasks containing 1200 ml of f/10 media and 25 $\mu g\ chl\ a\ l^{-1}$ each were prepared; one with no added ammonium, one with 1.2 µmol NH_4Cl added (1.0 µM), and one with 15 µmol NH_4Cl added (12.5 µM). After 24 h, the same amounts of ammonium were again added to each treatment yielding nominal ammonium concentrations of ≤ 0.1, ≤ 2.0, and ≤ 25.0 µM. Immediately, 60 μCi ^{3}H-MeA (2.86×10^{-3} µmol MeA) and 60 μCi ^{14}C-bicarbonate were added to each flask. Fifty ml were poured through three 3.0 µm PC filters from each flask at 0 (control), 5, 20, 40, 60, and 90 min. Samples were incubated in the dark for the first 25 min of the experiment to compare the effects of light on uptake of the 2 compounds by *T. weissflogii*.

Field experiments: particulate matter. During an August 1989 cruise to the Sargasso Sea (Lat. 31°40′ N, Long. 64° 15′ W), we ran time-course experiments to test for linear uptake of MeA by particulate matter. Water was collected with Niskin bottles from 2 m and the deep chlorophyll maximum and incubated on deck in 1 l polycarbonate bottles at the simulated isolume depth (neutral density screening) in flow-through (surface water) incubators. Fifty μCi of ^{3}H-MeA was added to each bottle and water from 3 bottles filtered through 0.22 and 2.0 µm PC filters at 0 (control), 15, 30, 60, and 90 min. Experiments were run with surface water during both day and night, and day only with water collected from the deep chlorophyll maximum.

Because isotope grazing measurements are potentially sensitive to differences in size-specific uptake of label by particulate matter, we measured uptake of ^{3}H-MeA into different size fractions of natural particles on cruises in the mesohaline portion of Chesapeake Bay, USA, from July to September, 1988. On one date (July 27), water was collected from the surface of 3 mid-channel stations along the axis of the bay with salinities ranging from 10 to 18 ppt. On all other occasions, water was collected from the surface at a mid-channel station in the mesohaline region of the bay (Lat. 38° 30′ N, Long. 76° 25′ W). Three 1 l polycarbonate bottles were filled with water and inoculated with 50 μCi ^{3}H-MeA and incubated on deck in flowing bay water under neutral density screen (60 % of full daylight). After 20, 40 and 60 min, subsamples from one bottle were filtered through 0.22 µm membrafil (MF) 1.0, 3.0, 5.0, 8.0, and 12.0 µm PC filters. Unlabelled water was filtered through the same pore sizes and the filters frozen for later fluorometric analysis of chlorophyll *a* (Parsons et al. 1984). For each size fraction, chlorophyll-specific uptake rates were calculated from the slope of the time series. Ammonium samples taken at the same stations were frozen and assayed by autoanalyzer (Solorzano 1969).

Field experiments: grazing. In order to compare zooplankton grazing rates measured with ^{3}H-MeA and ^{14}C-bicarbonate, we used in situ chambers (Roman & Rublee 1981) and 1 l shipboard bottles (Daro 1978). Fourteen experiments were run between February 1988 and May 1989 at the mid-channel station in Chesapeake Bay. In situ chambers were closed and incubated at 1 to 2 m depth in daylight for 50 to 60 min. For shipboard experiments, zooplankton were collected from the surface layer by oblique tows of a

200 µm, 0.5 m net fitted with a closed cod end. The catch (mostly copepods) was diluted and unsorted zooplankton were transferred into bottles filled with surface water (20 to 100 animals l^{-1}), inoculated with isotopes and incubated on deck in daylight as described above for particulate matter. For both procedures, zooplankton were separated from particulate matter using a 200 µm Nitex screen, washed onto preweighed 12.0 µm PC filters with filtered bay water and rinsed with 10 % HCl followed by distilled water to remove adsorbed ^{14}C-bicarbonate and salt respectively. Filters were then dried at 60 °C, weighed and analyzed for radioactivity. Filtration rates (F, ml (mg C)$^{-1}$ h^{-1}) were calculated for both isotope types using the equation of Daro (1978):

$$F = 2(q_z)/(q_p\ t) \quad (1)$$

where q_z = zooplankton activity per unit weight (dpm mg^{-1}); q_p = particulate activity per unit volume (dpm ml^{-1}); and t = experiment duration (h).

On May 3 to 5, 1989, we used ^{3}H-MeA to measure the grazing rate of zooplankton > 200 µm in the surface (2 m) and in the subsurface chlorophyll maximum (8 to 12 m), during both day and night at the Chesapeake Bay station. Grazing was measured using in situ chambers every 6 h for 3 d following the experimental protocol described above.

RESULTS

Particulate matter

In the laboratory, uptake (U) of ^{14}C-MeA by *Thalassiosira weissflogii* was linear with time in both light and dark during incubations of 120 and 90 min respectively (light: $U = 224t + 8598$, $r^2 = 0.92$; dark: $U = 305t + 3798$, $r^2 = 0.97$, where U has units dpm ml^{-1} and t is in min). Methylamine uptake rate was not significantly different between light and dark treatments (F-test for difference in slope: $F = 1.41$, $p < 0.01$; Sokal & Rolf 1981). For experiments run with Sargasso Sea water, uptake of ^{3}H-MeA by particles > 2.0 µm was linear for incubations up to 2 h in the light (surface) and dark (surface and chlorophyll maximum; Fig. 1).

We found a clear negative effect of ammonium on MeA uptake in the laboratory. Uptake of ^{3}H-MeA by *Thalassiosira weissflogii* was only slightly suppressed at a nominal ammonium concentration of 2.0 µM, while 25.0 µM NH_4^+ clearly suppressed uptake of ^{3}H-MeA compared to controls (< 0.1 µM NH_4^+; Fig. 2A). To test whether ammonium levels were affecting photosynthesis by the phytoplankton, we measured uptake of ^{14}C-bicarbonate simultaneously in all treatments. Carbon uptake did not differ between ammonium treatments (Fig. 2B). A 25 min initial dark period suppressed uptake of ^{14}C-HCO_3^- (Fig. 2B), but had no effect on ^{3}H-MeA uptake (Fig. 2A).

In order to determine conditions that favor uptake of MeA by natural particulate matter, we compared MeA uptake to ambient temperature and ammonium concentrations measured in the Chesapeake Bay (> 3.0 µm) and in the Sargasso Sea (> 2.0 µm; Fig. 3). We chose these size fractions because they contain the smallest particles copepods are able to ingest efficiently (Nival & Nival 1976) and are therefore relevant to ingestion experiments. The relationships between chlorophyll-specific uptake rate (CSUR), temperature and NH_4^+ concentration are shown in Fig. 3. CSUR increased abruptly with temperature above 25 °C such that log CSUR and temperature are positively correlated (r =

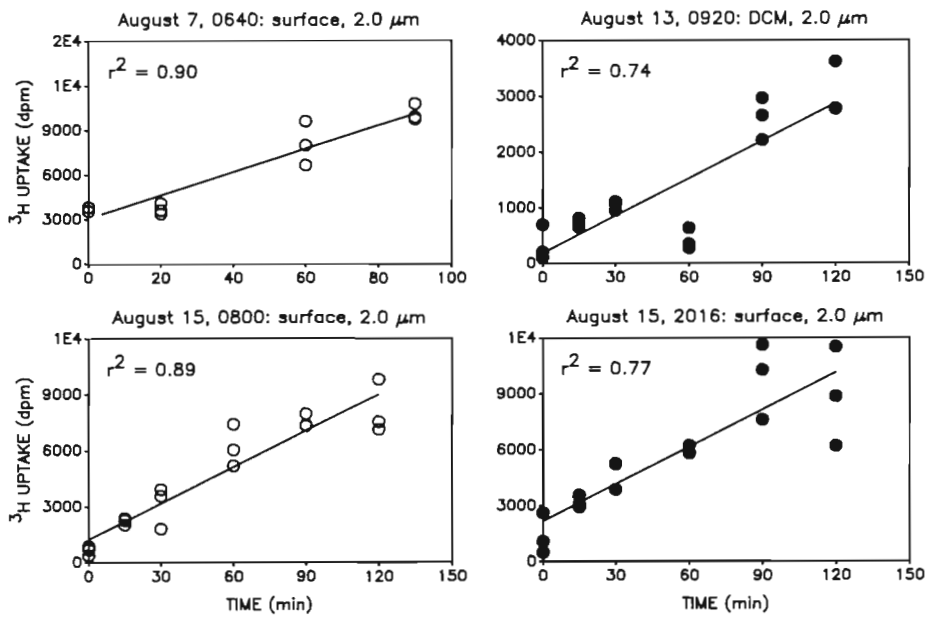

Fig. 1. Uptake of ^{3}H-methylamine by particulate matter in water collected from the Sargasso Sea in August 1989. All r^2 values are coefficients for least squares regression. (•) Samples incubated in dark

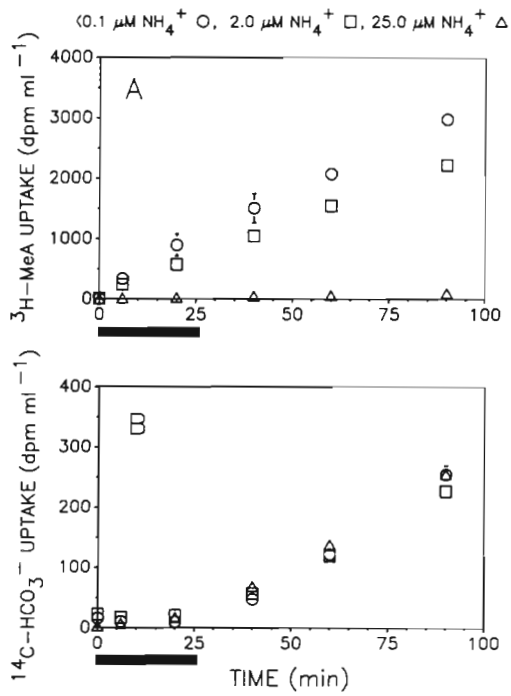

Fig. 2. *Thalassiosira weissflogii*. Effect of ammonium concentration and light on isotope uptake by a diatom. Uptake is for (A) ^{3}H-methylamine, and (B) ^{14}C-bicarbonate. Symbols represent mean of 3 replicates and error bars are 95 % confidence limits. Horizontal bar below *x*-axes shows dark period

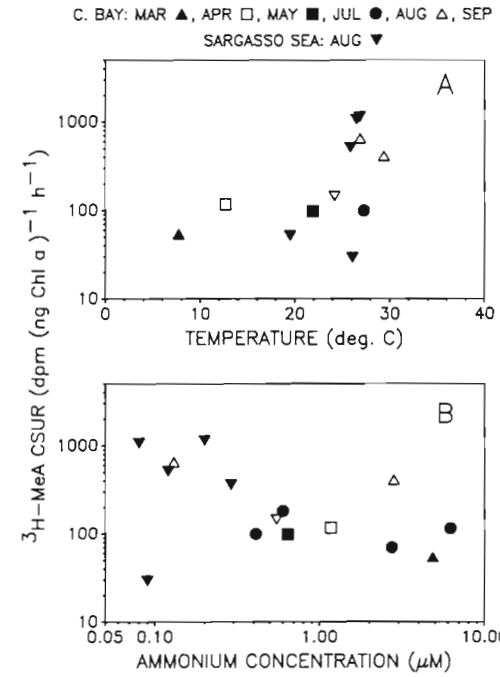

Fig. 3. Relationship between ambient temperature and ammonium concentration, and chlorophyll-specific methylamine uptake rate (CSUR) of particulate matter. Samples from the Sargasso Sea were collected on 2.0 μm polycarbonate (PC) filters, Chesapeake Bay samples on 3.0 μm PC filters

0.42, n = 12, partial correlation of log CSUR with temperature and log NH_4^+; Sokal & Rolf 1981). Methylamine uptake decreased exponentially with increasing NH_3 concentration and the logs of both variables show weak negative correlation (r = −0.04, n = 12). Removal of one uptake rate measurement made at low NH_4^+ concentration in the Sargasso Sea (Fig. 3b) improves this relationship considerably (r = −0.34, n = 11). For the same data, NH_4^+ concentration is negatively correlated with temperature (r = −0.58, p = 0.046, multiple correlation analysis; Sokal & Rolf 1981).

We also examined the dynamics of MeA uptake by measuring the activity of particulate material retained on different size filters. The uptake of ^{3}H-MeA in Chesapeake Bay samples was always highest in the size fraction containing all particles > 0.22 μm (Table 1, Fig. 4). In contrast, uptake of ^{14}C-bicarbonate was not significantly different for particles retained on 0.22 and 3.0 μm filters (F = 1.413, p = 0.2843, 1-way ANOVA and Table 1). When rates are standardized to chlorophyll, the size-related differences in uptake between the 2 compounds become even more pronounced. The ratio of CSUR between all particles > 0.22 and > 3.0 μm averages 19.0:1 for MeA, and 1.5:1 for bicarbonate (Table 1). However, the ratios of CSUR between the > 3.0 and > 8.0 μm size fractions were less different for the 2 compounds, averaging 3.6:1 and 3.0:1 for MeA and bicarbonate respectively (Table 1). These results suggest that MeA is taken up by non-pigmented particles (bacteria) in the smallest size fraction to a greater extent than bicarbonate. For particles retained on larger filters which do not efficiently retain free living bacteria, CSUR decreases slightly with increasing particle size in equal portions for the 2 compounds, indicating that uptake of MeA is principally by phytoplankton in this size range.

Particulate uptake of MeA was variable on both temporal and spatial scales. Variation in the CSUR of ^{3}H-MeA for all particle sizes was as large between different stations on the same date (Fig. 4A, B), as between dates at one station (Fig. 4C, D). Although the CSUR of ^{3}H-MeA was always greater for particles retained on 0.22 compared with 3.0 μm filters, this relationship was not consistent for particles retained on 3.0 to 12.0 μm filters either between stations (Fig. 4B) or between dates (Fig. 4D). Thus, for size ranges dominated by phytoplankton (> 3.0 μm) CSUR does not necessarily decrease with increasing particle size (3.0 to 12.0 μm).

Grazing

Zooplankton weight-specific filtration (F) rates (ml (mg C)$^{-1}$ h^{-1}), measured simultaneously using ^{14}C-bicarbonate and ^{3}H-MeA, were similar over a broad

Table 1. Size-fractionated uptake of ^{3}H-methylamine and ^{14}C-bicarbonate by Chesapeake Bay particulate matter. Uptake rates per unit volume, chlorophyll *a* concentration, and chlorophyll-specific uptake rate (CSUR) are given (mean ± SE) for 3 size classes of particulate matter collected from surface water on 2 dates in 1988

Date	Size fraction (μm)	Uptake ^{3}H-MeA (dpm ml^{-1} h^{-1})	Uptake ^{14}C-HCO_3^- (dpm ml^{-1} h^{-1})	Chl *a* (ng ml^{-1})	CSUR ^{3}H-MeA (dpm [ng chl *a*]$^{-1}$ h^{-1})	CSUR ^{14}C-HCO_3^- (dpm [ng chl *a*]$^{-1}$ h^{-1})
Aug 31	> 0.22	22.341 (6.574)	2.009 (872.7)	5.1	4.415 (1.299)	397 (172.5)
	> 3.0	2.318 (572.2)	888 (353.7)	3.6	647 (159.8)	248 (91.8)
	> 8.0	659 (215.5)	210 (102.0)	2.8	239 (78.1)	76 (37.0)
	> 0.22	27.189 (6.897)	347 (120.7)	6.0	4.532 (1.149)	58 (20.1)
Sep 14	> 3.0	403 (99.1)	114 (42.9)	2.8	145 (35.7)	41 (15.4)
	> 8.0	57 (9.5)	28 (10.6)	1.8	32 (5.3)	15 (5.9)

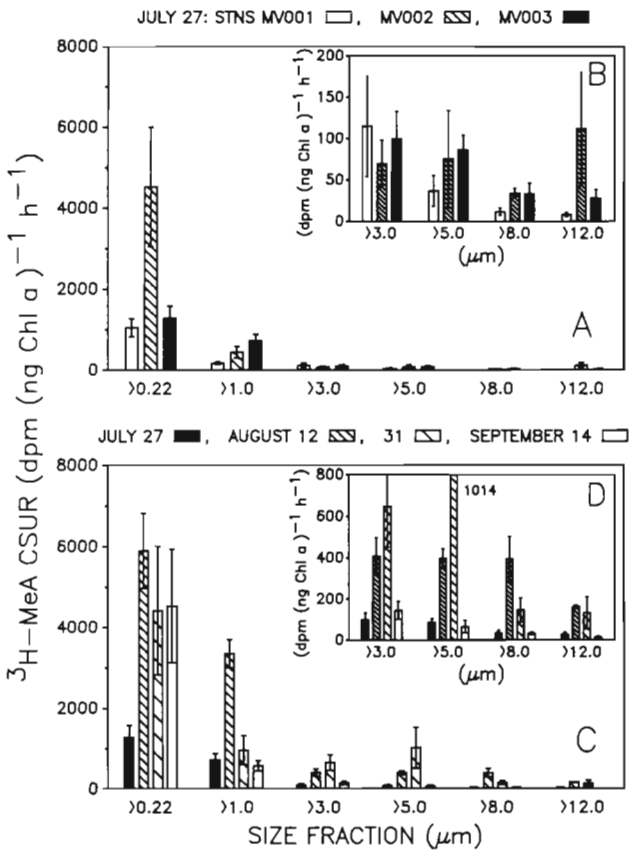

Fig. 4. Size-fractionated uptake of methylamine by Chesapeake Bay particulate matter. Values represent chlorophyll-specific uptake rates of particles collected on a given filter size. (A) and (B) Uptake by particles at different stations on the same date (MV001, lowest salinity; MV003, highest); (C) and (D) uptake at Stn MV003 on different dates. (B) and (D) show same data as (A) and (C) respectively, only with expanded *y*-axes. Error bars represent standard error of 3 measurements

range of conditions in Chesapeake Bay. There was a close relationship between filtration rates estimated using the 2 isotopic tracers ($\log F_{3H} = -0.41 + 1.24[\log F_{14C}]$, $n = 42$, $r^2 = 0.80$; Fig. 5). The slope of 1.24 is near to, but significantly different from 1 (outside 95 % confidence interval, Student's *t*-test, $p = 0.05$). Over the range of measured filtration rates, the MeA technique slightly overestimated F at higher values and underestimated F for low values, compared with the bicarbonate method.

Time series data of zooplankton grazing was collected over 3 d at the mid-bay station in May 1989 (Fig. 6). Values of chlorophyll $a > 3.0$ μm, F (not shown), and consequently ingestion ($I = F \times$ chl *a*) were consistently higher in the chlorophyll maximum than in surface waters. We found no clear diel trend in

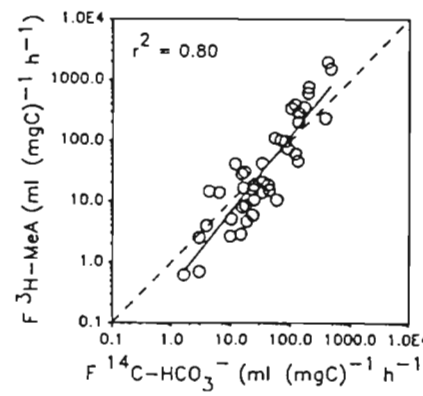

Fig. 5. Comparison of filtration rates measured using particulate matter labeled simultaneously with ^{3}H-methylamine and ^{14}C-bicarbonate. Rates are for zooplankton > 200 μm collected from Chesapeake Bay between February and October 1988 and during May 1989. Dashed line indicates 1 : 1 relationship, r^2 value is for log-log regression (n = 42)

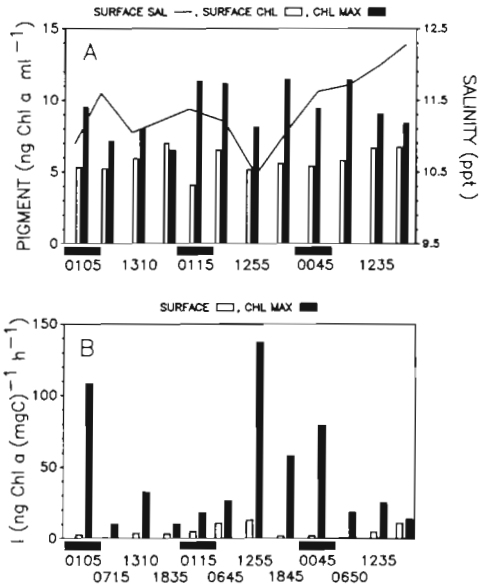

Fig. 6. Time series data for May 3 to 5, 1989. (A) Chlorophyll (ng ml^{-1}) samples, filtered on 3.0 μm PC filters, collected from surface (2 m) and chlorophyll maximum (8 to 12 m), salinity values obtained from surface water (B) Ingestion rates measured in situ for zooplankton > 200 μm in surface water and chlorophyll maximum

ingestion over the 3 d sampled with higher values occurring occasionally at both midnight and midday but rarely in the morning or evening (Fig. 6B).

DISCUSSION

Our finding that phytoplankton incorporate MeA in the light and dark and that uptake is linear over the first hour of incubation both confirms the results of Balch (1985) and supports its potential usefulness as a isotope carrier in zooplankton ingestion experiments. After 1 h, uptake of MeA may slow or stop due to saturation of cellular pools (Balch 1985) and/or equilibrium between uptake and loss of MeA by the cells (Balch 1986). Our experiments were not long enough to show this conclusively. However, this factor should be considered when setting the length of incubations in grazing experiments since the Daro (1978) equations assume linear uptake of label by the particulate fraction.

We confirmed in laboratory experiments that ammonium suppresses MeA uptake by phytoplankton (Wheeler & McCarthy 1982). In Chesapeake Bay and the Sargasso Sea, the highest rates of MeA uptake by particles > 2.0 μm occurred at the lowest ammonium concentrations and highest temperatures, although uptake was not closely correlated to either parameter. In addition, the CSUR of MeA varied as much between stations along the axis of the Chesapeake Bay on a single date, as between dates at a single station. This variability in uptake may be due to spatial and temporal heterogeneity in ammonium concentration (Wheeler & McCarthy 1982), nitrate concentration (Dortch 1990), temperature, or to variations in species composition of phytoplankton communities with differing relative affinities for MeA. Therefore, it may be difficult to predict the rate of MeA uptake by particulate matter prior to grazing experiments based on measured (or expected) hydrographic parameters. Nevertheless, we observed enough MeA uptake by naturally occurring particulate matter > 2.0 μm to measure grazing at ammonium concentrations ranging from 0.01 to 6.2 μM.

Radioisotope methods are potentially difficult to interpret if different-sized phytoplankton cells have different rates of isotope uptake, and if recycling of isotope occurs between storage pools (Conover & Francis 1973, Lampert 1975). Isotope recycling was minimized in our experiments by keeping experimental duration short (Daro 1978, Roman & Rublee 1981). Although particles retained on 0.22 μm filters always had higher CSUR values for MeA than particles retained on 3.0 μm filters in our Chesapeake Bay samples, we did not find a consistent inverse relationship between particle size and uptake for particles 3.0 to 12.0 μm. Therefore, in the range of particles most readily available to zooplankton > 200 μm during our study, differences in CSUR for ^{3}H-MeA related to particle size were small (1 to 4×). At other times, when large diatoms with relatively low CSUR values are present, zooplankton may select against small phytoplankton in favor of larger cells. This could lead to underestimates of actual clearence rate if the denominator in Eq. (1) includes small, high-activity cells that are not ingested by the zooplankton. For this reason, we suggest that size-fractionated uptake rates of isotope by particulate matter be determined whenever grazing experiments are conducted.

The large uptake of ^{3}H-MeA we observed on filters retaining small particles (0.2 and 1.0 μm) did not occur for ^{14}C-bicarbonate, indicating that small non-photosynthetic organisms incorporate MeA. This is consistent with evidence that bacteria assimilate ammonium (Wheeler & Kirchman 1986) and amino acids (Hanson & Snyder 1979, Hagström et al. 1984, Kirchman & Hodson 1986). Methylamine is intermediate in molecular size between ammonium and the amino acid glycine. It is possible that MeA provides a carbon source or acts as a precursor to amino acid synthesis in marine bacteria.

The fact that bacteria take up MeA points to a potential drawback of using this label to measure consumption of phytoplankton by zooplankton. A problem could arise when grazers feed on both phytoplankton and

bacteria-sized particles or when a large fraction of the available particulate matter consists of detritus with attached bacteria that are not ingested by copepods. In Eq. (1), activity of zooplankton is divided by activity of particulate matter. If the denominator contains material 'unavailable' for ingestion (i.e. detritus with high-activity bacteria attached), then filtration rates are underestimated. On the other hand, if zooplankton ingest labelled bacteria directly or small bactivores with label in their guts, but only larger particles (phytoplankton) with lower activity are included in the denominator, then filtration rates are overestimated. These scenarios may account for the slight deviation (slope = 1.24) from 1:1 correspondence between zooplankton filtration rates measured with ^{3}H-MeA and ^{14}C-bicarbonate (Fig. 5). For very low values of F, particles may have been labelled by MeA that were not ingested by zooplankton, underestimating values obtained for bicarbonate. For very high filtration rates, labelled particles ingested may not have been included in particulate calculations, overestimating F. Another possible explanation involves the ability to detect ^{3}H decay at low levels of activity in the presence of ^{14}C. Lower relative values of F reported for ^{3}H-MeA at very low absolute values of F may reflect reduced scintillation counting efficiency of ^{3}H compared to ^{14}C at low activities. To avoid this problem, care should be taken to label particulate matter with both compounds at similar activities.

The 2 methods gave similar results over the broad range of filtration rates we measured. The largest disparities occurred at the highest and lowest rates recorded where we also have the least data. It will be necessary to collect more measurements at these extremes of the filtering curve to determine if the disparities there are real. In general, the special situations described above should not present a serious problem for measuring grazing by larger zooplankton which feed mainly on cells > 3.0 µm (Nival & Nival 1976, Roman & Rublee 1981), but should be considered when designing grazing experiments with MeA.

Sampling intervals in our May 1989 field study were too long to determine whether there was any tidal influence on measured zooplankton feeding rates. There was no diel trend in feeding, at least discernible within the resolution of our sampling scheme. There was however a pronounced increase in ingestion rates measured for zooplankton in the chlorophyll maximum compared to the surface. These findings contrast with those of Stearns (1986) who found a distinct nighttime feeding maximum in non-migrating *Acartia tonsa* and Tiselius (1988) who found the gut fullness of *Acartia* sp. in the Skagerrak and Kattegat was highest at night and constant with depth between 3 and 12 m. During May in the mesohaline Chesapeake Bay, the bulk of the zooplankton > 200 µm consists of *A. tonsa* that remain in the subsurface chlorophyll maximum throughout the day (Roman et al. 1988b) and feed at rates much higher than animals at the surface. Given the apparent variability of zooplankton feeding behavior (Roman et al. 1988a), it is important to be able to measure zooplankton grazing activity in surface and deep waters, night and day, in order to accurately determine water column clearance rates and hence, carbon cycling.

In summary we have shown that: (1) Methylamine uptake by phytoplankton occurs in the light, dark and at depth, and is linear over the first hour of incubation; (2) Ammonium suppresses MeA uptake, but is not the only factor controlling uptake in field samples; (3) Chlorophyll-specific MeA uptake rates of different sized particles occurring within the size range available to zooplankton generally differed by no more than a factor of 4 and at times, not at all; (4) Filtering rates estimated using both tracers (^{3}H-MeA and ^{14}C-bicarbonate) were similar for zooplankton > 200 µm; (5) The methylamine technique can be used to measure grazing by zooplankton at night and at depth, which can be significantly different from rates at the surface during daylight.

Methylamine can be a useful isotope carrier for measuring ingestion of phytoplankton by larger zooplankton in situ or in the laboratory. For measuring grazing by smaller zooplankton that ingest both phytoplankton and bacteria, or in waters containing large amounts of detritus, isotopically labelled MeA may be useful as a tracer of total particulate matter ingested. When applying the methylamine technique in unfamiliar environments, we recommend dual labelling some samples in the light with both ^{14}C-bicarbonate and ^{3}H-MeA to check for agreement between the 2 methods.

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LITERATURE CITED

Baars, M. A., Oosterhuis, S. S. (1984). Diurnal feeding rhythms in North Sea copepods measured by gut fluorescence, digestive enzyme activity and grazing on labelled food. Neth. J. Sea Res. 18: 97–119

Balch, W. M. (1985). Lack of an effect of light on methylamine uptake by phytoplankton. Limnol. Oceanogr. 30: 665–674

Balch, W. M. (1986). Exploring the mechanism of ammonium uptake in phytoplankton with an ammonium analogue, methylamine. Mar. Biol. 92: 163–171

Boyd, C. M., Smith, S. L., Cowles, T. J. (1980). Grazing patterns of copepods in the upwelling off Peru. Limnol. Oceanogr. 25: 583–596

Conover, R. J., Francis, V. (1973). The use of radioactive isotopes to measure the transfer of materials in aquatic food chains. Mar. Biol. 18: 272–283

Dagg, M. J., Grill, D. W. (1980). Natural feeding rates of *Centropages typicus* females in the New York Bight. Limnol. Oceanogr. 25: 597–609

Daro, M. H. (1978). A simplified ^{14}C method for grazing measurements on natural planktonic populations. Helgoländer wiss. Meeresunters. 31: 241–248

Dortch, Q. (1990). The interaction between ammonium and nitrate uptake in phytoplankton. Mar. Ecol. Prog. Ser. 61: 183–201

Guillard, R. R. L. (1975). Culture of phytoplankton for feeding marine invertebrates. In: Smith, W. L., Chanley, M. H. (eds.) Culture of marine invertebrate animals. Plenum Press, New York, p. 29–60

Hagström, Å., Ammerman, J. W., Henrichs, S., Azam, F. (1984). Bacterial growth in seawater: II. Organic matter utilization during steady-state growth in seawater cultures. Mar. Ecol. Prog. Ser. 18: 41–48

Haney, J. F. (1971). An in situ method for the measurement of zooplankton grazing rates. Limnol. Oceanogr. 16: 970–977

Haney, J. F. (1973). An in situ examination of the grazing activities of natural zooplankton communities Arch. Hydrobiol. 72: 87–132

Hanson, R. B., Snyder, J. (1979). Microheterotrophic activity in a salt-marsh estuary, Sapelo Island, Georgia. Ecology. 60: 99–107

Kirchman, D. L., Hodson, R. E. (1986). Metabolic uptake of amino acid uptake in marine waters. Limnol. Oceanogr. 31: 339–350

Lampert, W. (1975). A tracer study on the carbon turnover of *Daphnia pulex* Verh. Int. Verein. Limnol. 19: 2913–2921

Lampert, W., Taylor, B. E. (1985). Zooplankton grazing in a eutrophic lake: implications of vertical migration. Ecology 66: 68–82

Napp, J. M., Brooks, E. R., Matrai, P., Mullin, M. M. (1988). Vertical distribution of marine particles and grazers. II. Relation of grazer distribution to food quality. Mar. Ecol. Prog. Ser. 50: 59–72

Napp, J. M., Long, D. L. (1989). A new isotopic method for measuring diel grazing rates of marine zooplankton in situ. Limnol. Oceanogr. 34: 618–629

Nival, P., Nival, S. (1976). Particle retention efficiencies of an herbivorous copepod, *Acartia clausii* (adult and copepodite stages): effects on grazing. Limnol. Oceanogr. 21: 24–38

Parsons, T. R., Maita, Y., Lalli, C. M. (1984). A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York

Roman, M. R., Ashton, C. A., Gauzens, A. L. (1988a). Day/night differences in the grazing impact of marine copepods. Hydrobiol. 167/168: 21–30

Roman, M. R., Malone, T. C., White, J. R. (1988b). Short-term variations in phytoplankton-zooplankton interactions in Chesapeake Bay. EOS 69: 1097

Roman, M. R., Rublee, P. A. (1981). A method to measure in situ zooplankton grazing rates on natural particle assemblages. Mar. Biol. 65: 303–309

Roman. M. R., Yentsch, C. S., Gauzens, A. L., Phinney, D. A. (1986). Grazer control of the fine-scale distribution of phytoplankton in warm-core Gulf stream rings. J. mar. Res. 44: 795–813

Sokal, R. R., Rohlf, F. J. (1981). Biometry. Freeman, New York

Solorzano, L. (1969). Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14: 799–801

Sorokin, Y. I. (1966). Carbon-14 method in the study of the nutrition of aquatic animals. Int. Rev. ges. Hydrobiol. 51: 209–244

Stearns, D. E. (1986). Copepod grazing behavior in simulated natural light and its relation to nocturnal feeding. Mar. Ecol. Prog. Ser. 30: 65–76

Tiselius, P. (1988). Effects of diurnal feeding rhythms, species composition and vertical migration on the grazing impact of calanoid copepods in the Skagerrak and Kattegat. Ophelia 28: 215–230

Wheeler, P. A. (1980). Use of methylammonium as an ammonium analogue in nitrogen transport and assimilation studies with *Cyclotella cryptica* (Bacillariophycae). J. Phycol. 16: 328–334

Wheeler, P. A., Kirchman, D. L. (1986). Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnol. Oceanogr. 31: 998–1009

Wheeler, P. A., McCarthy, J. J. (1982). Methylammonium uptake by Chesapeake Bay phytoplankton: evaluation of the use of the ammonium analogue for field uptake measurements. Limnol. Oceanogr. 27: 1129–1140

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