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Quantifying marine snow as a food choice for zooplankton using stable silicon isotope tracers

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Aggregates of biogenic origin >0.5 mm, known as marine snow, represent a concentrated potential source of food for zooplankton. Little is known, however, about whether aggregates are commonly grazed by zooplankton in the field. While previous laboratory studies have shown that the euphausiid Euphausia pacifica, and the copepod, Calanus pacificus, common crustacean zooplankters, consume marine snow if it is the only food source available, it is not known if euphausiids will select marine snow in the presence of edible dispersed cells, as readily occurs in nature. To examine this question, we offered E. pacifica the diatom, Nitzschia angularis in aggregated and dispersed form as prey simultaneously. Aggregates and dispersed food contained identical cell types so that differing prey quality, taste or motility would not be a factor. A new method was developed to track food sources by labeling the frustules of aggregated cells with different naturally occurring, but rare, stable isotopes of silicon, ^{30}Si and ^{29}Si. Food selection was then estimated by measuring the isotopic composition of silica within fecal pellets produced by animals feeding on mixtures of the two labeled foods. Results indicate that E. pacifica consumed both aggregates and dispersed cells, even when more cells were made available in dispersed form than in aggregated form. This suggests that aggregates may indeed be a food source in the field, even when dispersed cells are relatively abundant. The method of labeling diatom cells with stable isotopes of silica may prove useful for future grazing experiments to distinguish identical cell types.

INTRODUCTION

Marine snow, or organic detrital aggregates >0.5 mm in diameter, plays an important role in the marine carbon cycle due to its high abundance, relatively large size and rapid sinking rate (Fowler and Knauer, 1986; Alldredge and Silver, 1988). Although ubiquitous throughout the world's oceans, the flux of marine snow decreases exponentially with depth (Martin *et al.*, 1987). Several processes including grazing and disaggregation of marine snow by macrozooplankton have been hypothesized to decrease particulate flux with depth (Karl *et al.*, 1988; Dilling and Alldredge, 2000; Goldthwait *et al.*, 2004). Zooplankton are extensively associated with marine snow aggregates in the field and it has been estimated that 20–70% of aggregate carbon may be lost to consumption before leaving the euphotic zone (Kiørboe, 2000).

Many studies have suggested that aggregates are sources of food for zooplankton in the field (Alldredge 1972, 1976; Ohtsuka and Kubo, 1991; Suh *et al.*, 1991; Bochdansky and Herndl, 1992; Dagg, 1993; Lampitt *et al.*, 1993; Steinberg *et al.*, 1994, 1997; Green and Dagg, 1997; Dilling *et al.*, 1998; Schnetzer and Steinberg, 2002). Most laboratory studies have shown feeding on marine snow through single food type experiments. Often, studies of natural populations have speculated that guts of wild zooplankton have contained marine snow, yet direct confirmation is usually lacking since marine snow is often indistinguishable in the gut from

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cells captured individually (Ohtsuka and Kubo, 1991; Dagg, 1993). Progress has been made in certain regions where marine snow is distinguishable in gut contents from suspended cells due to the presence of a specific 'marine snow signature' composed of olive-green debris or when the zooplankter has been directly observed feeding on an aggregate (Lampitt *et al.*, 1993; Steinberg *et al.,* 1994; Schnetzer and Steinberg, 2002). The marine snow signature is not ubiquitous to all regions, however, as it has only been found thus far in the North Atlantic. It therefore remains difficult to quantify feeding on marine snow in the field and in laboratory experiments when such a signature is lacking, and other suspended cell food sources are present.

In the euphotic zone, marine snow almost always occurs along with many other potential food sources for omnivorous zooplankton, including individual phytoplankton cells. Marine snow is formed from the aggregation of smaller particles in the water column, including phytoplankton, Transparent Exopolymer particles (TEP) and fecal pellets, or from discarded mucous feeding structures (Alldredge and Silver, 1988; Passow, 2002). Additionally, it is often the case that the same species of phytoplankton may be present in the water column in both individual cell and aggregate form, such as during bloom conditions. During these situations, it is extremely difficult to determine what proportion of the diet is obtained through feeding on marine snow when other food types are available.

We therefore set out to develop and test a method that could conclusively distinguish between ingestion of aggregates and individual cells. To most closely simulate the aggregation conditions that might occur during a bloom, we chose to develop a method that could be used with the same species of diatom simultaneously, in both aggregate or individual cell form. We used two different stable isotopes of silicon to label diatom cells which could then be used to distinguish among food types eaten by the euphausiids. Since the silica present in diatom frustules is not assimilated to a significant degree by herbivores (Tande and Slagstad, 1985; Cowie and Hedges, 1996), the isotopic ratio of silicon within the fecal pellets was a quantitative measure of the relative proportions of the two food sources eaten.

Our test zooplankton species, *Euphausia pacifica*, is a common krill-type species off the California coast in upwelling regions and throughout the North Pacific (Mauchline and Fisher, 1969). With a large filter basket, it is morphologically suited for pelagic filter feeding as well as carnivorous feeding on relatively large organisms such as copepods (Ohman, 1984). *Euphasia pacifica* is known to consume its entire daily ration as aggregates in the absence of alternate prey (Dilling *et al.*, 1998). It is not known, however, if *E. pacifica* will consume marine snow in the presence of potentially preferred dispersed prey, such as phytoplankton. In addition to demonstrating a potential new technique for distinguishing food types, this study has implications for the selection of marine snow by zooplankton as a food item in the field.

METHOD

Overview

Dispersed and aggregated cells of the diatom *Nitzschia angularis* were obtained from replicate cultures that were in the same physiological state so that when a mixture of aggregated and dispersed cells was presented to the euphausiids, selective grazing on either type of food was due solely to the differences in the physical characteristics (aggregated versus dispersed) of the food rather than to differences in food quality. Because the same diatom clone was offered in both aggregated and dispersed form, we devised a method that could distinguish and quantify feeding on each food source despite their morphological and physiological similarity. Two separate cultures of *N. angularis* were each uniquely labeled with different rare heavy isotopes of silicon (^{29}Si or ^{30}Si). One culture was used to form aggregates, whereas the other was left as dispersed cells. Both food types were offered to the euphausiid, *E. pacifica*, simultaneously. At the conclusion of the experiment, the isotopic signature of fecal pellets was measured to determine the percentage of each food type consumed.

Pre-experiment procedures

Animals

Adult *E. pacifica* were collected from the Santa Barbara Channel, California, USA (34°20.0′N, 120°0.0′W), in the upper 80 m at night using a 1 m diameter plankton net with 333 μm Nitex mesh. The animals were maintained in a flow-through fiberglass tank at 15°C on a diet of the diatom *Thalassiosira weissflogii* and starved for 24 h prior to their use in experiments to allow their guts to clear.

Diatom cultures

Two stock cultures of the diatom *N. angularis* (length 42–45 μm, width 4–10 μm), established from the same clonal culture, were maintained at 12°C under constant light in polycarbonate flasks containing F/2 medium with added silicon (Guillard, 1975). The silicic acid in one of the cultures was enriched to ~10 atom% ^{29}Si [isotopically natural silicon contains 4.71 atom% ^{29}Si (Weast, 1980)] and the silicic acid in the other was enriched to ~10 atom% ^{30}Si [isotopically natural silicon contains 3.12 atom% ^{30}Si (Weast, 1980)]. Cell abundance in each culture was determined every other

day using a hemocytometer and compound microscope during the 10 days before the experiment. Both cultures had exhausted the silicic acid in the medium {[$Si(OH)_4$] < 0.1 μM} after 10 days and had entered stationary phase. The two cultures, therefore, had two distinct labels, but were otherwise composed of cells of the same species raised under identical conditions.

Each culture was then prepared for use in experiments. Aggregates were formed by dividing the stationary phase culture enriched to ~10 atom% ^{30}Si into several 285 mL cylindrical polycarbonate centrifuge bottles and rotating them at 2 r.p.m. on a roller table for ~4 h to aggregate the cells (Shanks and Edmondson, 1989). Dispersed cells were obtained from the ^{29}Si-enriched culture. Any clumps of cells that had formed naturally were disrupted by vigorous shaking just before being fed to the animals. Microscopic inspection revealed that the shaken culture consisted of only solitary cells and that the cells remained intact during agitation.

Subsamples of each culture were filtered onto Whatman GF/F filters and analyzed for chlorophyll content according to the methods of Strickland and Parsons (Strickland and Parsons, 1972). 20 mL aliquots of each culture were filtered onto separate 0.6 μm polycarbonate filters, dried at 65°C, and stored for analysis of siliceous biomass and silicon isotopes. Three subsamples from each of the two cultures were processed and analyzed for particulate organic carbon and nitrogen according to Sharp (Sharp, 1992) using a Leeman Labs. Inc. CE CHN Analyzer (Model 440) with acetanilide for calibration and NIST Citrus Leaves (SRM 1572) as control standard for the batch.

Preconditioning

We sought to minimize silicic acid uptake by the phytoplankton cells during the grazing experiments since uptake of isotopically natural Si during the experiment would alter the isotopic signature of each food type. The labeled diatom cultures were placed in the dark for the 24 h immediately preceding the experiment. Silicic acid transport is known to be inhibited by prolonged darkness with uptake ceasing after 24 h without light (Blank and Sullivan, 1979). However, uptake of isotopically natural Si from filtered sea water occurred during the first experiment (initial dispersed cell abundance = 520 cells mL^{-1}). Thus, additional precautions to minimize Si uptake were taken for the second experiment. The sea water used in the second experiment was first depleted of Si by filling 20 L polycarbonate bottles with raw seawater and inoculating them with *N. angularis*. The cultures were allowed to grow until the ambient silicic acid concentration was decreased to <0.2 μM. This water was pre-filtered through a 0.45 μm cartridge filter followed by filtration through a 0.6 μm polycarbonate filter to ensure all phytoplankton cells were removed, and then used for the feeding experiments.

Experimental design

Polypropylene bottles (4.3 L) were filled with filtered sea water. To avoid contamination by natural silicon, only plastic laboratory ware was used. Dispersed cell culture, individual aggregates and animals were added to experimental treatments. Aggregates were gently rinsed in filtered seawater before being added to the experimental bottles to decrease transfer of unaggregated cells with the aggregates. The concentration of both food types added to the bottles was more than the animals could consume in each 12 h experiment, and approximated a typical range of surface water concentrations in the Santa Barbara Basin. Experimental treatments had approximately 520 cells mL^{-1} (0.19 μg Chl L^{-1}) in experiment 1, and 1900 cells mL^{-1} (2.9 μg Chl L^{-1}) in experiment 2. Both experiments had about five or six aggregates per liter as well. Although an attempt was made to equalize the amounts of each food type added, it was not possible to directly assess the biomass of the aggregates prior to each experiment.

Additional bottles containing filtered sea water and either dispersed culture, aggregates or aggregates and dispersed algae served as controls. The control bottles containing only dispersed or aggregated cells allowed changes in the isotopic composition of the food sources due to the uptake of isotopically natural Si from the filtered seawater during the experiment to be quantified. The control containing both dispersed and aggregated cells served to quantify the extent to which dispersed cells were scavenged onto aggregates or the extent to which aggregates fell apart during the experiment.

Each experimental and control bottle containing both food and animals was filled to the brim with filtered sea water to exclude air bubbles and rotated at 2 r.p.m. Euphausiids were allowed to feed for 12 h overnight in darkness at 15°C. After feeding, the animals were removed from the bottles and their fecal pellets were gently concentrated onto a 35 μm Nitex screen, sorted from debris (such as small aggregates or molts) and placed into centrifuge tubes. Fecal pellets in the centrifuge tubes were rinsed twice with distilled water, dried at 65°C and stored at room temperature prior to biogenic silica and silicon isotopic analysis.

The contents of the dispersed control and aggregate control bottles were filtered onto 0.6 μm polycarbonate filters. In the mixture control bottle, aggregates were removed and filtered separately onto a 0.6 μm polycarbonate filter. The rest of the water was then filtered onto another 0.6 μm polycarbonate filter. These filters were

dried at 65°C and stored desiccated at room temperature prior to biogenic silica and silicon isotopic analysis. In the second experiment, water from the animal treatments was also filtered for analysis.

Analytical methods

Samples of dispersed cells, aggregates and fecal pellets were prepared for isotopic analysis by conversion to $BaSiF_6$ followed by solid phase mass spectrometry (Nelson and Goering, 1977). The biogenic silica on the filters containing dispersed and/or aggregated diatoms was dissolved using a 30 min digestion in 0.2 N NaOH at 96°C as described by Brzezinski and Nelson (Brzezinski and Nelson, 1989). The mass of biogenic silica in each sample was determined by diluting a small volume (10–50 μL) of the digest to 10 mL with deionized distilled water and then analyzing the mixture for dissolved silicon concentration according to Strickland and Parsons (Strickland and Parsons, 1972) modified to use the reagent blank of Brzezinski and Nelson (Brzezinski and Nelson, 1986). Three micromoles of silicon were added to the remainder of each digest as carrier to ensure a strong signal on the mass spectrometer. For digests of samples from the control bottles that contained cells labeled with ^{29}Si, ^{30}Si (95.28 atom% ^{30}Si) was added as a carrier. Digests of ^{30}Si-labeled cells were supplemented with 3 μmol of ^{29}Si (95.65 atom% ^{29}Si). The silicon in each sample (sample + carrier) was then precipitated as $BaSiF_6$ using the procedure of Nelson *et al.* (Nelson *et al.*, 1991).

The silicon in the samples of fecal pellets was converted to $BaSiF_6$ by adding 3 mL of 30% H_2O_2 to each sample to decompose organic matter. Then after 24 h each sample was centrifuged and rinsed twice with deionized distilled water to remove the H_2O_2. The biogenic silica within the fecal pellets was then dissolved by digestion in 0.2 N NaOH as described above. The biogenic silica content of a small subsample of each digest was measured to determine the total mass of biogenic silica in each sample. Since the fecal pellets contained an unknown mixture of cells labeled with both ^{30}Si and ^{29}Si, 3 μmol of isotopically natural silicon was added as carrier.

Preliminary experiments demonstrated that precipitation of $BaSiF_6$ from the mixture of digested fecal pellets and carrier was unreliable. Therefore, each sample and carrier mixture was reacted with acidified ammonium molybdate and the resulting silicomolybdic acid concentrated onto a 2 mL bed of Sephadex resin in a polypropylene chromatography column. The bound silicomolybdic acid on the column was rinsed with 0.01 N HCl containing 25 g L^{-1} NaCl to remove compounds which interfered with the precipitation of $BaSiF_6$. The silicomolybdic acid was removed from the column by decomposition in 0.4 N NaOH, cleaned of Mo by anion exchange, and precipitated as $BaSiF_6$ as described by Nelson *et al.* (Nelson *et al.*, 1991).

All isotopic analyses were performed on a Measurement and Analysis Systems 6–60 (6 inch radius, 60 degree deflection) magnetic sector mass spectrometer. The analytical precision of the measurement was 0.002 atom%. Each sample was corrected for the presence of the carrier using the equations of Nelson and Goering (Nelson and Goering, 1977) or by mass balance.

Calculations of percentage food type eaten

The proportion of each food type eaten was calculated from the isotopic composition of the fecal pellets, dispersed cells, and aggregated cells. Ideally, the ratio of silicon isotopes within the fecal pellets is a simple mixture of the unique isotopic signatures of the silica in the dispersed and aggregated diatom foods. However, some growth of the food sources during the experiment is inevitable. Diatoms growing in the experimental flasks would take up dissolved silicon from the filtered sea water used to fill the bottles. The Si in filtered seawater would have the isotopic signature of natural Si (92.18 atom% ^{28}Si, 4.71 atom% ^{29}Si, 3.12 atom% ^{30}Si). Thus, diatom growth and the consequent Si uptake would shift the isotopic signatures of each food source towards that of isotopically natural Si during the feeding experiment. Such shifts in the isotopic compositions of the food sources occurred over the course of the experiment due to cell growth and Si uptake (see Results). To account for this effect, the isotopic composition of the fecal pellets was assumed to be a mixture of the original and final isotopic compositions of both the dispersed and aggregate foods. The proportion of each of those four isotopic compositions–initial and final aggregates, and initial and final dispersed cells–in the fecal pellets can be calculated using the following system of linear equations (equations 1–4):

$$(atom\%^{28}Si_{Di} * d_i) + (atom\%^{28}Si_{Df} * d_f) + (atom\%^{28}Si_{Ai} * a_i) + (atom\%^{28}Si_{Af} * a_f) = atom\%^{28}Si_{FP} \quad (1)$$

$$(atom\%^{29}Si_{Di} * d_i) + (atom\%^{29}Si_{Df} * d_f) + (atom\%^{29}Si_{Ai} * a_i) + (atom\%^{29}Si_{Af} * a_f) = atom\%^{29}Si_{FP} \quad (2)$$

$$(atom\%^{30}Si_{Di} * d_i) + (atom\%^{30}Si_{Df} * d_f) + (atom\%^{30}Si_{Ai} * a_i) + (atom\%^{30}Si_{Af} * a_f) = atom\%^{30}Si_{FP} \quad (3)$$

$$d_i + d_f + a_i + a_f = 1 \quad (4)$$

where atom%, D, A and FP symbolize the measured atom percents of each isotope for dispersed cells, aggregates and fecal pellets, respectively. Initial and final values for the two food sources are denoted by i and f. The proportions of dispersed (d) and aggregate (a) food sources found in the fecal pellets are d_i, d_f, a_i and a_f, which sum to 1. The proportion of aggregated and dispersed cells consumed is then calculated as $(d_i + d_f)$ and $(a_i + a_f)$, respectively. These calculations are approximate in that they do not use the instantaneous change in the isotopic composition of each food through time during an experiment. The error introduced is not large because the actual measured changes in the isotopic composition of each food due to growth during each 12 h experiment were relatively small. More complicated calculations that attempt to account for the change in the isotopic composition of each food through time are not likely to improve the accuracy of the final result because it is not known when during the experiment individual aggregates or dispersed cells were eaten.

Table I: Summary of experimental set-up and initial conditions for experiments 1 and 2

	Experiment 1	Experiment 2
Dispersed cell conditions		
Cells mL^{-1}	520 ± 90	1900 ± 90
μg Chl *a* L^{-1}	1.8 ± 0.7	2.9 ± 0.4
μg C L^{-1}	77.9 ± 39.5	146.9 ± 18.8
C/N mass ratio	9.9 ± 0.5	7.9 ± 0.1
Aggregate conditions		
No. aggs L^{-1}	4.65	5.8
ng Chl *a* $aggregate^{-1}$	365.5 ± 340	270.6 ± 95.1
μg Chl *a* L^{-1}	1.7 ± 1.6	1.6 ± 0.5
μg C $aggregate^{-1}$	48.8 ± 5.5	ND (50 estimated)
μg C L^{-1}	226.9 ± 25.6	ND (290 estimated)
mm^2 $aggregate^{-1}$	1.01 ± 0.2	0.92 ± 0.1
C/N mass ratio	10.6 ± 1.9	8.0 ± 1.8
Treatments	(No. of replicates)	(No. of replicates)
Aggs + disp. + 4 animals	4	2
Controls	(No. of replicates)	(No. of replicates)
Aggregates alone	1	2
Dispersed cells alone	1	2
Aggregates + dispersed	1	2

Abbreviations: no. aggs L^{-1}, the number of aggregates available per liter of seawater, and disp., dispersed cells. Treatments were four euphausiids per 4.3 L container with aggregates and dispersed cells added as quantified above.

RESULTS

Experimental conditions

The initial experimental set-up was designed to present the euphausiids with an equal proportion of aggregate and dispersed food types measured as Chl *a* concentration. This is difficult to achieve since the aggregates are heterogeneous and the actual aggregates added to the experimental treatment are not easily quantified *a priori*. In practice, measurements are made on aliquots identical to, but separate from, those added to the treatments. In the first experiment, the goal was largely achieved, as measured by chlorophyll concentrations in the dispersed cells and the aggregates (Table I). Approximately the same amount of food as measured by chlorophyll was available in both types. In the second experiment, as measured by chlorophyll, more food was available in the form of dispersed cells than in the form of aggregates. Due to the presence of TEP, which is also consumed by euphausiids, chlorophyll is not the only measure of food available. Food available can also be estimated by carbon content. As measured by carbon content more material was available in both experiments in aggregates (Table I). This suggests the presence of non-chlorophyll containing material incorporated as part of the aggregate. The C/N ratio of cells in both dispersed and aggregates was the same within each experiment (although they differed between experiments).

The isotopic composition of the cells in the controls of each food type shifted toward that of natural silicon over the 12 h of the experiments, indicating that both food types actively took up dissolved Si of isotopically natural composition from the seawater during the course of the experiment (Figs 1 and 2; Tables II and III). The dispersed cells took up more natural Si per unit biomass than the aggregated cells, as indicated by the larger shift in their isotopic ratio towards that of isotopically natural Si. As dispersed cells shifted more strongly toward natural Si during the experiment, dispersed cells likely became proportionately more available in comparison with aggregates. The strategy of limiting growth in the second experiment by depleting the water of Si did limit growth, but some uptake still occurred (Fig. 2).

Mixing between aggregated and dispersed food sources was measured by analyzing the change in isotopic ratios of both sources in the mixture control from each experiment. The isotopic composition of the aggregates did not shift appreciably toward that of the dispersed cells, showing that the aggregates did not scavenge many dispersed cells during either experiment (Figs 1 and 2; Tables II and III). Likewise, cells from the aggregates did not appear to slough off into the dispersed pool as the isotopic composition of dispersed cells did not shift toward that of the aggregates (Figs 1 and 2; Tables II and III). In addition, the dispersed cells' isotopic

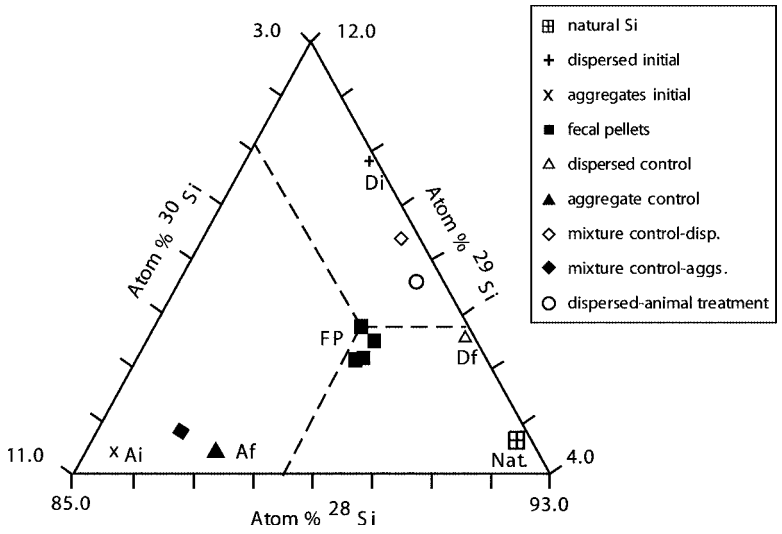

Fig. 1. Isotopic composition of samples collected in experiment 1. Each axis represents the percentage of each isotope present. The dotted lines indicate how to read the values of a data point on the graph (the example is 88.5 atom% ^{28}Si, 6.7 atom% ^{29}Si, and 4.8 atom% ^{30}Si). Note that each point represents a single replicate, not a mean.

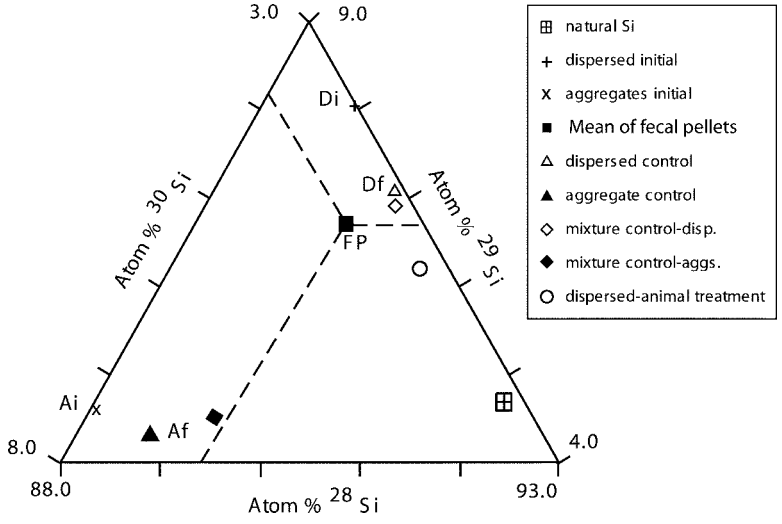

Fig. 2. Isotopic composition of samples analyzed in experiment 2. The scales are presented with a smaller range than in Fig. 1 to magnify the relevant portion of the plot. The dotted lines illustrate the example data point: 89.4 atom% ^{28}Si, 6.5 atom% ^{29}Si, and 4.1 atom% ^{30}Si. Symbols are the same as in Fig. 1 with the addition of open circles = leftover dispersed cells in the animal treatments. Note that each point represents a mean, not a replicate as in Fig. 1.

composition shifted toward that of natural Si, indicating growth, and not toward the aggregate ratio in the animal treatments, showing that in these experiments the animals did not break up aggregates without consuming them (Figs 1 and 2, open circles). *Nitzschia angularis* forms compact, durable aggregates, which may explain why cells did not slough off from aggregates during the experiment.

Feeding treatments

The isotopic signatures of the fecal pellets were intermediate between that of the dispersed and aggregated food sources indicating that the animals consumed both foods (Fig. 1; Table II). The solution of the system of equations 1–4 indicates that the animals consumed 66% dispersed cells and 34% aggregates (summing initial and final ratios).

Table II: Silica biomass and isotopic composition of fecal pellets, control aggregates and control dispersed cells in experiment 1. The initial isotopic composition of the aggregates and dispersed cells, and the composition of natural silicon are also given

Sample	Variable name	Biomass Si (μmol flask^{-1})	Atom% ^{28}Si	Atom% ^{29}Si	Atom% ^{30}Si
Natural Si		n.d.	92.1	4.8	3.1
Initial aggregates	A_i	n.d.	85.5	4.5	10.1
Initial dispersed	D_i	n.d.	87.0	9.8	3.2
Fecal pellets (mean ± S.D., n = 4)	FP	0.21 ± 0.04	88.7 ± 0.14	6.4 ± 0.3	4.9 ± 0.2
Aggregates from disp. + agg. Control		0.41	86.4	4.8	8.8
Dispersed from disp. + agg. Control		0.54	88.3	8.3	3.3
Dispersed only Control	D_f	1.38	90.3	6.6	3.1
Aggregate only Control	A_f	0.33	87.2	4.5	8.3

Abbreviations: aggs., aggregates; disp., dispersed cells. n.d., no data. Percentages do not always add to 100 due to rounding errors.

Table III: Biogenic silica biomass and isotopic composition of fecal pellets produced in feeding treatments, control aggregates and control dispersed cells in experiment 2. The initial isotopic composition of the aggregates and dispersed cells, and the composition of natural silicon are also given

Sample	Variable name	Biomass Si (μmol flask^{-1})	Atom% ^{28}Si	Atom% ^{29}Si	Atom% ^{30}Si
Natural Si		n.d.	92.1	4.8	3.1
Initial aggregates	A_i	n.d.	88.1	4.6	7.4
Initial dispersed	D_i	n.d.	88.9	8.1	3.0
Fecal pellets (mean ± SD, n = 2)	FP	0.22 ± 0.1	89.5 ± 0.2	6.7 ± 0.2	3.8 ± 0.4
Aggregates from disp. + agg. Controls (mean ± SD, n = 2)		0.34 ± 0.1	89.3 ± 0.7	4.5 ± 0.1	6.2 ± 0.6
Dispersed from disp. + agg. Controls (mean ± SD, n = 2)		1.22 ± 0.0	89.9 ± 0.4	6.9 ± 0.4	3.3 ± 0.0
Dispersed only Control (mean ± SD, n = 2)	D_f	1.3 ± 0.1	89.8 ± 0.1	7.1 ± 0.1	3.1 ± 0.1
Aggregate only Control (mean ± SD, n = 2)	A_f	0.27 ± 0.0	90.5 ± 1.9	4.4 ± 0.1	7.0 ± 0.6
Remaining disp. animal (mean ± SD, n = 3)		1.21 ± 0.2	90.6 ± 0.2	6.2 ± 0.2	3.3 ± 0.1

Abbreviations: aggs., aggregates; disp., dispersed cells. n.d., no data. Percentages do not always add to 100 due to rounding errors.

Only two of the four fecal pellet replicates were analyzed from the second experiment because of sample loss; one was lost due to a broken bottle, and one was thrown out due to isotopic results that indicated analysis error. The isotopic analysis of the fecal pellets replicated well and the ratios obtained were again intermediate between those of the two food sources (Fig. 2; Table III). As in the first experiment, the euphausiids consumed proportionately more dispersed (83%) than aggregated (17%) cells.

DISCUSSION

Euphausiid feeding

These experiments clearly show that euphausiids will consume aggregates even when dispersed cells are available. It is most common that aggregates in the field are present with many other types of particles, including dispersed algae and fecal pellets. We therefore suggest that marine snow is a viable food source for zooplankton in the field, even when dispersed phytoplankton is present. It remains to be tested whether marine snow is a preferred food when zooplankton prey are available. We speculate that aggregates may be preferred over zooplankton since animal prey such as copepods are known to be suboptimal prey for *E. pacifica* compared with phytoplankton (Ohman, 1984). Natural aggregates often contain polysaccharides or transparent exopolymer particles (TEP) or mucous fibers from larvacean houses (Alldredge and Silver, 1988; Passow and Alldredge, 1995). While some of these constituents as well as large size may make aggregates more difficult to handle for zooplankton grazers, TEP actually

enables feeding by euphausiids on otherwise unavailable nano-sized particles (Passow and Alldredge, 1999; Passow, 2002). It also remains to be tested whether or not zooplankton will feed on N-limited aggregates when N-replete dispersed cells are available, if for example cells in aggregates are in the stationary phase while some cells in the dispersed form remain in the exponential growth phase (Engel *et al.*, 2002). As our experiments were designed to solely test feeding on aggregated versus suspended cells, we were not able to examine issues of food quality or preference for different nutritive value—in fact our experiments specifically were designed to offer foods of very similar quality, with the only difference being aggregated or suspended diatom cells.

Euphausiids, like all other zooplankton, face a temporally and spatially patchy food environment. *Euphausia lucens* exploits both phytoplankton and zooplankton prey in order to survive in the fluctuating environment of the Southern Benguela upwelling region (Stuart and Huggett, 1992). *Euphasia pacifica* likely faces a similar fluctuating environment in the California Current. Marine snow may provide an additional food source that may aid survival in an unpredictable environment. Marine snow is ubiquitous in both the euphotic zone and in the deep ocean, although its abundance is highly variable (Alldredge and Silver, 1988). Dagg (Dagg, 1993) found that there was not enough phytoplankton in an ingestible size range to support the population of *Neocalanus cristatus* found in the subarctic Pacific and speculated that the animals were surviving on sinking aggregates.

Previous work has shown that euphausiids and copepods will consume aggregates in the laboratory, in both filtered and coarsely screened seawater (Dilling *et al.*, 1998). However, in those experiments, it was not possible to measure how much of the food was consumed as aggregates or as dispersed material in the seawater. Here we have measured the proportion of food offered in a mixture that was consumed as aggregates. Despite greater availability of dispersed cells by the end of each experiment, 30 or 17% of the diet in each experiment (respectively) was consumed in aggregate form.

Estimates of potential aggregate ingestion by euphausiids in the field using maximum laboratory-derived ingestion rates suggested that on many representative days, only 1–2% of aggregate standing stock would be consumed in an evening's feeding (Dilling and Alldredge, 2000). If euphausiids only consume 20% of their natural diet in aggregate form, as suggested by the current study, that estimate would be lower still.

While euphausiids may consume marine snow as part of their diet (Dilling *et al.*, 1998; Graham *et al.*, 2000), they may impact the flux of marine snow to depth more significantly by breaking apart large aggregates (Dilling and Alldredge, 2000; Goldthwait *et al.*, 2004). This process of fragmentation decreases the sinking rates of aggregates, potentially making them more available for other consumers and microbial processes. By consuming and fragmenting aggregates, euphausiids and other zooplankton may be responsible for some of the observed decrease in the flux of carbon with depth (Martin *et al.*, 1987).

Advantages of the stable Si isotope method

To our knowledge, this is the first time stable isotopes of silicon have been used as tracers in zooplankton feeding experiments. The method has several advantages over other techniques. Other methods based on pigment analysis, cell counts, or fecal pellet production, cannot easily distinguish among identical cell types offered simultaneously (Omori and Ikeda, 1984). In contrast, our method allows otherwise identical cells to be differentially labeled and distinguished. The proportion of different food types eaten can be discerned without having to count cells by species, either in the water or in the fecal pellets. Changes in diatom food sources such as uptake/growth can be detected easily and quantified. Interactions among food types, such as disaggregation of aggregates or scavenging of dispersed cells onto aggregates can be traced and quantified. Finally, because silicon is not digested and assimilated significantly by herbivores (Tande and Slagstad, 1985; Cowie and Hedges, 1996), the tracer is unchanged by passage through the animal's gut [unlike pigments (Head, 1992)]. One limitation of the method is that the only phytoplankton food that can be labeled are diatoms as other phytoplankton do not take up and deposit Si to any significant degree. However, the method would be appropriate for studies of feeding on siliceous organisms such as chaonoflagellates, chrysophytes, silicoflagellates and radiolarians.

Our technique using stable isotopes of silicon also has several advantages over techniques using stable isotopes of other elements (e.g. C, N, P). Since silicon is not assimilated it does not become fractionated by or involved in metabolic processes as are isotopes of C and P (Conover and Francis, 1973). With two isotopes that are stable (^{29}Si and ^{30}Si), the problem of differential leakage after sample preservation of radiotracers such as ^{14}C and ^{32}P is avoided (Sierszen and Watras, 1987). It would be possible to use the radioisotope ^{32}Si for feeding studies (Brzezinski and Phillips, 1997), but since only one food source would be labeled with the isotope calculating the proportions of each food type eaten would rely on determining the specific activity of ^{32}Si in the fecal pellets [i.e Bq ^{32}Si (μg Si)$^{-1}$]. This diminishes the sensitivity of the technique, as the requisite determinations of

biogenic silica concentration within fecal pellets are not as accurate as our determinations of the atom% ^{29}Si and ^{30}Si of the frustules within the pellets. Use of a single label technique also makes it difficult to distinguish diatom cell growth from the ingestion of a greater proportion of unlabeled cells. Finally, the high costs of handling radioactive isotopes and waste are eliminated.

The method of tracing feeding by labeling diatom cells with stable isotopes of silicon gives clear, consistent results. Care must be taken to avoid contamination of the samples with extraneous Si. Our method shares a common problem with all feeding experiments using phytoplankton prey in that the cells grow during the experiment. We largely solved this problem relative to Si uptake by limiting the amount of light given to the cells 24 h prior to and on the day of the experiment and by depleting the water used in the experiment of silicic acid. Even if uptake does occur, it can be accounted for very accurately by measuring the change in isotopic ratio of each food in control cultures.

Further research using this new method would be helpful in elucidating prey preferences of euphausiids and copepods between aggregates and suspended cells. Mixtures of varying concentrations of suspended cells and aggregates could be used to understand whether euphausiids might prey preferentially on aggregates despite their availability, or whether they prey on them strictly as a function of prey encounter rate. It may also be possible to devise diatom aggregates of varying food quality, by ageing cultures, for example, and thereby directly testing whether aggregate age will affect feeding preference.

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