

Absorption efficiencies and biochemical fractionation of assimilated compounds in the cold water appendicularian *Oikopleura vanhoffeni*

Alexander B. Bochdansky,¹ Don Deibel, and Richard B. Rivkin

Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7

Abstract

Using ⁶⁸Ge:¹⁴C dual-labeling, we investigated the absorption efficiency of diatom carbon for the cold water appendicularian *Oikopleura vanhoffeni*. The absorption efficiency of bulk carbon (mean = 67%) was not influenced by body size or ingestion rate. For the first time for a pelagic tunicate, food and feces were fractionated into their major biochemical constituents (i.e., low-molecular-weight compounds, lipid, protein, and polysaccharide), allowing calculation of absorption efficiencies for each fraction. Low-molecular-weight compounds and proteins were preferentially absorbed over lipids and polysaccharides. However, predicted C:N ratios of the fecal pellets of *O. vanhoffeni* were in the lower range of C:N ratios reported for zooplankton feces. The results are relevant for modeling biogeochemical cycles because pelagic tunicates contribute greatly to vertical particulate organic carbon flux.

Quantifying organic matter absorption is important to understanding feeding ecology. On an autecological level, the extent to which various organic nutrients are absorbed is important for growth and survival of individuals and may elucidate feeding and digestive mechanisms (Checkley 1980; Houde and Roman 1987; Kiørboe 1989; Roman 1991). On a synecological level, changes in the composition of particulate organic matter caused by ingestion and digestion by major grazers are important for modifying biogeochemical fluxes, since pellets of large zooplankton are a primary mode of particle flux (Suess 1980; Michaels and Silver 1988; Fortier et al. 1994).

Pelagic tunicates are among the major grazers that remove a significant fraction of primary production from the water column (Alldredge 1981; Deibel 1988; Knoechel and Steel-Flynn 1989); they also produce fast-sinking fecal pellets (Michaels and Silver 1988; Fortier et al. 1994) and, in the case of appendicularians, discard a large number of fast-sinking feeding webs or "houses" (Taguchi 1982). Although absorption efficiencies have been studied in great detail for crustacean zooplankton, especially copepods (Conover 1966 *a,b*) and krill (Lasker 1960), information pertaining to pelagic tunicates is sparse. Gorsky (1980) examined absorption efficiencies in the appendicularian *Oikopleura dioica*, Andersen (1986) in the salp *Salpa fusiformis*, and Madin and Purcell (1992) in the salp *Cyclosalpa bakeri*. Absorption efficiencies have not yet been determined for doliolids and pyrosomids (Madin and Deibel 1997).

In the present study, we investigated the absorption efficiencies for bulk carbon of the cold water appendicularian *Oikopleura vanhoffeni* feeding on the laboratory-grown diatom *Thalassiosira nordenskioldii*. We use the term "absorption" instead of "assimilation" because, strictly defined,

assimilation is absorption minus respiration, and assimilation is equivalent to the absorption of carbon available for growth and reproduction. For consistency, the term absorption is also used where the original authors used assimilation in the sense of absorption. For calculating absorption of bulk carbon, we employed a dual-labeling technique using the radioisotopes ¹⁴C and ⁶⁸Ge. In separate experiments, the incorporated ¹⁴C was fractionated into four major groups of biochemical constituents: low-molecular-weight compounds, lipids, proteins, and polysaccharides. This procedure allowed us to determine the absorption efficiencies of these four biochemical pools.

Materials and methods

Preparation of the experimental food source—The cold-water diatom *T. nordenskioldii* (equivalent spherical diameter = 15 μm) was grown under continuous light at 5°C in f/2+ medium. When a new medium was inoculated with algae, ⁶⁸Ge(OH)₄ (New England Nuclear) and NaH¹⁴CO₃ were added, and the flask was stoppered tightly to avoid any exchange with the atmosphere. The final concentrations were 100 μCi liter⁻¹ for ¹⁴C (Nielsen and Olsen 1989) and 60 μCi liter⁻¹ for ⁶⁸Ge (Penry and Frost 1991). At these levels, the isotopes have no detectable effects on growth rates (Nielsen and Olsen 1989; Penry and Frost 1991). The algae were harvested in late exponential phase, 10 to 17 d after transfer. The long incubation times with the radioisotopes ensured that the algae were uniformly labeled, because at least five cell doublings are recommended for uniform labeling (Nielsen and Olsen 1989). Before the diatoms were added to the experimental jars, dissolved label in the culture was removed by a series of reverse flow filtrations using a 7-μm screen, thereby diluting the label ~10,000-fold.

Absorption experiments with O. vanhoffeni—Animals were collected in 500-ml glass jars (one animal per jar) by shore-based scuba divers in front of the Ocean Sciences Centre (Logy Bay, Newfoundland, Canada) from January to June 1996. The jars were immediately transported into the laboratory and placed in flowing ambient-temperature seawater. Within 3 h of collection, jars containing filtering animals were put on ice, old houses were removed with a wide-

¹ Present address: Fish Ecology Lab, Biology Department, Queen's University, Kingston, Ontario, Canada K7L 3N6.

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mouthed pipette, and the labeled algae were added in various concentrations ranging from ~30 to 1,330 cells ml⁻¹ final concentration in order to test the effect of availability of food on absorption efficiency. The sealed jars were covered with ice and kept in the dark for the 2–4-h experiments. At the end of the incubation, we removed 200 ml of seawater from the jars using a syringe, carefully avoiding fecal pellet contamination, and passed the water through a glass fiber filter (GF/F). The animals were gently sucked into a wide-mouthed pipette through the escape chamber of the house (Flood 1991) and put into GF/F-filtered seawater on ice. They were then serially transferred through two washes in GF/F-filtered seawater to reduce the number of attached algae. The trunk length (excluding the gonads) of each animal was measured to the nearest 20 μm. Animals were removed by the tail from the filtered seawater by use of forceps and transferred into 7-ml glass scintillation vials. Fecal pellets were removed with Pasteur pipettes from the bottom of the jar and from the dissected houses. The fecal pellets were serially transferred twice into 10 ml of GF/F-filtered seawater to remove attached algae and were then pipetted onto a GF/F using as little water as possible. Care was taken that the fecal pellets reaching the vials were all intact and were not contaminated by attached algae or other debris from the house. Residual NaH¹⁴CO₃ was removed by adding 0.25 ml of 0.2 N perchloric acid to the samples and leaving the vials loosely capped for 12 h. Five milliliters of scintillation cocktail (Ecolume) was then added, and the vial was stored for at least 48 h before counting to ensure that ⁶⁸Ge attained a transient equilibrium with ⁶⁸Ga (Rivkin 1986). The samples were counted with a Packard Tri-Carb liquid scintillation spectrometer (Model TR 2500) and were corrected for background activity. Absorption efficiencies were calculated using the following formula (Tande and Slagstad 1985):

$$AE_T = \left(1 - \frac{{}^{68}\text{Ge}_d/{}^{14}\text{C}_d}{{}^{68}\text{Ge}_f/{}^{14}\text{C}_f} \right) \cdot 100 \quad (1)$$

where AE_T is the absorption of total or bulk carbon, ⁶⁸Ge_d and ⁶⁸Ge_f are the disintegrations per minute (dpm) of ⁶⁸Ge in the diatoms and feces, respectively, and ¹⁴C_d and ¹⁴C_f are the dpm of ¹⁴C in diatoms and feces, respectively, at the end of the experiment.

Test of ⁶⁸Ge as a conservative tracer—For this experiment, three groups of animals were compared. One group was allowed to feed on the dual-labeled algae (~50 cells ml⁻¹) and was then placed in filtered seawater to evacuate the guts of the animals before measurement in the scintillation counter. A second group was transferred with full guts into scintillation vials to determine how much ⁶⁸Ge is present in animals with full guts, and a third group, comprising animals with crippled tails, was kept in the experimental jars in order to provide nonfeeding controls. If ⁶⁸Ge is indeed a conservative tracer, the amount of tracer recovered from the first group should be equal to that from the third group.

Comparison with copepods—Applying a new method poses the risk that the values obtained will not be comparable with other studies. Since no information exists on the

absorption efficiencies of cold-water appendicularians, one experiment measured copepods, which have been studied in more detail. For this purpose, a mixture of calanoid copepods (mainly stage VI females of *Calanus finmarchicus* and *Calanus glacialis*) were collected with a 500-μm plankton net. After 2 d in buckets filled with ambient seawater, the copepods were divided into two groups. One group was fed an unlabeled suspension of *T. nordenskioldii*, and the other group was kept in filtered seawater overnight. The following day, the animals were transferred into suspensions of dual-labeled algae and were kept on a plankton wheel overnight (12 h) at 2.5°C. After settling for ~30 min, fecal pellets were removed from the bottom of the jar, transferred into well plates, and washed three times with 10 ml of GF/F-filtered seawater. At least 20 pellets were transferred onto each GF/F, and each filter was put into a glass scintillation vial. After adding 0.25 ml of 0.2 N perchloric acid and leaving the vials loosely capped for 12 h, we added scintillation cocktail. We removed 180 ml of the supernatant food suspension with a syringe (avoiding fecal pellet contamination) and then filtered the food suspension onto GF/F and processed it in the same manner as the water samples from the *O. vanhoeffeni* experiments.

Dual-labeling protocol—The maximum β energies are 156 keV and 1.9 MeV for ¹⁴C and ⁶⁸Ge, respectively. The ratio of these maximum energies (i.e., 12.2) is therefore high enough for complete separation of the two isotopes (Kobayashi and Maudsley 1974). For each isotope, standard quench curves were constructed using internal standards, with chloroform as the quenching agent. For the dual-labeling counting protocol, we followed the procedure outlined in the Packard Tri-Carb liquid scintillation analyzer operation manual. In order to test for accuracy of the technique, we prepared mixtures of known concentrations of ¹⁴C and ⁶⁸Ge and measured them with the new dual-label scintillation counting protocol.

Biochemical fractionation—In these experiments, diatoms labeled solely with ¹⁴C were fed to the animals. The remainder of the protocol was identical to the one for the dual-labeling experiments (see above). Algae, animals, and feces were fractionated into biochemical pools using the separation technique of Li et al. (1980) as modified by Rivkin (1985). Briefly, the four fractions were low molecular weight (LMW, water soluble), lipid (chloroform–methanol soluble), polysaccharides (hot trichloroacetic acid (TCA) soluble), and proteins (TCA insoluble). The samples were dried at 50–60°C, resuspended in 0.2 ml of distilled water, and counted in 5 ml of Ecolume scintillation cocktail. GF/F filters without biological material were processed in the same manner and served as blanks. Recovery of ¹⁴C after extraction (sum of ¹⁴C in all biochemical pools in proportion to unextracted samples) is usually >90% (Rivkin 1985).

A knowledge of the biochemical composition of food and feces, in conjunction with the absorption efficiency of bulk carbon, allows us to calculate absorption efficiencies for each biochemical compound (Fig. 4):

$$AE_x = 100 - (100 - AE_T)pf_x pd_x^{-1}, \quad (2)$$

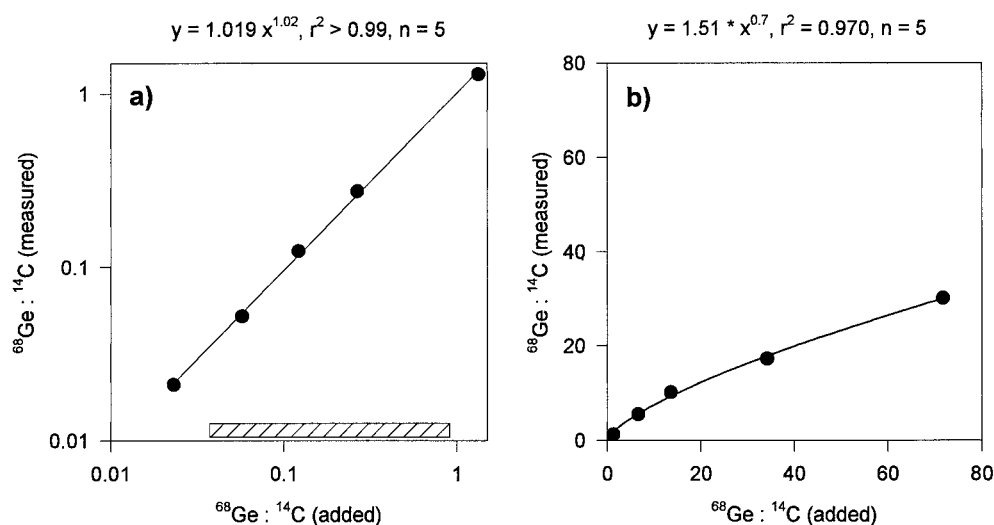


Fig. 1. Test of whether ^{68}Ge and ^{14}C can accurately be separated by the dual-labeling protocol. The lower range (a) is best described by a linear regression and departs slightly from the ideal 1:1 line. However, at higher ratios of ^{68}Ge : ^{14}C , the measured ratios depart significantly but predictably from the true ratios and would then have to be corrected by the formula shown in (b). All the experimental ratios were within the linear portion of the curve and no correction was required (shaded bar). Note that (a) is plotted on a log scale and (b) on a linear scale.

where AE_x is the absorption efficiency of the biochemical fraction (x) measured in percent, pf_x and pd_x are the proportions of fraction x in the feces and diatoms, respectively, and AE_T is the absorption efficiency of bulk carbon measured in percent, as measured by the dual-labeling protocol.

Results

Testing the dual-labeling protocol—A wide range of ratios of known activities of ^{14}C and ^{68}Ge was used to test whether the isotopes can be completely separated using liquid scintillation counting. The results (Fig. 1) were divided into two ranges, a lower range in which experiments were

performed (Fig 1a) and an upper range in which the measured ratios depart considerably from linearity at higher ^{68}Ge concentrations (Fig. 1b). We tested the sensitivity of the absorption efficiencies to slight departures from the ideal 1:1 relationship shown in Fig. 1a. The calculation error for absorption efficiencies was 2.5% in extreme cases and, considering other experimental sources of error, was thus negligible.

Absorption efficiencies for bulk carbon—Table 1 summarizes the five experiments with *O. vanhoeffeni* and the one experiment with copepods. The absorption efficiencies were calculated for each animal and were significantly different among experiments, with the means for each experi-

Table 1. Absorption experiments with *Oikopleura vanhoeffeni* and *Calanus* spp. using the ^{68}Ge : ^{14}C dual-labeling technique. Ambient temperature is the temperature at the collection site in Logy Bay (Newfoundland). Experimental temperature for *O. vanhoeffeni* was kept constant (i.e., 0°C) by covering the experimental jars with ice. The experimental temperature in the copepod experiments was +2°C. ^{68}Ge : ^{14}C *T. nord.* is the ratio of the two isotopes in *Thalassiosira nordenskioldii*; ^{68}Ge : ^{14}C feces is their ratio in the feces. Values are given as means (sample size; \pm standard deviation). Overall gives the mean of the means of each experiment with *O. vanhoeffeni* ($n = 5$). * indicates the date of collection in 1996 during the spring diatom bloom. Note that the final column is not derived directly from the third and fourth columns, but rather represents the arithmetic mean of assimilation efficiencies (AE) calculated for each individual animal.

Date (1996)	Ambient temperature (°C)	^{68}Ge : ^{14}C <i>T. nord.</i> [mean (n ; \pm SD)]	^{68}Ge : ^{14}C feces [mean (n ; \pm SD)]	AE% [% (n ; SD)]
<i>Oikopleura vanhoeffeni</i>				
13 Feb	-1.2	0.048 (12; 0.0041)	0.36 (10; 0.17)	83 (10; 7.7)
14 Feb	-1.2	0.047 (12; 0.0061)	0.26 (7; 0.18)	75 (7; 13)
6 May*	n.d.	0.069 (21; 0.0047)	0.30 (16; 0.19)	68 (16; 19)
31 May	+4.0	0.121 (10; 0.0038)	0.32 (7; 0.26)	42 (7; 31)
14 Jun	+4.5	0.063 (12; 0.0041)	0.23 (9; 0.14)	66 (9; 15)
Overall		0.070 (5; 0.030)	0.29 (5; 0.05)	67 (5; 15)
<i>Calanus finmarchicus</i> and <i>Calanus glacialis</i> :				
25 Apr	+1.5	0.084 (4; 0.003)	0.326 (8; 0.021)	74 (8; 1.8)

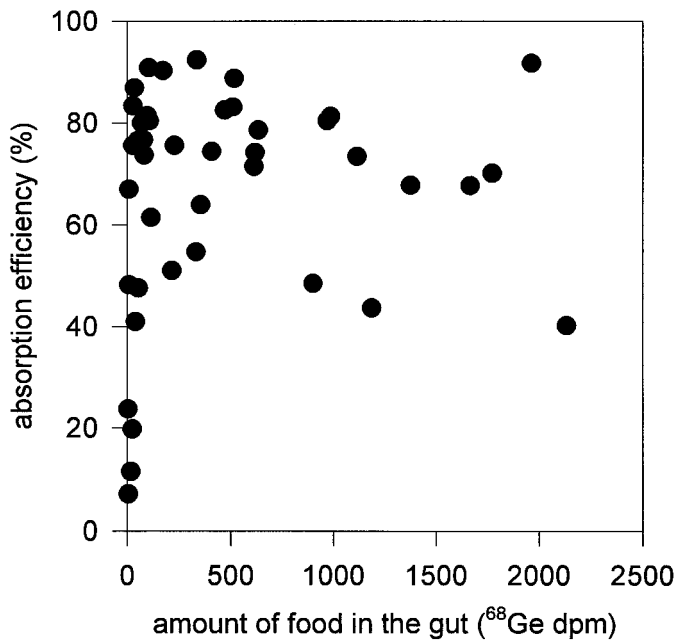


Fig. 2. Absorption efficiencies in *Oikopleura vanhoffeni* were independent of the amount of food in the gut ($n = 42$, $r^2 = 0.013$, $P = 0.47$). At constant gut passage times, gut fullness can be used as a proxy for ingestion rates (Bochdansky et al. 1998; Bochdansky and Deibel 1999).

ment ranging from 42 to 83% (one-way analysis of variance [ANOVA], Table 1). The mean of experimental means (i.e., 66.8%) was very similar to the mean calculated by pooling all absorption efficiency data (i.e., 67.5%). The differences in absorption efficiencies among experiments (Table 1) were caused by a significantly higher ratio of $^{68}\text{Ge} : ^{14}\text{C}$ in the algae in experiment 4 ($n = 67$, $r^2 = 0.97$, $P < 0.0001$). In contrast, the ratio of $^{68}\text{Ge} : ^{14}\text{C}$ in the fecal pellets was constant and not significantly different among experiments ($n = 49$, $r^2 = 0.05$, $P = 0.65$). The absorption efficiencies were related neither to trunk length, which ranged from 1.7 to 4.9 mm ($n = 43$, $r^2 = 0.081$, $P = 0.07$), nor to gut content of the animals (Fig. 2). The mean absorption efficiency for the copepods was 74% ($\pm 8\%$ SD) (Table 1). There was no significant difference between the means of prefed and starved copepods (one-way ANOVA, $n = 8$, 2 groups, $P = 0.33$).

Biochemical fractionation—The proportions of radiolabel in each biochemical fraction for each compartment (diatom, feces, and animal) are shown in Fig. 3. There was a significant difference in the proportions of all biochemical constituents between algae and fecal pellets (Wilcoxon two-sample test, Fig. 3). Nonparametric statistics were used since the residuals after pairwise comparison with a one-way ANOVA were not normally distributed, even after an arcsine transformation of the proportions (Shapiro–Wilk statistical test for normality, SAS statistical software).

Discussion

Test of assumptions—The following is a combination of conditions suggested by Calow and Fletcher (1972), Wight-

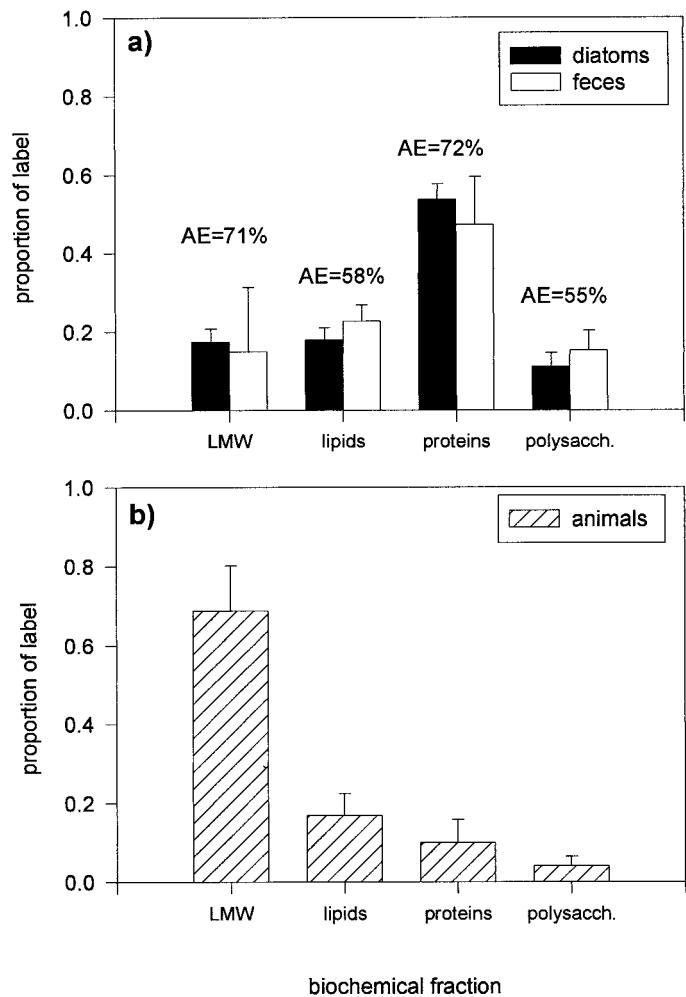


Fig. 3. (a) Proportions of each biochemical fraction for diatoms and feces. Bars indicate standard deviations. In pairwise comparisons, the proportions changed significantly between diatoms and feces, according to a Wilcoxon two-sample test ($\alpha = 0.05$). For LMW and proteins, the proportions significantly decreased from food to feces. For lipids and polysaccharides, the proportions increased from food to feces. Probabilities of the Wilcoxon two-sample analysis: LMW: $P = 0.034$; lipids: $P = 0.0012$; proteins: $P = 0.0063$; polysaccharides: $P = 0.005$. The sample size was 33 in all fractions. The calculated absorption efficiencies (AE_x , Eq. 2) for each biochemical pool are printed above the column pairs. (b) Accumulation of absorbed ^{14}C in the animals after incubation. Note the large amounts of ^{14}C -labeled LMW in contrast to the low amounts of ^{14}C in proteins and polysaccharides.

man (1975), and Tande and Slagstad (1985), which must be satisfied in order for a dual-labeling method to give reliable results:

Are ^{14}C and ^{68}Ge uniformly distributed throughout the food material? Since only uniformly labeled cell cultures were used in this study, this condition was met. The algae were uniformly labeled because of the extremely long incubation times with the labels (>10 d, Nielsen and Olsen 1989) and because the algae were grown in a closed system with no exchange of CO_2 with the atmosphere.

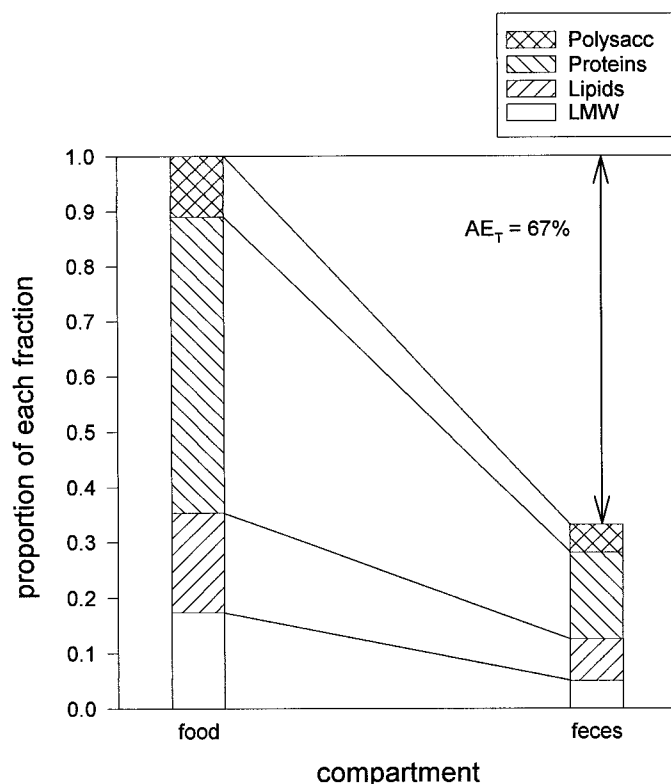


Fig. 4. Schematic diagram of the rationale for calculating absorption efficiencies for each biochemical fraction, knowing their proportion in food and feces and the absorption efficiency of bulk carbon (AE_T).

Does the digestible fraction of carbon derive entirely from food? In contrast to the Conover ratio technique, we used ^{14}C , not ash-free dry weight, to represent organic carbon. Therefore, all of the labeled carbon that was recovered in the feces derived from the diatoms and not from mucous and chitinous intestinal secretions (Johannes and Satomi 1967).

Does the $^{68}\text{Ge}:^{14}\text{C}$ ratio change in the food during the feeding experiment? To answer this question, subsamples of the cultures were taken before the experiments and compared with the water samples taken after the experiments. The ratios of the isotopes in *T. nordenskioldii* before and after the experiments were essentially identical, mainly because of the short incubation times (<4 h; data not shown). For comparison, Tande and Slagstad (1985) found that *Thalassiosira anguste-lineata* lost only 10% of the initial ^{14}C activity within the first 24 h in the dark and 0.5°C .

Are ^{14}C and ^{68}Ge moving through the gut at the same rates? Bricelj et al. (1984) have shown that the bivalve *Mercenaria mercenaria* has separate pathways for processing cell walls and cell contents of *Pseudoisochrysis paradoxa*: the cell contents are incorporated into the digestive gland, and they remain there for intracellular digestion. Thus ^{14}C is retained for a longer period of time than cell wall constituents, and because of this delay, complete recovery of the feces may be important. There is also strong indication for

intracellular digestion in copepods (Hassett and Blades-Eckelbarger 1995) and in some chordates such as *Amphioxus* sp. (Barnard and Prosser 1973). Although we do not know whether intracellular digestion occurs in pelagic tunicates, this potential problem was reduced by collecting almost all of the fecal pellets. The fecal pellets were therefore representative subsamples for the entire incubation period. Phagocytosis of whole cells, as reported by Hildreth (1980) for mussels, is unlikely because of the low amounts of ^{68}Ge that we found within the animals (Table 2).

Treatment	^{14}C (dpm) (n; \pm SD)	^{68}Ge (dpm) (n; \pm SD)	^{14}C : ^{68}Ge ratio
Crippled	111 (8; 138)	19 (8; 4.9)	6
Gut empty	14,113 (12; 12,556)	39 (12; 23)	357
Gut full	26,898 (19; 26,154)	746 (19; 688)	36

Is ^{68}Ge absorbed to any significant degree? The dual-labeling technique permits direct testing of one of the most fundamental assumptions of any technique that uses conservative tracers. Since individual animals can be measured, no indirect budget calculations based on assumptions of complete recovery, such as those performed by Tande and Slagstad (1985), were necessary to explore the potential absorption of ^{68}Ge . The amount of ^{68}Ge was approximately twice as high in animals after defecation than in the crippled controls (Table 2), but how do these values compare with the total amount of ^{68}Ge that passed through the gut? Using the amount of ^{14}C absorbed by the animals (14,113 dpm, Table 2), one has a minimum estimate of the amount of algae that must have passed through the gut during the incubation period. This is considered a minimum estimate because it does not account for respiration or incomplete digestion. Using an average ratio of ^{68}Ge and ^{14}C in the algae (i.e., 0.07; Table 1), the amount of ^{68}Ge that passed through the guts during the incubation period was 988 dpm. The amount retrieved in the animals after gut evacuation (39.48 dpm minus blank) was only 21 dpm, or 2% of 988 dpm (Table 2). This shows that even under the extreme case of 100% of carbon being absorbed with no respiration occurring, a negligible amount of ^{68}Ge was taken up by the animals. We therefore conclude that ^{68}Ge incorporated into the silica frustules of diatoms is a useful conservative tracer and that the 15% loss of biogenic silica as calculated by Tande and Slagstad (1985) is

probably attributable to incomplete recovery of feces in their experiments, as these authors already suspected.

Does ^{68}Ge or ^{14}C leak from the fecal pellets? Since ^{68}Ge is incorporated into the silica frustules that are wrapped within a fecal pellet membrane, it is unlikely that ^{68}Ge leaks from the pellets. Furthermore, unlike coprophagous copepods, *O. vanhoeffeni* can neither feed on nor mechanically rupture fecal pellets. On the other hand, dissolved ^{14}C can potentially pass the fecal pellet membrane. Of all the biochemical fractions, the LMW fraction is the most volatile. The relatively high proportions of LMW found in the fecal pellets (Fig. 3a), however, suggest that their leakage from the pellets was insignificant. We did not directly test leakage of ^{14}C from the pellets, but data in Tande and Slagstad (1985) indicate that no leakage occurred from copepod fecal pellets kept in suspension over a period of 34 h.

Does coprophagy occur? If, for a given animal, the probability of ingestion of its own feces is higher in an experimental condition than it is in nature, the absorption efficiencies could be artificially elevated. This is particularly a problem for copepods kept at high densities in incubation jars (compare Lampitt et al. 1990). *O. vanhoeffeni*, however, cannot reingest its fecal pellets because the inlet filter meshes exclude particles of this size (Deibel 1986).

Is the experimental situation representative of the field? Absorption efficiencies are affected by the quality of the food (Houde and Roman 1987) and by acclimation of animals to the quantity of food (Landry et al. 1984). Maintaining animals in experimental conditions inevitably introduces the risk of producing results that are not representative of the field. However, for absorption studies in general, the potential for laboratory artifacts is usually outweighed by the advantage of more controlled conditions in laboratory studies. To minimize experimental artifacts, we processed *O. vanhoeffeni* within hours of collection, thereby ensuring that the animals were healthy and fed. We know that diatoms constitute a major part of the diet of *O. vanhoeffeni* because large amounts of chlorophyll *c* are found in their guts throughout the field season (Bochdansky et al. 1998).

Comparison of the ^{68}Ge : ^{14}C dual-labeling technique with other methods—A dual-labeling technique has many advantages over the conventional gravimetric Conover-ratio technique. It is more sensitive and therefore more useful for small sample sizes such as individual fecal pellets, and it is not influenced by the problem of excretion of substances into the feces. A dual-labeling (or twin tracer) technique for absorption experiments, with all of its advantages, has already been described by Calow and Fletcher (1972) using ^{51}Cr as the indigestible fraction. The advantage of ^{51}Cr is that it is not restricted to diatoms and can be used for a variety of food sources (Wang et al. 1996). However, considerable amounts of tracer can be lost since ^{51}Cr is not incorporated into the cells like ^{68}Ge but is absorbed to the surface (Stuart et al. 1982). This is particularly problematic in filter feeders like bivalves, in which losses onto the large surface areas of ctenidia, palps, and digestive glands have been reported to

be as high as 17 to 45% (Stuart et al. 1982) and 14% (Bricelj et al. 1984) of the ingested amount.

Biogenic silica has been used as a conservative tracer for absorption studies (Tande and Slagstad 1985; Head 1992; Cowie and Hedges 1996) as well as to investigate pigment destruction (Conover et al. 1986; Head 1988). However, measuring biogenic silica instead of the incorporated ^{68}Ge has several disadvantages. First, samples must be split into two parts: one part is used for analysis of the organic or pigment fraction and the other part is hydrolyzed prior to performing the silica assay. Therefore, more material is required and more analytical steps are involved. In the dual-labeling approach with ^{68}Ge , the feces, as well as the filtered algae, are simply transferred into scintillation vials. Fewer analytical steps mean less chance for contamination or loss of material. One could argue that carbon-to-biogenic silica ratios could be studied in the field to estimate absorption efficiencies, as attempted by Head (1992), but the ratios of food and feces can only be compared if feeding is unselective. Because feeding is rarely unselective in copepods, one of the fundamental assumptions (*see above*) is therefore violated. For animals with some degree of selectivity, including various retention efficiencies of mucous filters, the biogenic silica assay and the ^{68}Ge : ^{14}C dual-labeling approach are therefore limited to laboratory studies with diatoms.

Absorption of bulk carbon—The absorption efficiencies of *O. vanhoeffeni* varied significantly among experiments (Table 1). It is remarkable that this difference of absorption efficiencies was not caused by a change in isotope ratios in fecal pellets but rather by the isotope ratios of the diatoms. This fact may be explained by a change in the organic composition of the cells. For example, diatoms can accumulate polysaccharides such as acid-soluble β -1,3 glucan (i.e., chrysolaminaran; Darley 1977) during the later stages of their growth (Myklestad 1974). According to Reinfelder and Fisher (1991), the cell plasma rather than the cell wall components of diatoms are digested by copepods. If a similar mechanism holds true for *O. vanhoeffeni* and the high ratio of ^{68}Ge : ^{14}C in experiment 4 was a reflection of relatively small amounts of digestible carbon in the cell plasma, then the low absorption rates can be explained. As a consequence, it is mainly the cell wall constituents that would be defecated; since these constituents are less variable than the plasma carbon (Handa 1969), the ^{68}Ge : ^{14}C in the feces would be predictably less variable as well (Table 1). However, to test this hypothesis, the contribution of cell wall constituents to the polysaccharide fraction needs to be analyzed in more detail.

Comparison with other zooplankton—Absorption efficiencies have been calculated by Gorsky (1980) for *Oikopleura dioica* by using the Conover ratio (1966a) and assuming that various food particles were distributed in the same proportion in the gut as in the water. Three different food types were offered: *Isochrysis galbana*, a mixture of the diatom *Thalassiosira pseudonana* and the flagellate *Platymonas sueica*, and natural seawater filtered through a 50- μm screen. The absorption efficiencies varied: 17% for *I. galbana*, 88% for the mixture of diatom and flagellate, and 79% for the

natural seawater. In comparison, the range of average absorption efficiencies from 47–80% in *O. vanhoeffeni* is considerably lower than the range calculated for the mixture containing the diatom in the *O. dioica* experiments (i.e., 88%). For *Salpa fusiformis* fed with the diatom *Phaeodactylum tricornerutum* and the flagellate *Hymenomonas elongata*, Andersen (1986) determined absorption efficiencies of 32% and 64%, respectively. The data in Gorsky (1980) and Andersen (1986) show opposite trends. Whereas the diatom was assimilated most efficiently and the flagellate least efficiently in the appendicularian, the opposite was true for the salp. Madin and Purcell (1992) calculated absorption efficiencies of 61% for carbon and 71% for nitrogen for *Cyclosalpa bakeri* feeding on mixed phytoplankton containing diatoms. Since chlorophyll *a* (Chl *a*) is efficiently digested in the tunicate gut (Bochdansky et al. 1998), Chl *a* concentration was corrected for pigment loss by counting fragments of silica frustules in the feces (Madin and Purcell 1992). Assuming that most nitrogen is found only in the protein fraction (see below), the absorption efficiency for nitrogen in this study was ~72% (Fig. 3a) and therefore similar to the efficiency measurement reported by Madin and Purcell (1992). The absorption efficiency obtained for the copepods (i.e., 74%, Table 1) not only fits well within the range reported in the literature (summary table 7.4 in Omori and Ikeda 1984) but also compares well with the absorption efficiencies usually assumed for herbivorous zooplankton in ecosystem models (e.g., 75% for nitrogen [Fasham et al. 1990]; 70% for carbon [Falkowski et al. 1988; Frost 1993]). Absorption efficiencies of ¹⁴C in copepods calculated by Wang et al. (1996) were significantly influenced by the type of algae, ranging from 69 to 97%. Although an overall constant absorption efficiency may be justified as a first approximation in ecosystem models, researchers should not ignore potentially important sources of variability, since little is known about the factors affecting absorption efficiencies in the field. Though outside the scope of this study, a more detailed examination of the influence of food type on absorption efficiencies is important for pelagic tunicates.

No effect of levels of ingestion on absorption efficiencies—Beklemishev (1962) hypothesized that zooplankton would use food inefficiently when food was offered at high concentrations, and indeed some digestion models predict that absorption efficiencies decrease with increasing gut fullness (Slagstad and Tande 1981). However, in many experiments, ingestion rates have exhibited little or no effect on absorption rates (Conover 1966a,b; Tande and Slagstad 1985; Wang et al. 1996). The absorption efficiencies in this study were not affected by the amount of material in the guts, measured as the amount of *T. nordenskioldii* recovered in the animals (Fig. 2b). Although natural seston was still present in the jars, it was greatly reduced by the filtering activity of the animals in the few hours before *T. nordenskioldii* was added. The size and color of the fecal pellets containing *T. nordenskioldii* were markedly different (brown-green) from the smaller black fecal pellets produced before the addition of the algae. One can therefore conclude that the amount of available food was closely related to the amount of *T. nordenskioldii* added to the suspension at the

beginning of the experiments. Since gut passage times remain constant irrespective of the amount of food ingested, the gut content can be used as a proxy for ingestion rates (Bochdansky et al. 1998; Bochdansky and Deibel 1999). Therefore, absorption efficiencies were also not related to ingestion rates. However, the lowest and some of the highest absorption efficiencies were found in the lower gut content range (Fig. 2b) simply because of decreasing accuracy of data at values close to zero (compare Conover 1966b). When we excluded gut content levels less than 25 dpm above zero, the low absorption values were eliminated, although absorption efficiency and gut content remained independent ($n = 35$, $r^2 = 0.03$, $P = 0.32$). Also, the removal of these data points would have little effect on the overall absorption efficiency, since the absorption efficiency calculated as the grand mean of the experimental means would only increase from 67% (shown in Table 1) to 70%. A constant absorption efficiency at various ingestion rates has two important implications: using only one value for absorption efficiency simplifies ecosystem models, and it shows that neither enzyme systems nor absorption processes in the guts of zooplankton show signs of saturation with substrate, even at high ingestion rates (Bochdansky and Deibel 1997). For appendicularians, as well as for copepods, these high levels of enzyme activity therefore ensure that incoming substrate is efficiently utilized whenever available (Hassett and Landry 1983).

Biochemical composition of T. nordenskioldii—The four fractions extracted represent a very crude biochemical characterization of the pools of cell carbon. The LMW fraction consists of a mixture of water-soluble metabolites such as monosaccharides, organic acids, amino acids, and lipid precursors (Laws 1991; Roman 1991). The polysaccharide fraction, on the other hand, ranges in its composition, from structural polysaccharides to nucleic acids, which are also TCA soluble (~5% of cell carbon; Laws 1991). In diatoms, the protein fraction may contain as much as 33% chitin, as demonstrated for *T. pseudonana* (Smucker and Dawson 1986). In uniformly labeled cells, the proportion of total carbon that is labeled is the same in all biochemical fractions, and the distribution of ¹⁴C among fractions should reflect the proportions of biochemical fractions in the cell. In this study, *T. nordenskioldii* allocated an average of 17% of ¹⁴C in the LMW fraction, 18% in the lipid fraction, 54% in protein, and 11% in polysaccharides (Fig. 3a). Assuming that most of the LMW compounds are simple sugars (Laws 1991), one arrives at 54% protein, 28% carbohydrate, and 18% lipid, which is similar to the 60% protein, 34% carbohydrate, and 7% lipid given in Parsons (1961). However, lipid levels in diatoms may be as high as 30% (table 4 in Laws 1991). Using the same extraction technique, Rivkin (1985) arrived at 15–30% LMW, 22–35% lipid, 33–48% protein, and 7–10% polysaccharides for three diatoms. In summary, the partitioning of carbon among the various pools in *T. nordenskioldii* is representative for a wide variety of diatoms.

Absorption efficiencies of various biochemical fractions in O. vanhoeffeni—The absorption efficiencies calculated using Eq. 2 were highest for LMW and proteins, which is similar

Table 3. Estimated C:N ratios for *Thalassiosira nordenskioldii* and for the feces of *Oikopleura vanhoeffeni* feeding on this diatom, calculated from proximate biochemical composition using three different methods, those of Gnaiger and Bitterlich (1984) of Laws (1991), and of Anderson (1994). For comparison, C:N ratios are given both by weight and by atoms.

	% protein	C:N by weight (atoms)		
		Gnaiger (1984)	Laws (1991)	Anderson (1994)
<i>T. nordenskioldii</i>	54	5.9 (6.9)	5.2 (6.1)	6.3 (7.3)
Feces of <i>O. vanhoeffeni</i>	47	6.9 (8.1)	6.0 (7.0)	7.2 (8.4)

to the findings for copepods (Landry et al. 1984; Hassett and Landry 1988; Roman 1991). Copepods may maximize protein uptake because protein could be limiting to growth and egg production (Checkley 1980; Houde and Roman 1987), although higher absorption efficiencies for proteins may simply reflect the digestibility of nitrogenous compounds (Anderson and Hessen 1995). For lipids, the absorption efficiencies were lower than for mean carbon in *O. vanhoeffeni*. Since this species does not accumulate lipids as storage products (Deibel et al. 1992), it may incorporate some lipids into somatic tissue, use lipids for maintaining membrane fluidity (Deibel et al. 1992), or catabolize lipids as krill do (Pond et al. 1995). The high amounts of ^{14}C in the LMW fraction of the animals (Fig. 3b) cannot entirely be explained by the preferential uptake of LMW; the high amount may be attributable to the action of exoenzymes on polysaccharides and proteins before the metabolites were absorbed by the animals. Finally, polysaccharides were expected to be the least digestible fraction, since a major portion of undigestible cell wall compounds would be located in this fraction (McClintock 1986; Reinfelder and Fisher 1991).

C:N ratios of T. nordenskioldii and fecal pellets—One of the benefits of biochemically fractionating phytoplankton and feces in absorption studies is the ability to predict the C:N ratios of sinking material. According to Laws (1991), the N:C ratio (by weight) can be calculated by dividing the proportion of ^{14}C allocated to protein by 2.8. An alternative calculation is to use mass fractions of standard carbohydrate, lipid, and proteins as given in Gnaiger and Bitterlich (1984). However, for this calculation it must be assumed that all nitrogen is in the protein fraction. This assumption is disproved by the fact that amino acids can be found in the LMW fraction and nucleic acids can be found in the polysaccharide fraction (see above), but this problem may be of little significance. Gnaiger and Bitterlich (1984) have calculated a new nitrogen mass fraction of 0.173 for standard proteins of bacteria, algae, and aquatic animals, a value that is higher than the traditionally used mass fraction of 0.16. A third formula is given by Anderson (1994), who suggested dividing the C:N ratio of proteins (i.e., 3.96 by atoms) by the proportion of proteins in the sample. The results of the three calculations are given in Table 3 for diatoms and feces.

The C:N ratios ranged from 5.2 to 6.3 by weight for *T. nordenskioldii*, which is similar to the Redfield ratio of 5.7 (6.6 by atoms, DiTullio and Laws 1986) and the predicted ratio for phytoplankton of 6.5 by weight (Laws 1991). However, the C:N ratios of the fecal pellets of *O. vanhoeffeni* ranged from 6.0 to 7.2 by weight (depending on the type of calculation) and were at the lower end of the range measured

for zooplankton feces (i.e., 6.7–19, Anderson 1994). This suggests that although there was a preferential absorption of proteins over other fractions in *O. vanhoeffeni* (Fig. 3a), there would only be a small increase in C:N ratios during digestion (Table 3). When generalizing the dynamics of C:N ratios that are caused by the feeding activity of zooplankton, it is important to keep in mind that in about half of the studies cited by Anderson (1994, his table 3), C:N ratios either did not change much or decreased from food to feces.

Conclusions

O. vanhoeffeni assimilates bulk carbon of the diatom *T. nordenskioldii* with an efficiency of 67%, independent of body size and ingestion rate. This was similar to an assimilation efficiency measurement of 74% as determined for a mixture of copepods (*C. finmarchicus* and *C. glacialis*) with the same technique. Although one must exercise some caution when comparing these values with other studies that use different techniques, the absorption efficiencies fit well within the ranges reported for both herbivorous copepods and pelagic tunicates. There is no a priori reason to assume that absorption efficiencies are different in crustaceans and tunicates when the same material is digested, since physiological processes are very similar in terms of carbon-based budget analyses (Schneider 1992). Although *O. vanhoeffeni* preferentially assimilates proteins and LMW compounds over lipids and carbohydrates, the difference is small. The estimated C:N ratios of fecal pellets are not much lower than those of the diatoms, which puts them into the lower range of C:N ratios of fecal material determined for other zooplankton.

References

- ALLDREDGE, A. L. 1981. The impact of appendicularian grazing on natural food concentrations in situ. *Limnol. Oceanogr.* **26**: 247–257.
- ANDERSEN, V. 1986. Effect of temperature on the filtration rate and percentage of assimilation of *Salpa fusiformis* Cuvier (Tunicata: Thaliacea). *Hydrobiologia* **137**: 135–140.
- ANDERSON, T. R. 1994. Relating C:N ratios in zooplankton food and faecal pellets using a biochemical model. *J. Exp. Mar. Biol. Ecol.* **184**: 183–199.
- , AND D. O. HESSEN. 1995. Carbon or nitrogen limitation in marine copepods? *J. Plankton Res.* **17**: 317–331.
- BARNARD, E. A., AND C. L. PROSSER. 1973. Comparative biochemistry and physiology of digestion, p. 133–164. *In* C. L. Prosser [ed.], *Comparative Animal Physiology*. Saunders.
- BEKLEMISHEV, C. W. 1962. Superfluous feeding of marine herbiv-

- orous zooplankton. Rapp. P.-V. Reun. Cons. Perm. Int. Explor. Mer. (ICES) **153**: 108–113.
- BOCHDANSKY, A. B., AND D. DEIBEL. 1997. Destruction of chlorophylls in copepod guts: Comment. Mar. Ecol. Prog. Ser. **147**: 301–303.
- , AND ———. 1999. Functional feeding response and behavioral ecology of *Oikopleura vanhoeffeni* (Appendicularia, Tunicata). J. Exp. Mar. Biol. Ecol. **233**: 181–211.
- , ———, AND E. A. HATFIELD. 1998. Chlorophyll *a* conversion and gut passage time for a non-selective mucous net filter feeder: Testing the assumptions of the gut pigment technique for *Oikopleura vanhoeffeni* (Appendicularia, Tunicata). J. Plankton Res. **20**: 2179–2197.
- BRICELJ, V. M., A. E. BASS, AND G. R. LOPEZ. 1984. Absorption and gut passage time of microalgae in a suspension feeder: An evaluation of the ^{51}Cr : ^{14}C twin tracer technique. Mar. Ecol. Prog. Ser. **17**: 57–63.
- CALOW, P., AND C. R. FLETCHER. 1972. A new radiotracer technique involving ^{14}C and ^{51}Cr , for estimating the assimilation efficiencies of aquatic, primary consumers. Oecologia **9**: 155–170.
- CHECKLEY, D. M. 1980. The egg production of a marine planktonic copepod in relation to its food supply: Laboratory studies. Limnol. Oceanogr. **25**: 430–446.
- CONOVER, R. J. 1966a. Assimilation of organic matter by zooplankton. Limnol. Oceanogr. **11**: 338–345.
- . 1966b. Factors affecting the assimilation of organic matter by zooplankton and the question of superfluous feeding. Limnol. Oceanogr. **11**: 346–354.
- , R. DURVASULA, S. ROY, AND R. WANG. 1986. Probable loss of chlorophyll-derived pigments during gut passage through the gut of zooplankton, and some of the consequences. Limnol. Oceanogr. **31**: 878–887.
- COWIE, G. L., AND J. I. HEDGES. 1996. Digestion and alteration of the biochemical constituents of a diatom (*Thalassiosira weissflogii*) ingested by a herbivorous zooplankton (*Calanus pacificus*). Limnol. Oceanogr. **41**: 581–594.
- DARLEY, W. M. 1977. Biochemical composition, p. 198–223. In D. Werner [ed.], The biology of diatoms. Univ. of California Press.
- DEIBEL, D. 1986. Feeding mechanism and house of the appendicularian *Oikopleura vanhoeffeni*. Mar. Biol. **93**: 429–436.
- . 1988. Filter feeding by *Oikopleura vanhoeffeni*: Grazing impact on suspended particles in cold ocean waters. Mar. Biol. **99**: 177–186.
- , J. F. CAVALETTO, M. RIEHL, AND W. S. GARDNER. 1992. Lipid and lipid class content of the pelagic tunicate *Oikopleura vanhoeffeni*. Mar. Ecol. Prog. Ser. **88**: 297–302.
- DITULLIO, G. R., AND E. A. LAWS. 1986. Diel periodicity of nitrogen and carbon assimilation in five species of marine phytoplankton: Accuracy of methodology for predicting N-assimilation rates and N/C composition ratios. Mar. Ecol. Prog. Ser. **32**: 123–132.
- FALKOWSKI, P. G., C. N. FLAGG, G. T. ROWE, S. L. SMITH, T. E. WHITLEDGE, AND C. D. WIRICK. 1988. The fate of a spring phytoplankton bloom: Export or oxidation? Cont. Shelf Res. **8**: 457–484.
- FASHAM, M. J. R., H. W. DUCKLOW, AND S. M. MCKELVIE. 1990. A nitrogen-based model of plankton dynamics in the oceanic mixed layer. J. Mar. Res. **48**: 591–639.
- FLOOD, P. R. 1991. Architecture of, and water circulation and flow rate in, the house of the planktonic tunicate *Oikopleura labradoriensis*. Mar. Biol. **111**: 95–111.
- FORTIER, L., J. L. FEVRE, AND L. LEGENDRE. 1994. Export of biogenic carbon to fish and to the deep ocean: The role of large planktonic microphages. J. Plankton Res. **16**: 809–839.
- FROST, B. W. 1993. A modelling study of processes regulating plankton standing stocks and production in the open subarctic Pacific Ocean. Prog. Oceanogr. **32**: 17–56.
- GNAIGER, E., AND G. BITTERLICH. 1984. Proximate biochemical composition and caloric content calculated from elemental CHN analysis: A stoichiometric concept. Oecologia **62**: 289–298.
- GORSKY, G. 1980. Optimisation des cultures d'Appendiculaires. Approche du métabolisme de *O. dioica*. Ph. D. thesis, L'Université Pierre et Marie Curie, France.
- HANDA, N. 1969. Carbohydrate metabolism in the marine diatom *Skeletonema costatum*. Mar. Biol. **4**: 208–214.
- HASSETT, R. P., AND P. BLADES-ECKELBARGER. 1995. Diel changes in gut-cell morphology and digestive activity of the marine copepod *Acartia tonsa*. Mar. Biol. **124**: 59–69.
- , AND M. R. LANDRY. 1983. Effect of food-level acclimation on digestive enzyme activities and feeding behavior of *Calanus pacificus*. Mar. Biol. **75**: 47–55.
- , AND ———. 1988. Short-term changes in feeding and digestion by the copepod *Calanus finmarchicus*. Mar. Biol. **99**: 63–74.
- HEAD, E. J. H. 1988. Copepod feeding behavior and the measurement of grazing rates *in vivo* and *in vitro*. Hydrobiologia **167**: 31–41.
- . 1992. Comparison of the chemical composition of particulate material and copepod faecal pellets at stations off the coast of Labrador and the Gulf of St. Lawrence. Mar. Biol. **112**: 593–600.
- HILDRETH, D. I. 1980. The passage of two species of micro-algae through the gut of *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. **48**: 17–22.
- HOUDE, S. E. L., AND M. R. ROMAN. 1987. Effects of food quality on the functional ingestion response of the copepod *Acartia tonsa*. Mar. Ecol. Prog. Ser. **40**: 69–77.
- JOHANNES, R. E., AND M. SATOMI. 1967. Measuring organic matter retained by aquatic invertebrates. J. Fish. Res. Bd. Can. **24**: 2467–2471.
- KIØRBOE, T. 1989. Phytoplankton growth rate and nitrogen content: Implications for feeding and fecundity in a herbivorous copepod. Mar. Ecol. Prog. Ser. **55**: 229–234.
- KNOECHEL, R., AND D. STEEL-FLYNN. 1989. Clearance rates of *Oikopleura* in cold coastal Newfoundland waters: A predictive model and its trophodynamic implications. Mar. Ecol. Prog. Ser. **53**: 257–266.
- KOBAYASHI, Y., AND D. V. MAUDSLEY. (1974). Biological applications of liquid scintillation counting. Academic.
- LAMPITT, R. S., T. NOJI, AND B. VON BODUNGEN. 1990. What happens to zooplankton faecal pellets? Implications for material flux. Mar. Biol. **104**: 15–23.
- LANDRY, M. R., R. P. HASSETT, R. P. FAGERNESS, J. DOWNS, AND C. J. LORENZEN. 1984. Effect of food acclimation on assimilation efficiency of *Calanus pacificus*. Limnol. Oceanogr. **29**: 361–364.
- LASKER, R. 1960. Utilization of organic carbon by a marine crustacean: Analysis with carbon-14. Science **131**: 1098–1100.
- LAWS, E. A. 1991. Photosynthetic quotients, new production and net community production in the open ocean. Deep-Sea Res. **38**: 143–167.
- LI, W. K. W., H. E. GLOVER, AND I. MORRIS. 1980. Physiology of carbon photoassimilation by *Oscillatoria thiebautii* in the Caribbean Sea. Limnol. Oceanogr. **25**: 447–456.
- MADIN, L., AND D. DEIBEL. 1997. Feeding and energetics of Thaliaceans, p. 81–103. In Q. Bone [ed.], The biology of pelagic tunicates. Oxford Univ. Press.
- , AND J. E. PURCELL. 1992. Feeding, metabolism, and growth of *Cyclosalpa bakeri* in the subarctic Pacific. Limnol. Oceanogr. **37**: 1236–1251.

- McCLINTOCK, J. B. 1986. On estimating energetic values of prey: Implications in optimal diet models. *Oecologia* **70**: 161–162.
- MICHAELS, A. F., AND M. W. SILVER. 1988. Primary production, sinking fluxes and the microbial food web. *Deep-Sea Res.* **35**: 473–490.
- MYKLESTAD, S. 1974. Production of carbohydrates by marine planktonic diatoms. I. Comparison of nine different species in culture. *J. Exp. Mar. Biol. Ecol.* **15**: 261–274.
- NIELSEN, M. V., AND Y. OLSEN. 1989. The dependence of the assimilation efficiency in *Daphnia magna* on the ^{14}C -labeling period of the food alga *Scenedesmus acutus*. *Limnol. Oceanogr.* **34**: 1311–1315.
- OMORI, M., AND T. IKEDA. 1984. *Methods in marine zooplankton ecology*. Wiley.
- PARSONS, T. R. 1961. On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Bd. Can.* **18**: 1001–1016.
- PENRY, D. L., AND B. W. FROST. 1991. Chlorophyll *a* degradation by *Calanus pacificus*: Dependence on ingestion rate and digestive acclimation to food resources. *Limnol. Oceanogr.* **36**: 147–159.
- POND, D. W., J. PRIDDLE, J. R. SARGENT, AND J. L. WATKINS. 1995. Laboratory studies of assimilation and egestion of algal lipid by Antarctic krill—methods and initial results. *J. Exp. Mar. Biol. Ecol.* **187**: 253–268.
- REINFELDER, J. R., AND N. S. FISHER. 1991. The assimilation of elements ingested by marine copepods. *Science* **251**: 794–251.
- RIVKIN, R. B. 1985. Carbon-14 labelling patterns of individual marine phytoplankton from natural populations. *Mar. Biol.* **89**: 135–142.
- . 1986. Radioisotopic method for measuring cell division rates of individual species of diatoms from natural populations. *Appl. Environ. Microbiol.* **51**: 769–775.
- ROMAN, M. R. 1991. Pathways of carbon incorporation in marine copepods: Effects of developmental stage and food quality. *Limnol. Oceanogr.* **36**: 796–807.
- SCHNEIDER, G. 1992. A comparison of carbon-specific respiration rates in gelatinous and non-gelatinous zooplankton: A search for general rules in zooplankton metabolism. *Helgol. Meeresunters.* **46**: 377–388.
- SLAGSTAD, D., AND K. S. TANDE. 1981. A mathematical model of the assimilation process in the copepod *Calanus finmarchicus* (Gunnerus): Computer simulations discussed in relation to experimental results. *Kiel. Meeresforsch. Sonderh.* **5**: 229–239.
- SMUCKER, R. A., AND R. DAWSON. 1986. Products of photosynthesis by marine phytoplankton: Chitin in TCA “protein” precipitates. *J. Exp. Mar. Biol. Ecol.* **104**: 143–152.
- STUART, V., J. G. FIELD, AND R. C. NEWELL. 1982. Evidence for absorption of kelp detritus by the ribbed mussel *Aulacomys ater* using a new ^{51}Cr -labelled microsphere technique. *Mar. Ecol. Prog. Ser.* **9**: 263–271.
- SUESS, E. 1980. Particulate organic carbon flux in the oceans—surface productivity and oxygen utilization. *Nature* **288**: 260–263.
- TAGUCHI, S. 1982. Seasonal study of fecal pellets and discarded houses of Appendicularia in a subtropical inlet, Kaneohe Bay, Hawaii. *Estuar. Coast. Shelf Sci.* **14**: 545–555.
- TANDE, K. S., AND D. SLAGSTAD. 1985. Assimilation efficiency in herbivorous aquatic organisms—the potential of the ratio method using ^{14}C and biogenic silica as markers. *Limnol. Oceanogr.* **30**: 1093–1099.
- WANG, W.-X., J. R. REINFELDER, B.-G. LEE, AND N. FISHER. 1996. Assimilation and regeneration of trace elements by marine copepods. *Limnol. Oceanogr.* **41**: 70–81.
- WIGHTMAN, J. A. 1975. An improved technique for measuring assimilation efficiency by the ^{51}Cr - ^{14}C twin tracer method. *Oecologia* **19**: 273–284.

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