A Method to Determine *in situ* Zooplankton Grazing Rates on Natural Particle Assemblages

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Abstract:

In situ zooplankton grazing rates on natural particle assemblages were estimated by measuring zooplankton uptake of labelled autotrophic (with $Na^{14}CO_3$) and heterotrophic (with [methyl-^{3}H]-thymidine) particulate matter in 1-h incubations in clear, Plexiglas, Haney chambers. The *in situ* grazing rates are in the same range as those measured for zooplankton in the laboratory using standard particle counting techniques. A negative selection coefficient for ^{3}H-labelled particles indicated a lower filtration efficiency or avoidance of these particles by zooplankton.

Article:

Introduction

Experimental marine zooplankton studies have been pre-dominantly concerned with the feeding behaviour of herbivorous copepods (see reviews of Marshall, 1973; Steele and Mullin, 1976), and have been conducted in the laboratory, where many environmental variables of ecological importance cannot be simulated. The most studied variables for herbivores feeding on phytoplankton have been food density and size, because they are easy to manipulate and measure quantitatively. Even here, however, from the first work of Fuller (1937) to recent papers (e.g., Lam and Frost, 1976; Reeve and Walter, 1977; Donaghay and Small, 1979) our concepts of the response of copepods to these variables continue to evolve, and these subtle changes in turn modify theoretical approaches (Steele and Frost, 1977). Other factors such as prior feeding history and enzyme adaptation (Mayzaud and Conover, 1976; Mayzaud and Poulet, 1978) and the "taste" of food particles (Poulet and Marsot, 1978; Donaghay and Small, 1979) are important in determining copepod feeding rates, thereby questioning the relevance of many early feeding studies. More realistic feeding experiments must be performed to extrapolate laboratory results to explain or predict natural phenomena.

In the natural environment marine seston is a complex mixture of phytoplankton, bacteria, detritus, and inorganic particles present in varying proportions, chemical compositions and size distribution. The main approach to study the natural grazing behavior of marine zooplankton has been to enclose a sample of natural particulate material with either mixed zooplankton or selected copepod species, and measure the change in particle concentration with an electronic particle counter (e.g. Parsons et al., 1969; Hargrave and Green, 1970; Poulet, 1973; Richman et al., 1977). However, because of removal from the environment, these experiments using natural particle suspensions and collected zooplankton often do not reflect ambient conditions of feeding, light, temperature or pressure. In addition, over long incubations (24 h) "bottle effects" and improper controls which do not correct for nutrient regeneration by zooplankton result in an underestimation of grazing rate and a misinterpretation of selective feeding by zoo-plankton (Harbison and McAlister, 1980; Deason, 1980; Roman and Rublee, 1980). *In situ* experiments offer a more realistic alternative to these artificial conditions.

The *in situ* method of choice is a Niskin-type bottle developed by Haney (1971) which both closes to trap zoo-plankton and simultaneously releases a substance (isotope, labelled food or an inhibitor) into the grazing chamber. The Haney *in situ* chamber avoids the handling of zoo-plankton associated with the technique of

Porter (1972) and does not require the diver-operated remote controlled chamber proposed by Zillioux (1973). Although *in situ* experiments have primarily been confined to limnetic studies where high plankton densities occur, by using radioactive tracers of high specific activity to increase the sensitivity of grazing estimates, the chambers may be used in the marine environment.

Radioactive tracers are a powerful tool for the study of the nutrition of marine zooplankton (Chipman, 1959; Rice, 1965; Sorokin, 1966). However, many of the early isotope studies were not accurate because of recycling of the isotopes as a result of the excretion and respiration by phytoplankton and zooplankton (Conover and Francis, 1973). With proper labelling techniques and controls to account for all of the "pools" of the isotope, labelling experiments can provide accurate, quantitative feeding data which, be-cause of the sensitivity of measuring isotopes, are particularly useful for studying the feeding behavior of marine zooplankton (Griffiths and Caperon, 1979; Smith et al., 1979). Few attempts have been made to use tracers in feeding experiments at natural plankton concentrations. Chmyr (1967) added ^{14}C (200 μCi) to 15 to 20-1 carboys containing unconcentrated phytoplankton and zooplankton collected in surface water at stations along the equator and 5' and 10'W. Unfortunately Chmyr used long incubation times (24-48 h) without agitating the samples to keep phytoplankton in suspension and did not give data on the specific activity of the labelled particulate matter so that ingestion rates cannot be quantitatively interpreted (Shushkina and Sorokin, 1969). Nevertheless, Chmyr demonstrated significant uptake of the labelled food at natural concentrations by oceanic copepods. Recently, Daro (1978) improved Chmyr's technique by using shorter incubation times to avoid isotope recycling in experiments under more natural conditions. Daro added ^{14}C to unconcentrated North Sea water samples containing phytoplankton and zooplankton and found significant uptake of labelled cells in only 1-h incubations. Copepod filtration and ingestion rates determined by ^{14}C uptake were comparable to replicate experiments based on differences in particle concentration (Coulter counts) (Hargis, 1977; Daro, 1978). Although this ^{14}C technique does not provide information on size selective grazing, it does give data on copepod grazing on natural phytoplankton populations (Daro, 1980).

We have improved upon the Daro technique by: (1) Using formalin controls to correct for the adsorption of isotopes to particulate matter, zooplankton and filters. (2) Labelling both autotrophic (with $NaH^{14}CO_3$) and heterotrophic (with [methyl-^3H]-thymidine) food components so that ingestion of phytoplankton, bacteria, and detritus (by labelling the attached bacteria) can be simultaneously measured. (3) Fractionating both the particulate matter and zooplankton for isotope uptake measurements. (4) Expressing grazing rate as a function of zooplankton biomass (mg ash-free dry weight) which reduces analysis time and data variation. (5) Conducting the grazing experiments *in situ* using the Haney chamber so that grazing rates of zooplankton are made at ambient food concentrations, light levels, and temperatures. We describe the details of this refined experimental technique and report *in situ* zooplankton grazing rates measured at several stations, in Biscayne Bay, Florida, Bimini Harbor, Bahamas, and in the Gulf Stream off Miami.

Materials and Methods

Daro (1978) chose a stationary 3-compartment model to express the exchange of radioactive tracers in her grazing experiments. This is a reasonable assumption considering the large magnitude of the initial isotope pool in the sea-water relative to that taken up in the particulate matter and zooplankton as well as the brevity of the experimental incubation (1 h). The short incubation also avoids feedback to the compartment via excretion and respiration by the phytoplankton and zooplankton. Isotope exchange is followed in the 3-compartment model immediately after adding the tracer to the water.

$$\underset{q\,1}{\text{Water}} \quad \lambda 1 \quad \underset{q2}{\text{Phytoplankton}} \quad \lambda 2 \quad \underset{q3}{\text{Zooplankton}} \quad \lambda 3,$$

where q 1, q 2, and q 3 are the concentrations of ^{14}C in water, phytoplankton, and zooplankton respectively and $\lambda 1$ is the rate of ^{14}C uptake by the phytoplankton, $\lambda 2$ the rate of ^{14}C-labelled phytoplankton uptake by zooplankton, and $\lambda 3$ the rate of ^{14}C excretion by zooplankton. The calculations of isotope exchange between the compartments can be found in Daro (1978). The grazing rate, $\lambda 2 = 2 \bullet q^3/ q^2 \bullet$ time.

This equation is similar to the equation used by Haney (1971) with the exception of the multiplication by 2. This is a result of the addition of label directly instead of pre-labelling cells prior to incubation. Daro assumed a linear uptake of label by particulate matter, thus the dpm $m1^{-1}$ suspension measured at the end on the incubation is halved to estimate the "average" dpm • 1^{-1} suspension over the experiment. We conducted time-series control experiments and verified Daro's assumption of linear uptake by phytoplankton over short incubations and also found linear uptake of thymidine by bacteria (Fig. 1). We also confirmed Daro's assumption that isotope uptake by zoo-plankton is a parabolic function of time (Fig. 2).

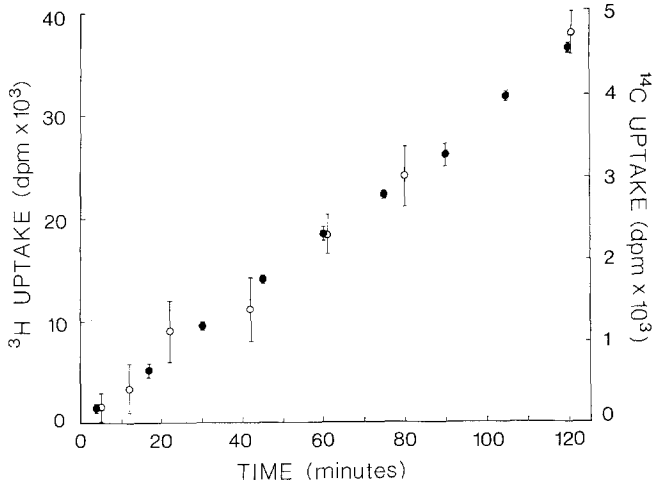

Fig. 1. Uptake (DPM× 10^3) of [methyl-^{3}H]-thymidine: closed circles (●) and $^{14}CO_3$: open circles (○) by particulate matter (>3 μm) over time. Each point is the mean of three replicates with bars indicating the standard deviation

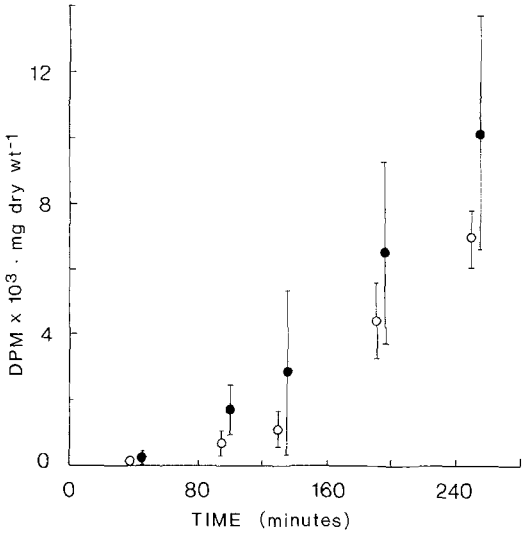

Fig. 2. Uptake (DPM× 10^3 · mg zooplankton dry weight^{-1}) of ^{3}H: closed circles (●) and ^{14}C: open circles (○) over time. Each point is the mean of three replicates with bars indicating the standard deviation

In 1-h incubations the probability of loss of label via fecal pellet production is slight. Reeve and Walter (1977) found that *Acartia tonsa* produced from less than 1 to 6 fecal pellets • 24 h^{-1} over 3 orders of magnitude difference in food concentration. Honjo and Roman (1978) measured fecal pellet production of *A. clausi* in natural seawater with 1.7×10^5 particles ml^{-1} and in suspensions of coccolithophores at 1.6×10^5 and 2.0×10^4 cells • ml^{-1} and found respective egestion rates of 24.5 ($s_{\bar{x}}$ = 4.1), 90.7 ($s_{\bar{x}}$ =7.6), and 8.0 pellets • copepod^{-1} • 24 h^{-1}. Paffenhöfer and Knowles (1979) measured somewhat higher rates of egestion for *Temora turbinata* (10 to 169 pellets • copepod^{-1} 24 h^{-1}) and *Eucalanus pileatus* (55 to 160 pellets cope-pod^{-1} • 24 h^{-1}). Direct measurement of gut passage time for *Centropages typicus* averaged 1.5 h (Dagg and Grill, 1980). Mixed copepod species from the North Pacific Central Gyre had gut passage times on the order of 1 to 3 h (Hayward, 1980). Thus although it is possible that in high food concentrations egestion of labelled food in the chambers could occur in 1 h, in most situations this probability is small.

Ingested label may also be lost through excretion (Lampert, 1975) and "leaking" (Pechenik, 1979) of non-assimilated dissolved compounds. The extent of this loss can vary with feeding time, zooplankton species, diet, food density, and environmental conditions. In our 1-h incubations starting with non-labelled food, this loss of label is assumed to be a negligible fraction of the final isotope concentration in the zooplankton.

Bacteria (Watson et al., 1977) and detritus (Parsons, 1963; Roman, 1978) can comprise a considerable portion of the particulate biomass available to zooplankton grazers. Labelling only phytoplankton for *in situ* grazing experiments would thus overlook these two important trophic components. We employed the thymidine method recently described by Hollibaugh et al. (1980). The method reduces loss of label during short-term feeding experiments because thymidine, due to its incorporation into DNA, is metabolically conservative. We found that like $NaH^{14}CO_3$ uptake by phytoplankton, ^{3}H thymidine uptake by >3 μm particulate matter is linear over short-term incubations (Fig. 1). Incorporation of ^{3}H-labelled particulate matter by copepods follows a parabolic function (Fig. 2). We partitioned thymidine uptake by particulate matter and assumed uptake by >3 μm particles

is predominantly the result of epiphytic detrital bacteria. Bacteria counts by the AODC method (Watson et al., 1977) on these >3 μm particles ranged from 13.0 to 17.4 cells per 100 μm^2 of detritus. Thymidine uptake in formalin controls was generally less than 10% of experimental values suggesting that ad-sorption of thymidine to particles is small. We tested possible uptake of thymidine by phytoplankton by using cultures of the diatom *Skeletonema costatum* and cyanobacteria *Synococcus* sp. and found that uptake was negligible relative to bacterial uptake of thymidine. These findings are consistent with those of Fuhrman and Azam (1980) and Hollibaugh et al. (1980).

We measured *in situ* grazing rates off the laboratory dock, various stations in Biscayne Bay, Bimini Harbor, and in the Gulf Stream off Miami. The 3.5 1 Haney (25 cm × 14 cm-diameter) chambers were lowered to the desired sampling depth and a messenger was released which both closed the chamber and released the radioactive isotopes into the chamber. We repeated Haney's (1971) dye study and found that mixing in the chambers is rapid, due to the agitation caused by the closing mechanism. In oligotrophic waters such as central Biscayne Bay, Bimini Harbor, and the Gulf Stream we concentrated zoo-plankton into the chambers. A 64 μm-mesh was attached to the bottom of the chamber so that we could raise the chamber through the water column to increase the number of zooplankton in the chamber. The chambers were raised slowly to prevent injury to the zooplankton, over no more than 3 m which resulted in as great as a 5-fold concentration of zooplankton above ambient density. We released $NaH^{14}CO_3$ (20-40 μCi • l^{-1}) and [methy1-^{3}H]-thymidine (10-20 μCi • l^{-1}) into the chambers and incubated replicate chambers at depth for 1 h. Air bubbles were released into the chambers with the isotope. Observations of incubations at sea by SCUBA verified that oscillations of the chambers due to the ship's roll were sufficient to agitate these air bubbles and thus keep particles in suspension. In calm seas or from stable structures, one might follow the procedure of Gliwicz (1968), who attached lines to an *in situ* chamber and turned it upside down every 20 min to keep particles in suspension. Incubations off the laboratory dock were conducted during mean ebb or flood tides, when strong tidal currents (3-4 kn) agitated the Haney chambers. Controls to correct for adsorption of label to particulate matter and zooplankton were run as above with 5% formalin.

After the 1-h incubation the chambers were retrieved and the zooplankton collected on 64 μm-mesh or, in several experiments, partitioned through 200 and 64 μm-mesh. Juvenile stages of small copepod species as well as protozoa may pass through the 64 μm-mesh and thus not be measured. The zooplankton were filtered onto tared 8 μm Nucleopore filters and rinsed with 10% HCl and filtered seawater to remove surface adsorbed ^{14}C and ^3H. The zooplankton samples were dried at 60 °C, weighed and then placed in scintillation fluor (Aquasol).

Inclusion of large phytoplankton and detritus (>64 μm) with zooplankton for isotope measurement is a potential source of error in our recovery method. We examined this possible source of contamination by measuring chlorophyll a in non-labelled chamber zooplankton samples collected by our incubation procedure, including the concentration of zooplankton into the chamber with a 64 μm bottom screen. Chlorophyll *a* in the zooplankton fraction (>64 μm) ranged from 0.54 to 2.23% of the ambient chlorophyll *a* concentration (filtered on GF/C). We therefore assume a negligible contribution of phytoplankton ^{14}C to zooplankton ^{14}C in these conditions. In blooms of long-chain diatoms or *Trichodesmium* sp., this error increases and it is necessary to isolate zooplankton for isotope measurements.

The labelled particulate matter in the chambers was filtered onto 0.4 and 3 μm Nucleopore filters, so that isotope uptake by the various size fractions of particulate food could be assayed. Isotope uptake of both zooplankton and particulate matter was measured in a liquid scintillation counter and the counts corrected for machine efficiency and sample quench.

Zooplankton were collected by replicate tows with a 0.5 m-diameter 64 μm-mesh net for biomass determination. At several stations the zooplankton catch was partitioned into >200 μm and 64-200 μm fractions. The per-cent ash-free dry weight of the collected zooplankton was used to convert the dpm • mg zooplankton dry $weight^{-1}$ to dpm • mg zooplankton ash-free dry $weight^{-1}$. This conversion reduced errors in grazing estimates per unit biomass due to suspended inorganic material. Thus grazing rate, $\lambda 2$, = 2 dpm • mg zooplankton ash-free

dry weight/dpm • liter suspension^{-1} = liters filtered • mg zooplankton ash-free dry weight^{-1} • h^{-1}.

Results and Discussion

Measurable uptake of ^{3}H and ^{14}C by zooplankton (formalin control subtracted) occurred in the 1 h incubations (Table 1). Isotope counts were normalized per 1 • mg zoo-plankton ash-free dry weight. Formalin controls were generally less than 10% of the experimental values. The coefficient of variation of grazing rates between replicates ($s_{\bar{x}}/\bar{x}$) averaged 48% (n=10; range 5 to 81%) for grazing rates computed from ^{14}C ratios and 38% (n=10; range 7 to 75%) for grazing rates calculated from ^{3}H uptake. Daro (1978) recommended using five replicates, however, we had only two Haney chambers and Limited shiptime. We recommend at least three replicates.

Grazing rates computed from ^{14}C uptake were greater than λ2 calculated from ^{3}H uptake. Among the 10 pairs of incubations the mean ratio of grazing rate on heterotrophs (detritus and free-living bacteria) to grazing rate on autotrophs was 0.27 (n = 10 , $s_{\bar{x}}$ = 0.27; range =0.05 to 0.98). Lampert (1974) fed *Daphnia pulex* a mixture of ^{14}C-labelled algae and ^{3}H-labelled bacteria. He calculated selection, S, for the bacteria by:

$$\frac{^{14}C \text{ (suspension)} \times {}^{3}H \text{ (animal)}}{^{3}H \text{ (suspension)} \times {}^{14}C \text{ (animal)}}$$

A coefficient of 1 indicates that the animals feed non-selectively, less than 1 indicates an avoidance and a coefficient greater than 1 indicates a selection for the bacteria. Lam-pert found that adult *D. pulex* filtered both bacteria and algae in proportion to their abundance in the medium (i.e., nonselective feeding behavior). In contrast, we calculated negative S coefficients for >3 μm ^{3}H-labelled particles in all *in situ* incubations. Because the thymidine was incorporated into bacterial DNA and is therefore relatively "conservative" as a label, we do not believe the difference in selection between ^{14}C- and ^{3}H-labelled particulate matter is the result of a more rapid exchange of ^{3}H in the guts of zooplankton. Therefore the lower filtration efficiency of >3 μm ^{3}H-labelled detrital particles presumably indicates avoidance, or particle rejection. Poulet and Marsot (1978) found that copepods (*Acartia clattsi* and *Eurytemora herdmani*) ingested greater numbers of microcapsules that were enriched with phytoplankton homogenate as compared to non-enriched controls. Donaghay and Small (1979) have demonstrated that the copepod *A. clausi* discriminates between similar-sized live and dead particles (plastic spheres) and can reject many of the non-nutritious particles. Thus, possibly because of a different nutritional value, "taste", or size, labelled detrital particles are not taken up in the same proportion as phytoplankton. It should be noted that although λ2, ^{3}H is less than λ2, ^{14}C (Table 1), the zooplankton ingest significant quantities of detritus. Extrapolated to a daily rate, grazing calculated by the ^{3}H ratio ranged from 1.92 to 97.68 1 • mg zooplankton AFDW^{-1} • d^{-1}.

A portion of the ^{3}H uptake by zooplankton may be due to ingestion of free-living bacteria and small detritus (<3 μm). Peterson et al. (1978) found that *Daphnia* spp. ingested free-living bacteria at rates averaging 30% that of yeast (maximum retention efficiency). The extent to which marine copepods, the dominant zooplankton group in the *in situ* incubations, ingest free-living bacteria is not known. A lower size limit of particle retention based on the intersetule spacing of the filtering maxilla of adult copepods of *Paracalanus parvus* and *Acartia tonsa*, which dominate the zooplankton of Biscayne Bay, is 3 to 5 μm (Nival and Nival, 1976). Thus the probability of their capturing free-living bacteria is low. Direct impaction (Rubenstein and Koehl, 1977) and possible adhesion of bacteria to mucus on cope-pod mouth parts may both allow copepods to ingest some free-living bacteria, but these amounts are probably small compared to the amount of bacteria ingested that are attached to detritus particles. Micro-zooplankton, here dominated by- copepod nauplii, may be more efficient at direct utilization of free-living bacteria, but the extent is unknown and still probably less than the ingestion of bacteria associated with particles.

In three incubations we partitioned isotope uptake into >200 and 64 to 200 μm zooplankton fractions (Table 1). The average ratio of grazing rate of 64 to 200 μm zoo-plankton : grazing rate of >200 μm zooplankton was: 0.86, 5.30, and 0.96 when calculated from ^{14}C ratios and 1.88, 1.90, and 18.0 when computed from ^{3}H uptake.

The major size fraction (>200 μm or 64 to 200 μm) of zooplankton biomass varied at the three stations. When the biomass of zooplankton is multiplied by their weight-specific grazing rate (Table 2), the grazing impact of the two size fractions differed among the three stations. Thus another benefit of this *in situ* grazing technique is to compare weight-specific grazing rate of various size fractions of zooplankton to their field abundance.

Table 1. Station; zooplankton biomass; concentration of ^3H and ^{14}C in zooplankton and particulate matter (> 3 μm); and zooplankton grazing rates in replicate Haney chambers (A and B) incubated in the Gulf Stream, Bimini Harbor and at various stations in Biscayne Bay, Florida, USA

Station	Zooplankton biomass · chamber^{-1} (mg · AFDW)	dpm · $^3H \times 10^3$ · mg zooplankton AFDW^{-1}	dpm · $^{14}C \times 10^3$ · mg zooplankton AFDW^{-1}	dpm · $^3H \times 10^3$ · liter^{-1}	dpm · $^{14}C \times 10^3$ · liter^{-1}	Grazing rate liter · mg · zooplankton AFDW^{-1} · h^{-1}	
						G, ^3H	G, ^{14}C
Gulf Stream							
A	0.13	4.38	8.19	2.58	0.69	3.39	23.62
B	0.06	12.54	7.85	5.28	1.43	4.75	10.97
Biscayne Bay #1							
A	0.31	44.27	44.65	35.72	9.14	2.48	9.77
B	0.23	43.26	34.50	18.58	3.07	4.66	22.49
Biscayne Bay #2							
A	0.56	27.85	17.59	28.70	2.52	1.94	13.96
B	0.23	13.96	12.68	20.19	2.61	1.38	9.72
Biscayne Bay #3							
A	0.58	1.73	1.11	56.14	15.34	0.06	0.14
B	0.48	1.52	1.15	56.39	15.47	0.05	0.15
Biscayne Bay #4							
200 μm A	0.09	1.76	2.03	15.42	2.99	0.23	1.36
200 μm B	0.36	0.57	1.72	11.40	1.80	0.10	1.91
64 – 200 μm A	0.25	3.32	2.92			0.43	1.95
64 – 200 μm B	0.64	1.10	0.74			0.19	0.85
Biscayne Bay #5							
200 μm A	0.08	2.62	0.93	74.02	5.50	0.07	0.34
200 μm B	0.57	0.86	0.16	62.14	3.10	0.03	0.10
64 – 200 μm A	0.19	3.36	1.37			0.09	0.50
64 – 200 μm B	0.29	3.11	2.83			0.10	1.83
Bimini Harbor							
200 μm A	0.07	0.45	2.39	6.80	2.23	0.13	2.15
200 μm B	0.35	0.12	2.12	6.15	3.82	0.04	1.11
64 – 200 μm A	0.08	6.18	2.53			1.82	2.27
64 – 200 μm B	0.07	3.83	1.62			1.24	0.85

The *in situ* grazing rates are in the same range as those measured by standard particle counting techniques for copepods feeding on laboratory monocultures of phytoplankton (e.g. Paffenhöfer, 1976; Heinbokel, 1978; Fernandez, 1979). Zooplankton grazing rates are affected by the concentration of particles, chemical composition of particles, particle size distribution, and the particle retention efficiency of the animals feeding, thus direct comparisons of grazing rates for different zooplankton species under various environmental conditions is of limited value. Our grazing estimates assume an "availability" of only >3 μm particles, even though smaller particles are probably ingested. This somewhat arbitrary cutoff is roughly the 50% size filtration efficiency of *Acartia clausi* (Nival and Nival, 1976) and thus may underestimate maximum grazing rate for similar size copepods in the in situ chambers, but may give a reasonable estimate of maximum micro-zooplankton grazing rates.

Another source of error which may result in under-estimating grazing rate will occur if zooplankton select large phytoplankton which may have a lower specific activity than smaller species. Thus, if the estimated "available" labelled phytoplankton pool (>3 μm) is biased by smaller "hotter" cells and the zooplankton selectively ingest larger cells with less isotope per cell volume, grazing rate will be underestimated. We performed two time-series (5, 15, 30, 45, 60 and 90 min samples) experiments and measured ^{14}C uptake in natural water samples on 0.5 μm, 3 μm and 8 μm filters to estimate ^{14}C uptake rates by various phytoplankton size fractions. We found no significant differences (95% confidence level) between the uptake slopes (all uptake slopes are linear over 90 min) of the >0.5 μm (412 to 563 dpm • min^{-1}), >3 μm (449 to 678 dpm • min^{-1}) and >8 μm (322 to 423 dpm • min^{-1}) fractions in a neritic water sample. In a Gulf Stream sample we found no significant difference between the > 0.5 μm (34 to 42 dpm • min^{-1}) and >3 μm (24 to 48 dpm • min^{-1}) fractions, however, the >8 μm fraction uptake slope was less by roughly 3 (7 to 9 dpm • min^{-1}). Thus, the Gulf Stream >8 μm phytoplankton would

have one-third the specific activity of 3 to 8 μm phytoplankton during a 1-h incubation, resulting in the underestimation of grazing rate if these larger cells are selectively ingested. Two factors mitigate against this potential error. The first being that in waters like the Gulf Stream, where isotope uptake rates of smaller phytoplankton may be greater than larger cells, the smaller cells are much more numerous in number and total biomass than larger cells (Mullin, 1965). Thus selective feeding on larger cells may not outweigh the energetic cost of searching. Secondly, in oligotrophic waters zooplankton the *in situ* chambers have been dominated by micro-zooplankton (Table 2) which ingest the smaller cells more efficiently than macrozooplankton (Fernandez, 1979; Heinbokel, 1978).

Table 2. Zooplankton biomass in > 200 μm and 64 to 200 μm fractions and estimated *in situ* zooplankton grazing rate calculated by multiplying G, ^{14}C = liter · mg zooplankton AFDW^{-1} · h^{-1} (Table 1) × zooplankton mg AFDW · m^{-3}

Station	Zooplankton biomass mg AFDW · m^{-3}					Grazing rate liter · m^{-3} · h^{-1}				
	64 – 200 μm	% total	> 200 μm	% total	total	64 – 200 μm	% total	> 200 μm	% total	total
Bicayne Bay 4	29.31	27	79.83	73	109.14	69.30	42	97.73	58	167.03
	41.77	40	63.88	60	105.65	29.14	18	137.25	82	166.39
	35.54	33	71.86	66	107.40	49.22	30	117.49	70	166.71
Biscayne Bay 5	660.61	57	502.60	43	1163.21	254.38	61	165.45	39	419.83
	356.89	43	470.63	57	827.52	931.01	95	48.66	5	979.67
	508.75	50	486.62	50	995.36	592.90	78	107.06	22	699.75
Bimini Harbor	23.54	88	3.06	12	26.60	78.69	93	6.07	7	84.76
	45.72	95	2.60	5	48.32	29.45	90	3.14	10	32.59
	34.63	92	2.83	8	37.46	54.07	92	4.60	8	58.68

This modified *in situ* grazing method is rapid (1-h incubation); uses natural food concentrations; measures grazing on both autotrophic and heterotrophic particulate matter; and by fractionating zooplankton for isotope uptake measurements, assesses the grazing impact of various zooplankton size groups. One shortcoming of the method is that it cannot be used at night to label phytoplankton although it can be used to measure grazing rate on ^{3}H thymidine-labelled particles. Daro (1980) measured night grazing rates shipboard by pre-labelling natural phytoplankton in the light then introducing zooplankton in the dark. Potential errors in this method due to recycling of isotopes (excretion and egestion) and selective feeding on larger phytoplankton with a lower specific activity both result in the underestimation of zooplankton grazing rate. In 1 h incubations with mixed zooplankton species we believe these errors are small. This *in situ* method has potential for water column trophic studies and as a bioassay tool. The sensitivity of this method may prove valuable in open ocean studies.

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