

Interstitial dissolved organic carbon (DOC) concentrations within sinking marine aggregates and their potential contribution to carbon flux

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Abstract

Accurate estimates of the quantity of organic carbon sedimenting to the sea floor are important in evaluating the rate at which carbon is sequestered in the deep sea and the impact of the ocean on the global carbon cycle. However, extensive studies quantifying marine sedimentation over the past decades have considered only the particulate fraction of sinking material. Dissolved organic carbon (DOC) carried along in the interstices of sinking marine snow, the particles that comprise the bulk of particle flux throughout most of the ocean, has not been included previously. Empirical measurements of the interstitial DOC concentrations of individual aggregates of marine snow from coastal California and Washington revealed high values ranging from 8.9 to 140 mg L⁻¹ that were significantly correlated with aggregate size, decreasing as aggregate size increased. Solubilization of particulate matter within aggregates by associated bacteria and reduced diffusion rates due to the fractal geometry of aggregates help maintain these high interstitial concentrations against diffusive processes. Although interstitial DOC concentrations were one to two orders of magnitude higher than ambient DOC concentrations in the surrounding seawater, the cumulative interstitial DOC in aggregates contributed <2.5% to total DOC in the water column. However, DOC comprised up to 31% of the total organic carbon in aggregates, averaging about 20%, indicating that previous measurements of sedimenting carbon in the ocean that have included only the particulate fraction have significantly and systematically underestimated the vertical flux of organic carbon.

Most of the nonliving organic matter sedimenting from the surface ocean to the deep sea sinks in the form of large detrital aggregates >0.5 mm in diameter, known as marine snow (Fowler and Knauer 1986; Silver and Gowing 1991). Marine snow is formed largely from the aggregation of smaller particles in the water column including phytoplankton, bacteria, fecal pellets, zooplankton feeding webs, and miscellaneous organic debris. Particles of marine snow are fractal in their geometry (Logan and Wilkinson 1990; Kilps et al. 1994) and very porous; >99% of their volume consists of interstitial fluid (Alldredge and Gotschalk 1988). Because marine snow harbors highly concentrated microbial communities engaged in photosynthesis, microbial decomposition, and remineralization at elevated levels relative to those in the surrounding seawater (Alldredge and Silver 1988; Simon et al. 1990; Smith et al. 1992), the chemical microenvironment within this interstitial fluid is also unique. Oxygen may be significantly depleted within aggregates (Alldredge and Cohen 1987; Ploug and Jorgensen 1999), while nutrients, especially nitrate, phosphate, and ammonia, reach concentrations one to two orders of magnitude higher than found in the surrounding seawater (Shanks and Trent 1979; Alldredge and Gotschalk 1990; Kaltenbock and Herndl 1992). Interstitial dissolved carbohydrates and free amino acids can

also be enriched above ambient concentrations (Herndl 1988; Muller-Niklas et al. 1994).

Although the term particulate carbon flux is used to refer to the sedimentation of carbon in particulate form, sinking aggregates may generate a significant downward flux of dissolved as well as particulate matter by sweeping their interstitial microenvironments along with them as they sink. The fractal geometry of marine aggregates greatly reduces diffusion of dissolved substances from their interstices (Meakin 1988; Brzezinski et al. 1997), while the high mucus content of aggregates may retard advective flow through them (Alldredge and Crocker 1995). Dissolved organic carbon (DOC) associated with sinking particles has received almost no attention in considerations of the carbon cycle of the ocean despite the strong interest in oceanic DOC as a component of the biological pump (Longhurst and Harrison 1989; Siegenthaler and Sarmiento 1993; Toggweiler 1999). This is primarily because the material falling into sediment traps has been considered to be overwhelmingly particulate in nature. Moreover, measurement of particle-associated DOC has been problematic because commonly used organic preservatives, such as Formalin, preclude the analysis of DOC in sediment trap samples, and direct measurements of DOC within the interstices of sinking particles have been difficult to obtain from natural samples. Herndl (1992) reported the only direct measurement of interstitial DOC known for marine snow. He found DOC concentrations enriched up to 25 times above ambient within large mucus aggregates from the Adriatic Sea. Noji et al. (1999) estimated that the DOC originating from sinking particles was similar in magnitude to the particulate component of sediment trap samples, suggesting that interstitial DOC could be exceptionally important. But they were unable to differentiate between interstitial DOC sinking with the particles and that derived from conversion of POC

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Table 1. The average concentrations (\pm standard error [SE]) of dissolved (DOC), particulate (POC), and total organic carbon (TOC) within marine snow at eight stations in the Santa Barbara Channel, California (1997 dates) and East Sound, Washington (1998 dates). C:N is the ratio of organic carbon to organic nitrogen.

	Type	Depth	Mean aggregate volume (mm ³)	$\mu\text{g organic carbon aggregate}^{-1}$			DOC as %		C:N ratio	
				POC	DOC	TOC	of TOC	POC:DOC	Seawater	Snow
14 May 97	Diatom	10	66	23.1 \pm 0.5	2.1 \pm 0.4	25.2	8	11	7.6	7.1
25 Jun 97	Larvacean	15	22	3.3 \pm 0.2	0.7 \pm 0.1	4.0	17	4.7	5.8	7.6
23 Jul 97	Larvacean	10	25	3.0 \pm 0.5	0.7 \pm 0.1	3.7	20	4.3	5.7	6.5
13 Jun 98	Diatom/debris	2–7	14	4.4 \pm 0.4	2.0 \pm 0.1	6.3	31	2.2	5.2	4.8
13 Jun 98	Debris	12	25	5.5 \pm 0.5	0.7 \pm 0.1	6.2	11	7.9	5.2	5.0
15 Jun 98	Debris	16	28	5.5 \pm 0.3	0.8 \mp 0.1	6.3	13	6.9	5.1	5.5
18 Jun 98	Diatom/debris	14	588	19.9 \pm 4.4	4.9 \pm 2.6	24.8	20	4.1	5.2	5.5
19 Jun 98	Diatom/debris	10	49	13.5 \pm 5.6	2.4 \pm 0.8	15.9	15	5.6	5.5	5.1
24 Jun 98	Debris	12	71	6.4 \pm 2.4	2.1 \pm 1.0	8.5	25	3.0	5.4	5.3
Mean \pm SE			98 \pm 62	9.4 \pm 2.5	1.8 \pm 0.5	11.2 \pm 2.8	18 \pm 2	5.5 \pm 0.9	5.6 \pm 0.3	5.8 \pm 0.3

to DOC through leaching and other processes once particles entered the traps.

In the following study, interstitial concentrations of DOC within natural marine snow were measured empirically in order to determine if significant DOC could flux to the sea floor within sinking particles. The contribution of the interstitial DOC within aggregates to total DOC in the water column was also determined for the first time. Finally, the relationship between interstitial DOC concentrations and bacteria abundance and division rate within aggregates was investigated.

Methods

Field Methods—Aggregates of marine snow were hand-collected at depths of 2–20 m by SCUBA divers at three stations in the Santa Barbara Channel (34°18'N, 119°50'W) in the early summer of 1997, and at six stations in East Sound, a shallow (30 m bottom depth) fjord of Orcas Island in the San Juan Islands, Washington, (48°40.6'N, 122°53.5'W) in June 1998. Because most individual aggregates were too small for accurate chemical analysis, we collected pooled samples of 45–300 aggregates in each of three size classes (small, medium, and large) on each dive using 60-ml syringes as described in detail in Alldredge (1998) for measurements of particulate organic carbon and nitrogen (POC and PON), DOC, mass, and bacteria content. Ten to eleven aggregates as close as possible in size to the prototype aggregate in each size class were also photographed underwater on each dive with a Nikonos IV in order to estimate average aggregate size in each size class. The camera system included a strobe and 1:1 close-up attachment and used Tri-X 400 black-and-white film. Aggregate sizes varied considerably among stations and aggregates considered to be large on one dive might be classified as small on another. What was important was that on any one dive each size category collected corresponded with the photographs of that same size class taken on the same dive, thus allowing size and chemical content of aggregates of the same size to be compared. Aggregates were photographed in a plane parallel to their direction of sinking. Bulk seawater containing aggre-

gates at natural concentrations was also collected by divers in 2-liter polyethylene bottles for similar analysis.

Marine snow was classified into three general categories based on the identity of the dominant particles composing the aggregates as revealed by microscopy. Larvacean houses consisted of the discarded houses of larvaceans in the family Oikopleuridae. Diatom aggregates were composed primarily of living, chain-forming diatoms, empty frustules, setae, and other phytoplankton detritus. Miscellaneous aggregates were composed primarily of unidentifiable detritus, naked flagellates, and occasional fecal pellets (see Alldredge and Gotschalk [1990] for additional descriptions of these aggregate classes). The vast majority of aggregates at any one station were of the same type, consistent with the findings of Alldredge and Gotschalk (1990). Two stations were dominated by larvacean houses, four by diatom flocs or diatom flocs containing a high percentage of detritus, and three by miscellaneous aggregates (Table 1).

In East Sound, profiles of the sizes and abundance of aggregates >0.5 mm in size with depth were obtained with an in situ still camera package as described in MacIntyre et al. (1995). This package also contained a CTD (conductivity/temperature/depth) and fluorometer. Aggregates were photographed in an illuminated volume $35 \times 25 \times 5$ cm (4.4 liters) in dimension, and four to six photographs were taken in each meter of depth as the camera descended.

Laboratory analysis for chemical content and size—Within 2 h of collection the aggregates in each size class were pooled in an acid-washed beaker and the total sample volume and number of aggregates collected were recorded. Aggregate concentrations ranged from 0.5 to 1.9 aggregates ml⁻¹ in these pooled samples with samples of large aggregates having lower concentrations than those of small ones. This generated about equal concentrations of particulate matter per ml among size classes and insured that filtering efficiencies would be similar. (The mean POC per filter across all three size classes at each station varied by $<33\%$.)

We wished to compare DOC and POC in the same samples. Thus, we defined DOC operationally as the organic carbon passing through a Whatman GF/F glass fiber filter.

Three 30-ml replicate subsamples of each size class were filtered through muffled glass fiber filters. The filters were dried and analyzed using standard methods for POC and PON using a Leeman Labs model CE 440 CHN analyzer according to Sharp (1992). The filtrate was captured in 30-ml muffled glass vials equipped with acid-washed, Teflon-lined caps and immediately frozen for later analysis for DOC. DOC was analyzed in the analytical lab of the Virginia Institute of Marine Science with a Shimadzu 5000 Analyzer using high temperature catalytic (HTC) techniques described in Williams et al. (1993) and Buesseler et al. (1996). DOC calibrations were done with 10–100- μ l injections of glucose in UV-oxidized Q-water. All HTC carbon data were corrected for an instrument blank of 22 μ M, and each determination represents the mean of three sample injections.

Three replicate aggregate samples in each size class were also filtered for dry mass (5–10-ml samples) using 0.4- μ m Nuclepore filters and a Cahn Electrobalance model 4600 according to Sharp (1992). Three replicates of seawater were filtered and similarly analyzed for POC and PON (500-ml samples), dry mass (50-ml samples), and DOC (30-ml samples), using the methods described above for aggregate samples.

Surrounding seawater is invariably included in the samples when marine snow is collected in situ, and measurements of aggregates must be corrected for this dilution effect. The volume of the aggregate samples occupied by seawater was estimated knowing the average volume per aggregate in the sample (from in situ photographs) and the aggregate concentration. Background seawater values were then subtracted from the aggregate samples to obtain the chemical content of the aggregates alone. The volume occupied by seawater in the aggregate samples ranged from 89 to 91%. Seawater blanks were not corrected for contributions from aggregates potentially included in the bottles during seawater collection because the small seawater samples analyzed were unlikely to contain enough aggregates to alter the results significantly.

The abundance of bacteria and the frequency of dividing cells in the marine snow slurries and seawater samples were enumerated from samples preserved in 2% buffered Formalin using epifluorescence microscopy and 4',6-diamidino-2-phenylindole (DAPI) staining (Porter and Feig 1980). In all cases duplicate filters (0.22 μ m black polycarbonate, Millipore) were prepared for each sample, and 10 fields (averaging >30 cells field⁻¹) were counted per filter.

The size and abundance of the aggregates in each frame of still film from the in situ camera package was determined using computerized image analysis of the developed film with a Megavision 1024 XM Image Analysis System containing a 1,000-line video camera as described in MacIntyre et al. (1995). The area of each aggregate on the still film (a two-dimensional measurement) was converted mathematically to equivalent spherical volume (ESV, a three-dimensional value). This conversion was chosen because ESV is the standard and most reasonable way of presenting data from in situ camera systems (see for example Honjo et al. 1984; Gardner and Walsh 1990; MacIntyre et al. 1995). Marine snow in nature tends to be symmetrical around the axis

parallel to the direction of sinking, which increases the accuracy of this conversion (Alldredge and Gotschalk 1988).

The contribution of DOC within marine snow to the DOC of the water column was calculated only for East Sound because in situ profiles of aggregate size and abundance were only available at this site. The quantity of DOC in each aggregate in each frame from the in situ camera was calculated by applying the regression equation of DOC as a function of aggregate volume determined in this study to each aggregate in the camera profiles. The total DOC contained within aggregates was then accumulated for each liter photographed in nature, averaged over the 30-m water column, and this average compared to the average DOC concentration in the seawater.

Results

Marine snow collected in the Santa Barbara Channel was dominated by houses of the larvacean *Oikopleura dioica* at two stations while the third station was dominated by diatom aggregates containing primarily numerous species of *Chaetoceros* and some *Nitzschia* spp. Some of the marine snow in East Sound contained abundant diatoms especially *Chaetoceros* (*Chaetoceros radicans*, *Chaetoceros socialis*, *Chaetoceros decipiens*, *Chaetoceros debilis*, *Chaetoceros vanhoeffii*, and many others), and some *Ditylum* spp., *Eucampia* spp., *Skeletonema* spp., *Thalassiosira* spp., as well as many other less abundant genera. At many stations, however, the aggregates were primarily detrital and contained relatively few identifiable phytoplankton.

Despite their disparate locations of collection and their different origins, the aggregates from both the Santa Barbara Channel and East Sound, Washington, displayed relationships between aggregate volume and both POC and mass content that were nearly identical to that which has been reported previously for aggregates from coastal surface waters (Fig. 1A,C). Because aggregate type and geographical location did not affect the relationship between aggregate volume and POC or mass content, data were pooled from all stations in order to examine relationships among the various aggregate parameters. The aggregates investigated had POC:PON ratios ranging from 5.0 to 7.6, averaging 5.8. These ratios were similar to that of C:N ratios of the particulate matter in the surrounding seawater that averaged 5.6 (Table 1), suggesting that the aggregates were relatively newly formed and contained labile particulate organic matter.

DOC in aggregates—The concentration of DOC within the interstices of aggregates of marine snow at our two study sites varied as a function of aggregate size (Fig. 2A) and was very high, ranging from 8.9 to 140 mg L⁻¹ (Table 2). Corresponding DOC concentrations in the surrounding seawater only ranged from 1.3 to 1.8 mg L⁻¹. Thus, the DOC concentration in aggregates was enriched over that of the surrounding seawater by a factor of 16–78 times, one to two orders of magnitude higher than ambient concentration (Table 2).

Interstitial DOC concentrations decreased exponentially as aggregate volume increased with aggregates 1,000 μ l in vol-

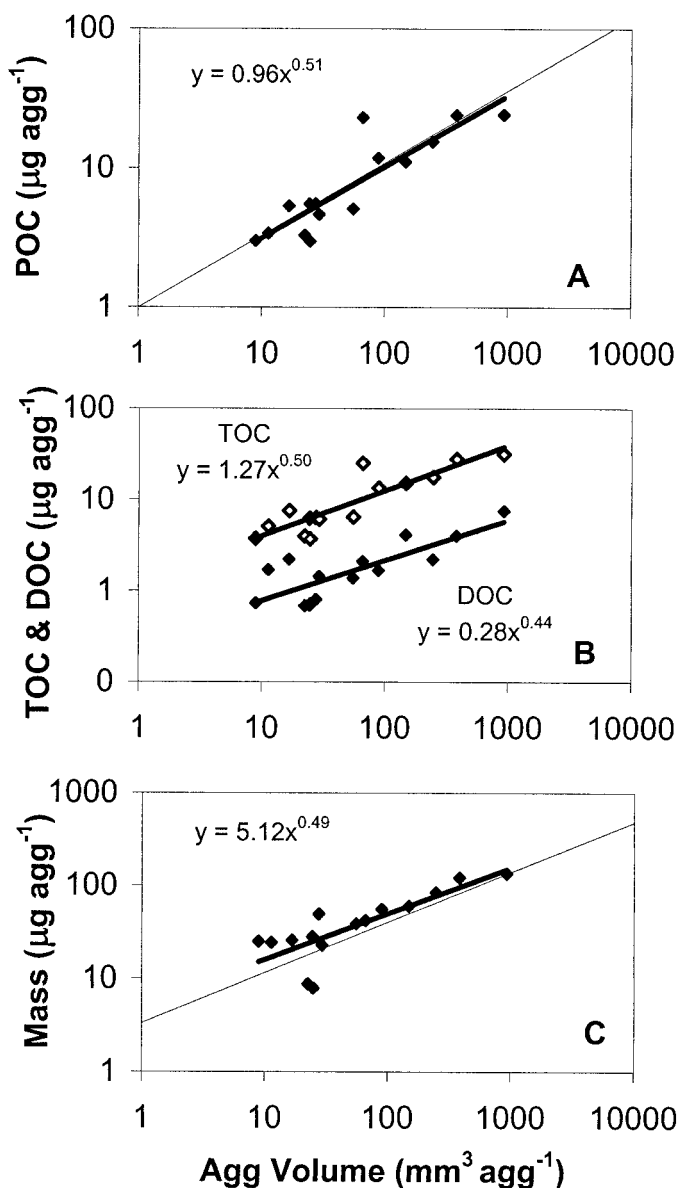


Fig. 1. The organic matter content of marine snow as a function of aggregate (Agg) size. (A) Particulate organic carbon (POC) of aggregates (thick line, diamonds; $r^2 = 0.73$, $P < 0.001$). The thin line is the regression of a much larger data set of coastal marine snow from Alldredge (1998) shown for comparison where $y = 0.99x^{0.52}$. (B) Open diamonds are the total organic carbon content (TOC; $r^2 = 0.75$; $P < 0.001$) and closed diamonds are the dissolved organic carbon (DOC; $r^2 = 0.65$, $P < 0.001$) content of aggregates. (C) Dry mass of aggregates from this study (thick line, closed diamonds; $r^2 = 0.65$, $P < 0.001$). The thin line is the regression of a much larger data set of coastal marine snow from Alldredge (1998) shown for comparison where $y = 3.33x^{0.53}$.

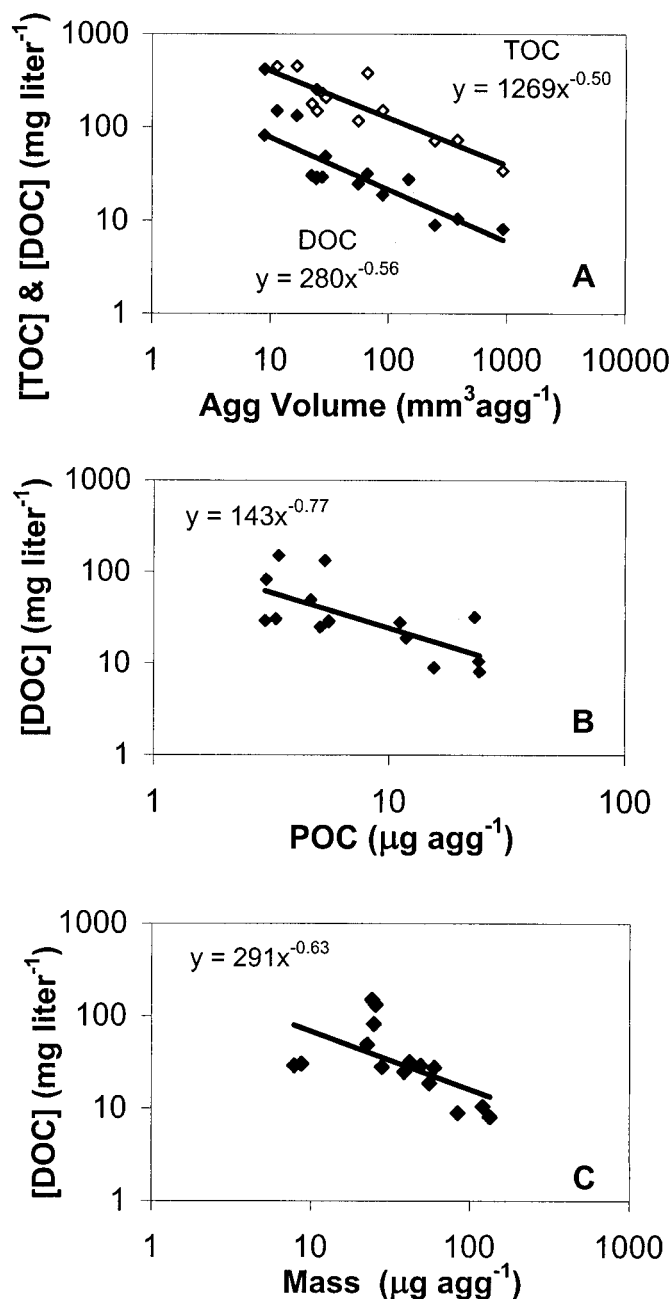


Fig. 2. The dissolved organic carbon (DOC) concentration within marine snow aggregates as a function of aggregate (Agg) size measured as volume, mass, and particulate organic carbon (POC) content. (A) Total organic carbon (TOC; $r^2 = 0.79$, $P < 0.001$) and DOC concentration ($r^2 = 0.65$, $P < 0.001$) versus aggregate volume. (B) DOC concentration versus aggregate POC content ($r^2 = 0.45$, $P < 0.01$). (C) DOC concentration versus aggregate mass ($r^2 = 0.36$, $P < 0.02$).

ume having an interstitial DOC concentration an order of magnitude lower than that of aggregates $10 \mu\text{l}$ in volume (Fig. 2A). Aggregate size (volume) was an excellent predictor of interstitial DOC concentration yielding a highly significant regression ($r^2 = 0.75$; $P < 0.001$; Fig. 2A). Interstitial DOC concentration also significantly decreased as the

POC and mass content of the aggregates increased, although these regressions were not as robust as that of DOC concentration versus aggregate volume ($P < 0.01$ and $P < 0.02$, respectively; Fig. 2B,C).

Because POC and DOC were measured on the same sample the total organic carbon (TOC) per aggregate, the TOC

Table 2. Comparison of the mean concentration of dissolved organic carbon (DOC) within aggregates to the concentration in the surrounding seawater at each station. The enrichment factor is (DOC in aggregates)/(DOC in surrounding seawater). The frequency of bacteria cells dividing on aggregates from East Sound is also shown. SE = standard error.

Station	Snow type	Depth (m)	[DOC] in aggregates (mg L ⁻¹)	[DOC] in seawater (mg L ⁻¹)	DOC enrichment factor in snow	% dividing cells (bacteria)	
						Snow	Seawater
14 May 97	Diatom	10	31.7	1.4	22	—	—
25 Jun 97	Larvacean	15	30.3	1.3	23	—	—
23 Jul 97	Larvacean	10	29.1	1.4	21	—	—
13 Jun 98	Diatom/debris	2–7	140.8	1.8	78	6.6	5.0
13 Jun 98	Debris	12	28.1	1.8	16	1.7	0.7
15 Jun 98	Debris	16	30.8	1.4	20	7.3	2.9
18 Jun 98	Diatom/debris	14	8.9	1.5	21	9.3	2.3
19 Jun 98	Diatom/debris	10	48.9	1.6	31	6.9	3.0
24 Jun 98	Debris	12	24.8	1.3	20	5.4	0.7
Mean ± SE			41.5 ± 12.8	1.5 ± 0.1	28 ± 6	5.6 ± 0.9	2.4 ± 0.5

concentration, and the contribution of DOC to TOC, considered both as absolute magnitude and as concentration, could be calculated. TOC and DOC concentrations within aggregates decreased with increasing aggregate size (Fig. 2A). Interstitial DOC concentrations were a constant proportion of TOC concentrations within aggregates regardless of aggregate size, as indicated by the similar slopes of the re-

gressions of TOC and DOC concentrations against aggregate volume (Fig. 2A). A comparison of the Y-intercepts of these regressions indicates that DOC concentrations were 22% of TOC concentration across all sizes of aggregates.

Although the concentration of TOC and interstitial DOC decreased with aggregate size, the total DOC within an aggregate increased significantly as aggregate size increased. Larger aggregates had significantly more total DOC per aggregate than smaller ones regardless of whether aggregate size was measured as volume (Fig. 1B), POC (Fig. 3A), or mass (Fig. 3B). This calculation is reported in Table 1 for each station. The aggregates investigated averaged from 14 to 588 mm³ in volume and averaged 3.0–23.1 μg POC and 3.7–25.2 μg TOC per aggregate. The quantity of DOC per aggregate comprised 7–31% of TOC, averaging 18 ± 2%, very similar to the contribution determined using the regression relationship in Fig. 2A. As with TOC concentrations, the magnitude of the average contribution of DOC to TOC per aggregate was consistent over all size ranges of aggregates as indicated by the similarity in the slopes of the regressions of interstitial DOC and TOC versus aggregate volume (Fig. 1B). The ratio of POC to DOC in aggregates ranged from 2.2 to 11.0, averaging 5.3 (Table 1).

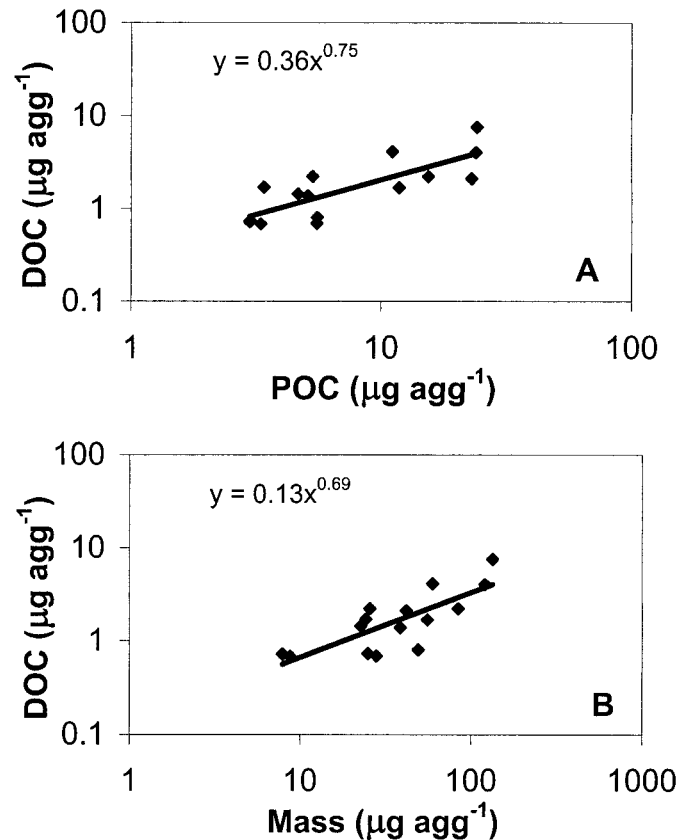


Fig. 3. The total dissolved organic carbon (DOC) content per aggregate as a function of aggregate size measured as mass and particulate organic carbon (POC) content. (A) Total DOC versus POC ($r^2 = 0.60$, $P < 0.001$). (B) Total DOC versus mass ($r^2 = 0.60$, $P < 0.001$).

Contribution of interstitial DOC to total DOC in the water column—Although marine snow was exceptionally abundant in East Sound, aggregates still occupied a very small percentage of the water column by volume, ranging from 0.1 to 0.4% (Table 3). Thus, despite the fact that the interstitial DOC concentration within aggregates was one to two orders of magnitude higher than that of ambient seawater, the contribution of interstitial DOC to total DOC was quite small. In the 30-m water column investigated, the average total cumulative volume of aggregates ranged from 109 to 457 μl L⁻¹ and the mean cumulative DOC in aggregates averaged 30.9 μg L⁻¹. This represented only 1.4–2.4% of the average total DOC concentration per liter in the water column (Table 3).

Bacteria—Bacterial abundance ranged from 10⁵ to 10⁷ cells per aggregate and larger aggregates contained significantly more total bacteria per aggregate than smaller ones

Table 3. The mean percent contribution of DOC within aggregates to total DOC in the water column in East Sound, Washington. SD = standard deviation.

Station	Snow type	Mean cumulative aggregate volume ($\mu\text{L L}^{-1}$)	Mean cumulative DOC in aggregates ($\mu\text{g L}^{-1}$)	[DOC] in bulk seawater (mg L^{-1})	% total DOC occurring in aggregates
13 Jun 98	Diatom/debris	224	34.2	1.81	1.9
15 Jun 98	Debris	259	32.5	1.45	2.3
18 Jun 98	Diatom/debris	356	35.9	1.45	2.4
19 Jun 98	Diatom/debris	457	34.0	1.57	2.1
24 Jun 98	Debris	109	18.0	1.25	1.4
Mean \pm SD		281 \pm 132	30.9 \pm 7.3	1.49	2.0 \pm 0.4

(Fig. 4). However, the abundance of bacteria on marine snow at the stations investigated were somewhat lower than those reported previously by Alldredge and Gotschalk (1990) for near-surface coastal aggregates of similar mass, especially for smaller aggregates (Fig. 4B). In contrast to abundance, the concentration of bacteria per unit volume of aggregate declined significantly with aggregate size (Fig. 5A). This finding was also consistent with previous studies (Alldredge and Gotschalk 1990).

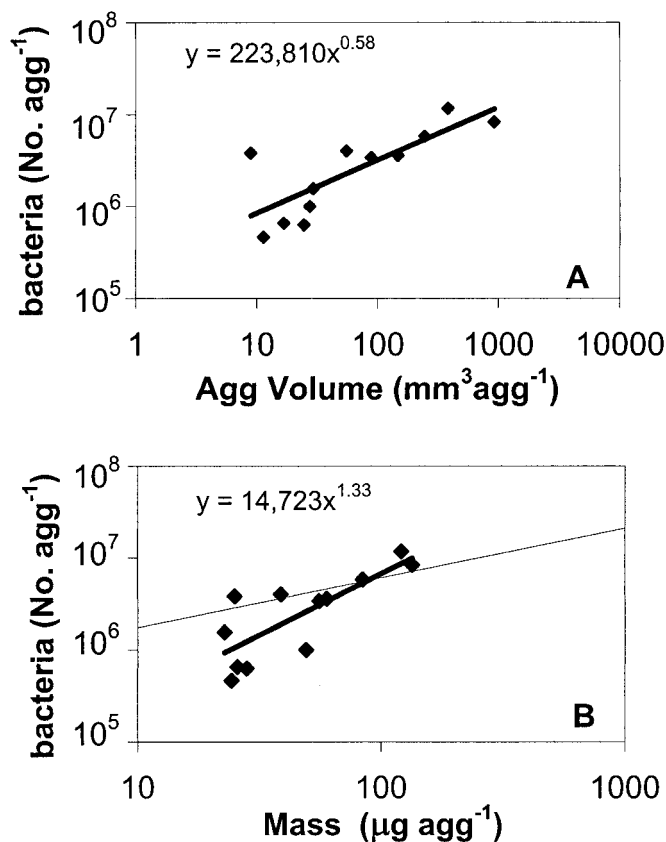


Fig. 4. Total number of bacteria per aggregate (agg) as a function of aggregate volume and mass. (A) Total bacteria per aggregate versus aggregate volume ($r^2 = 0.62$, $P < 0.001$). (B) Total bacteria per aggregate versus aggregate mass ($r^2 = 0.62$, $P < 0.001$). The thin line, shown for comparison, is from a much larger data set of coastal marine snow investigated by Alldredge and Gotschalk (1990).

Bacteria both produce DOC from POC through solubilization processes and take up DOC to grow. These competing processes probably resulted in the lack of a significant relationship between DOC concentration and bacteria concentration within aggregates (Fig. 5B). However, higher bacteria concentrations were significantly correlated with higher POC concentrations within aggregates (Fig. 5C) as might be expected because POC is the primary substrate for heterotrophic bacteria. Bacteria on marine snow at East Sound were growing actively and had frequencies of dividing cells averaging 5.6%. This was over twice as high as that for free-living bacteria in the surrounding seawater, where the frequency of dividing cells averaged 2.4%.

Discussion

DOC within aggregates—Herndl (1992) found DOC concentrations ranging from 3 to 25.5 mg L^{-1} within large mucus aggregates in the Adriatic Sea. I found concentrations of interstitial DOC from the coastal eastern Pacific to be an order of magnitude higher than this, up to 141 mg L^{-1} . Herndl (1992) assumed no dilution of aggregate samples by surrounding seawater during aggregate collection, although some dilution would be unavoidable. He also sampled aggregates considerably larger than those found in this study. Because interstitial concentrations of DOC decrease with increasing aggregate size, both dilution of samples with surrounding seawater during sample collection and larger aggregate size probably contributed to the lower interstitial concentration of DOC reported in his study.

Interstitial DOC concentrations of aggregates were up to two orders of magnitude higher than observed in the surrounding seawater. Several processes contribute to the production of these very high interstitial DOC concentrations within marine snow. Exudation from both living algal and bacterial cells and cell lysis of dying cells (Baldi et al. 1997) may be significant sources. However, the most important source is probably the hydrolytic activity of bacterial exoenzymes that break down and solubilize the particulate organic components of the aggregates (Cho and Azam 1988; Urban-Rich 1999). On a per cell basis, the hydrolytic activity of bacteria associated with marine snow is considerably higher than that of free-living bacteria in some studies (Karner and Herndl 1992; Smith et al. 1992) and similar to free-living bacteria in others (Karner and Herndl 1992; Muller-

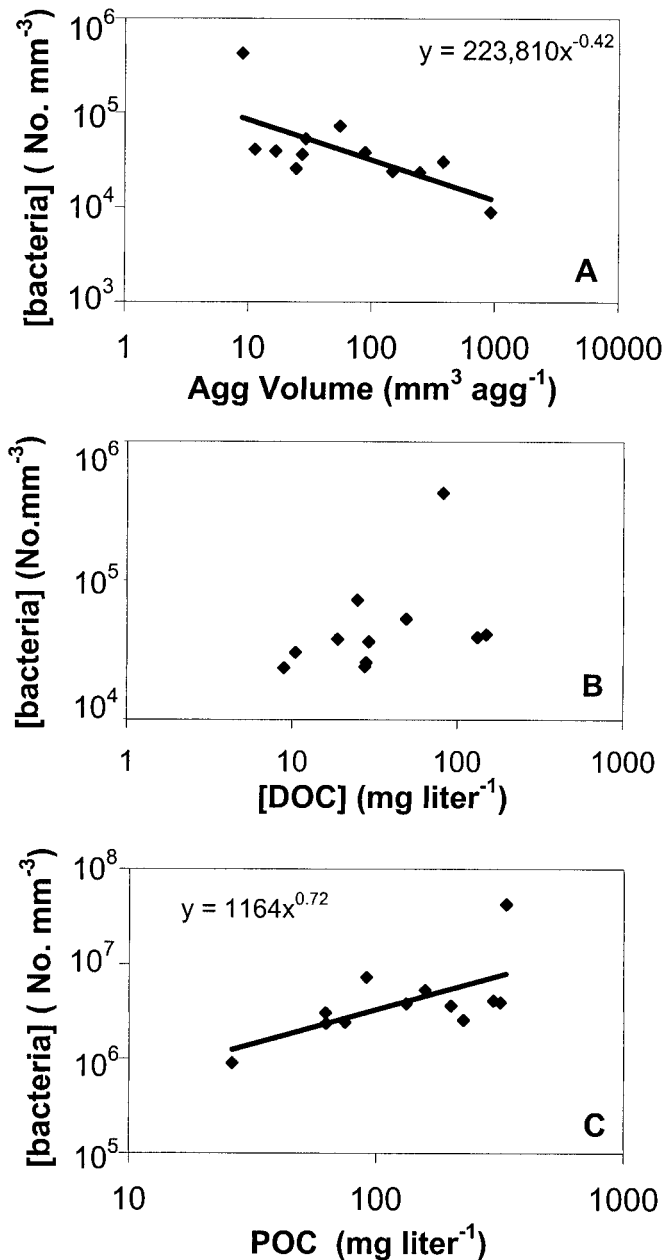


Fig. 5. The concentration of bacteria in aggregates as a function of aggregate volume, dissolved organic carbon (DOC), and particulate organic carbon (POC) content. (A) Bacteria concentration versus aggregate volume ($r^2 = 0.47$, $P < 0.001$). (B) Bacteria concentration versus interstitial DOC concentration ($r^2 = 0.30$, not significant, $P < 0.1$). (C) Bacteria concentration versus POC concentration in aggregates ($r^2 = 0.40$, $P < 0.05$).

Niklas et al. 1994; Unanue et al. 1998). However, the extremely high concentrations of bacteria within aggregates in general (two to four orders of magnitude higher than in the surrounding seawater; see Alldredge and Silver [1988] for review) facilitate the production of large amounts of DOC within a relatively small interstitial volume, leading to the potential for high concentrations in aggregates.

Once produced, DOC has several possible fates within an

aggregate that also affect the final interstitial concentration. DOC may be taken up by bacteria, adsorbed to surfaces, or diffuse out of the particle to the surrounding seawater. Unanue et al. (1998) reported lower uptake rates of hydrolyzed protein by attached bacteria relative to free-living forms, suggesting that bacterial uptake of dissolved substances could be lower on a per cell basis in aggregates and possibly contribute to greater accumulation of DOC. However, the high frequency of dividing cells in attached bacteria observed in this study suggests that bacteria were, in fact, growing rapidly and likely to have correspondingly high uptake rates of DOC.

Diffusion rates may be the most significant factor counterbalancing high production rates of DOC and regulating final interstitial concentrations of DOC within aggregates. Although the rate of diffusion of any dissolved substance from marine snow has not been measured directly, diffusion coefficients for dissolved silica have been calculated to be two orders of magnitude lower in aggregates than in pure seawater (Brzezinski et al. 1997). Diffusion rates of solutes from marine snow are likely to be much slower than diffusion in seawater because the fractal geometry of aggregates retards diffusion of solutes within their interstices. The blind passages and mazelike nature of fractals tends to retain randomly moving substances such as diffusing molecules (Meakin 1988), resulting in retention of DOC. Diffusion may also be retarded by the presence of abundant mucus and gel-like particles such as transparent exopolymer particles (Alldredge et al. 1993) within marine snow that reduce aggregate porosity and the exchange of solutes (Alldredge and Crocker 1995). Despite the factors that retard diffusion rates, release of DOC from aggregates via diffusion certainly occurs and is potentially significant for organisms in the surrounding seawater.

Several factors contribute to the observed decrease in interstitial DOC concentration with increasing aggregate size. Lower densities of bacteria in larger aggregates produce fewer hydrolytic enzymes resulting in lower overall solubilization of POC. Aggregate porosity also increases with increasing aggregate size (Alldredge and Gotschalk 1988), potentially increasing both diffusion rates and the advection of seawater through channels in the interstices. Although probably slow (because of mucus content), such advection would tend to dilute interstitial DOC in large aggregates more rapidly than in smaller ones. The higher porosity of larger aggregates may also increase diffusion rates of solutes from the center of the particles to their surfaces, allowing for greater mass transfer to the surrounding seawater. Finally, the higher sinking velocities of larger aggregates (Alldredge and Gotschalk 1988) results in lower boundary layer thickness around them (Ploug and Jorgensen 1999), facilitating higher diffusion rates in these particles.

Contribution of interstitial DOC to total DOC in the water column—The majority of aggregates investigated in this study came from East Sound, Washington, an area of extremely high marine snow abundance relative to most coastal and open ocean sites. For example, the total volume of marine snow in East Sound ranged from 100 to $\sim 500 \mu\text{L L}^{-1}$, representing only 0.01–0.05% of the water column, but this

occupied volume was two orders of magnitude higher than that measured in the Gulf of Mexico ($1.3\text{--}5\ \mu\text{L}^{-1}$; Walsh and Gardner 1992) and 2–50 times higher than that measured in the Southern California Bight (MacIntyre et al. 1995; Graham et al. 2000). However, despite the very high marine snow abundance in East Sound, aggregates still contributed <2.4% to the total DOC in the water column, suggesting that interstitial DOC is unlikely to be a significant component of total DOC in the water column throughout most of the ocean. However, the DOC diffusing from aggregates may be significantly more labile than the largely refractory DOC found in seawater. Fecal pellets are known to release mostly highly labile DOC as they decay (Urban-Rich 1999), suggesting that DOC diffusing from the phytoplankton-rich aggregates investigated here might also be readily usable by suspended microbes. Thus, although it is only a small percentage of the total DOC, the DOC within aggregates may be disproportionately important for biological processes in the water column.

One region where interstitial DOC may also be more significant is the Adriatic Sea. Assuming that aggregates occupied 5% of the water column by volume ($1,000\times$ higher than East Sound), Herndl (1992) calculated that interstitial DOC contributed 40% to total water column DOC. The northern Adriatic experiences periods when gelatinous aggregates are so large and abundant that they form an opaque layer, or false bottom, at the pycnocline (Stachowitsch et al. 1990). In these layers interstitial DOC may make up the bulk of total DOC. However, quantitative measurements of aggregate abundance in the Adriatic Sea are needed to estimate accurately the contribution of interstitial DOC to total water column DOC in this region.

The relatively inconsequential contribution of interstitial DOC to total DOC in the water column reported here indicates that DOC is unlikely to be underestimated in the ocean due to exclusion of relatively rare marine snow particles from the small seawater samples used for DOC analysis. Interstitial DOC contributes relatively little to total DOC primarily because aggregates occupy such a small volume of the water column. However, marine snow does make a significant contribution to other chemical constituents in seawater, including POC and nutrients (Shanks and Trent 1979; Alldredge and Silver 1988) that are rarer in the surrounding seawater than DOC.

Interstitial DOC and carbon flux—Interstitial DOC in sinking particles has not been included in measurements of particulate carbon flux. Oceanographers have tended to discount DOC in sediment traps because particle flux has appeared to be mediated primarily by particles, and interstitial DOC was assumed to be inconsequential. Moreover, most traps use Formalin as a preservative, thereby precluding direct measurement of DOC in trap samples. Finally, even in cases where DOC can be measured directly in trap samples, the DOC actually sedimenting into the traps cannot be separated from DOC arising from conversion of POC once particles enter the trap. Substantial DOC is released from leaching of dead zooplankton swimmers and detrital particles captured by sediment traps and may also originate from degradation processes related to the enzymatic activity of mi-

crobes within traps (Noji et al. 1999). Noji et al. (1999) estimated that particle-associated DOC in sediment trap samples was about the same order of magnitude as the particulate fraction itself and suggested that failure to measure particle-associated DOC in sediment trap samples constitutes a serious error in estimates of carbon sedimentation. My measurements indicate that interstitial DOC comprised up to 31% of TOC in sinking aggregates, averaging 18–22% (depending upon whether one uses absolute averages or regression relationships). Aggregates contained about six times more POC than interstitial DOC on average. This suggests that the high DOC values of sediment trap samples reported by Noji et al. (1999) were largely derived from POC to DOC conversions once particles entered the traps.

Aggregates of marine snow are the major component of particulate flux in most regions of the ocean (Fowler and Knauer, 1986). For example marine snow contributed >70% of the sinking carbon flux at most depths along the five-station VERTEX study transect stretching from coastal California to near Hawaii. At many depths and stations the contribution was >85% (Silver and Gowing 1991). Fecal pellets contributed <10% to mass flux at three tropical and subtropical sites in the Atlantic and Pacific with the majority of flux composed of amorphous aggregated material (Pilskaln and Honjo 1987). Given that marine snow is a substantial portion of sinking particles, this study suggests that measurements of organic carbon flux based on POC alone underestimate the amount of total organic carbon actually exported from surface waters by about 20%. While this underestimation may not seem large, it is a systematic error that may have a significant impact on results of global carbon models utilizing rates of oceanic carbon sequestration based on POC flux. The measurements reported here pertain to relatively fresh, young aggregates (as indicated by low C:N ratios), with high microbial and hydrolytic enzyme activity. The DOC content of older aggregates sinking into deep water is not known but could be lower than those of near-surface aggregates if microbial activity decreases substantially as aggregates age and decompose.

References

- ALLDREDGE, A. L. 1998. The carbon, nitrogen, and mass content of marine snow as a function of aggregate size. *Deep-Sea Res.* **45**: 529–541.
- , AND Y. COHEN. 1987. Can microscale chemical patches persist in the sea? Microelectrode study of marine snow, fecal pellets. *Science* **235**: 689–691.
- , AND K. M. CROCKER. 1995. Why do sinking mucilage aggregates accumulate in the water column? *Sci. Total Environ.* **165**: 15–22.
- , AND C. GOTSCHALK. 1988. In situ settling behavior of marine snow. *Limnol. Oceanogr.* **33**: 339–351.
- , AND ———. 1990. The relative contribution of marine snow of different origins to biological processes in coastal waters. *Cont. Shelf Res.* **10**: 41–58.
- , U. PASSOW, AND B. LOGAN. 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Res.* **40**: 1131–1140.
- , AND M. W. SILVER. 1988. Characteristics, dynamics and significance of marine snow. *Prog. Oceanogr.* **20**: 41–82.
- BALDI, F., A. MINACCI, AND A. MALEJ. 1997. Cell lysis and release

- of particulate polysaccharides in extensive marine mucilage assessed by lipid biomarkers and molecular probes. *Mar. Ecol. Prog. Ser.* **153**: 45–57.
- BRZEZINSKI, M. A., L. M. O'BRYAN, AND A. L. ALLDREDGE. 1997. Silica cycling within marine snow. *Limnol. Oceanogr.* **42**: 1706–1713.
- BUESSELER, K. O., AND OTHERS. 1996. An intercomparison of cross-flow filtration techniques used for sampling marine colloids: Overview and organic carbon results. *Mar. Chem.* **55**: 1–31.
- CHO, B. C., AND F. AZAM. 1988. Major role of bacteria in the biogeochemical fluxes in the ocean's interior. *Nature* **332**: 441–443.
- FOWLER, S. W., AND G. A. KNAUER. 1986. Role of large particles in the transport of elements and organic compounds through the oceanic water column. *Prog. Oceanogr.* **16**: 147–194.
- GARDNER, W. D., AND I. D. WALSH. 1990. Distribution of macroaggregates and fine-grained particles across a continental margin and their potential role in fluxes. *Deep-Sea Res.* **37**: 401–411.
- GRAHAM, W., S. MACINTYRE, AND A. L. ALLDREDGE. 2000. Diel patterns in the concentration of marine snow and particle flux in surface waters. *Deep-Sea Res.* **47**: 367–395.
- HERNDL, G. J. 1988. Ecology of amorphous aggregates (marine snow) in the Northern Adriatic Sea. II. Microbial density and activity in marine snow and its implications to overall pelagic processes. *Mar. Ecol. Prog. Ser.* **48**: 265–275.
- . 1992. Marine Snow in the Northern Adriatic Sea: Possible causes and consequences for a shallow ecosystem. *Mar. Microb. Food Webs* **6**: 149–172.
- HONJO, S., K. W. DOHERTY, Y. C. AGRAWAL, AND V. L. ASPER. 1984. Direct optical assessment of large amorphous aggregates (marine snow) in the deep ocean. *Deep-Sea Res.* **31**: 67–76.
- KALTENBOCK, E., AND G. J. HERNDL. 1992. Ecology of amorphous aggregates (marine snow) in the Northern Adriatic Sea. IV. Dissolved nutrients and the autotrophic community associated with marine snow. *Mar. Ecol. Prog. Ser.* **87**: 147–159.
- KARNER, M., AND G. J. HERNDL. 1992. Extracellular enzymatic activity and secondary production in free-living and marine snow associated bacteria. *Mar. Biol.* **113**: 341–347.
- KILPS, J. R., B. E. LOGAN, AND A. L. ALLDREDGE. 1994. Fractal dimensions of marine snow determined from image analysis of in situ photographs. *Deep-Sea Res.* **41**: 1159–1169.
- LOGAN, B., AND D. B. WILKINSON. 1990. Fractal geometry of marine snow and other biological aggregates. *Limnol. Oceanogr.* **35**: 130–136.
- LONGHURST, A. R., AND W. G. HARRISON. 1989. The biological pump: Profiles of plankton production and consumption in the upper ocean. *Prog. Oceanogr.* **22**: 47–123.
- MACINTYRE, S., A. L. ALLDREDGE, AND C. C. GOTSCHALK. 1995. Accumulation of marine snow at density discontinuities in the water column. *Limnol. Oceanogr.* **40**: 449–468.
- MEAKIN, P. 1988. Fractal aggregates. *Adv. Colloid Interface Sci.* **28**: 249–331.
- MULLER-NIKLAS, G., S. SCHUSTER, E. KALTENBOCK, AND G. J. HERNDL. 1994. Organic content and bacterial metabolism in amorphous aggregations of the northern Adriatic Sea. *Limnol. Oceanogr.* **39**: 58–68.
- NOJI, T., K. Y. BORSHEIM, F. REY, AND R. NORTVEDT. 1999. Dissolved organic carbon associated with sinking particles can be crucial for estimates of vertical carbon flux. *Sarsia* **84**: 129–135.
- PILSKALN, C. H., AND S. HONJO. 1987. The fecal pellet fraction of biogeochemical particle fluxes to the deep sea. *Global Biogeochem. Cycles* **1**: 31–48.
- PLOUG, H., AND B. B. JORGENSEN. 1999. A net-jet flow system for mass transfer and microsensor studies of sinking aggregates. *Mar. Ecol. Prog. Ser.* **176**: 279–290.
- PORTER, G. K., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25**: 943–948.
- SHANKS, A. L., AND J. D. TRENT. 1979. Marine snow: Microscale nutrient patches. *Limnol. Oceanogr.* **24**: 850–854.
- SHARP, J. H. 1992. Total mass and particulate carbon, nitrogen and phosphorus, p. 87–91. *In* D. C. Hurd and D. W. Spencer [eds], *Marine particles: Analysis and characterization*. Geophysical Monograph Am. Geophys. Union.
- SIEGENTHALER, U., AND J. L. SARMIENTO. 1993. Atmospheric carbon dioxide and the ocean. *Nature* **365**: 119–125.
- SILVER, M., AND M. M. GOWING. 1991. The "particle" flux: Origins and biological components. *Prog. Oceanogr.* **26**: 75–113.
- SIMON, M., A. L. ALLDREDGE, AND F. AZAM. 1990. Bacterial carbon dynamics on marine snow. *Mar. Ecol. Prog. Ser.* **65**: 205–211.
- SMITH, D. C., M. SIMON, A. L. ALLDREDGE, AND F. AZAM. 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* **359**: 139–141.
- STACHOWITSCH, M., N. FUNUKO, AND M. Y. RICHTER. 1990. Mucus aggregates in the Adriatic Sea: An overview of stages and occurrences. *PSZNI: Mar. Ecol.* **11**: 327–350.
- TOGGWEILER, J. R. 1999. An ultimate limiting nutrient. *Nature* **400**: 511–512.
- UNANUE, M., I. AZUA, J. M. ARRIETA, A. LABIRUA-ITURBURU, L. EGEA, AND J. IRIBERRI. 1998. Bacterial colonization and ectoenzymatic activity in phytoplankton-derived model particles: Cleavage of peptides and uptake of amino acids. *Microbial. Ecol.* **35**: 136–146.
- URBAN-RICH, J. 1999. Release of dissolved organic carbon from copepod fecal pellets in the Greenland Sea. *J. Environ. Mar. Biol. Ecol.* **232**: 107–124.
- WALSH, I. D., AND W. D. GARDNER. 1992. A comparison of aggregate profiles with sediment trap fluxes. *Deep-Sea Res.* **39**: 1817–1834.
- WILLIAMS, P. M., J. E. BAUER, K. J. ROBERTSON, D. M. WOLGAST, AND M. L. OCCCELLI. 1993. Report on DOC and DON measurements made at Scripps Institution of Oceanography, 1988–1991. *Mar. Chem.* **41**: 271–281.

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