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Bathypelagic marine snow: deep-sea algal and detrital community

by Mary Wilcox Silver¹ and Alice L. Alldredge²

ABSTRACT

Using the research submersible ALVIN, we have observed and collected marine snow (fragile macroscopic aggregates), in two basins off Southern California. Marine snow was present throughout the water column, with several layers of higher concentrations in the mesopelagic and bathypelagic zones. We collected the first intact specimens from the deep sea at 1000 and 1650 m depths and examined these by light and electron microscopy.

Flocculent marine snow consisted of mucous sheets from larvacean houses and distinctive but unknown additional sources. The origin of the more common but smaller, flake-like marine snow is unknown. Both flocculent and flake-like marine snow had large numbers of associated cells and biogenic debris, with similar concentrations of associated materials in all samples. The organic carbon content of the aggregates exceeds that of surrounding water by at least three orders of magnitude, with most organic material on marine snow, and in surrounding water consisting of waste products. However, large numbers of apparently healthy cells also occur on marine snow, and over 3/4 of the biomass in these microenvironments consists of phytoplankton. Even within the picoplankton ($< 2 \mu\text{m}$) fraction, structural photoautotrophs contribute 1/3 to 1/2 of the total biomass. In the larger size categories, intact photoautotrophs consist primarily of diatoms, while picoplankton-sized forms consist of cyanobacteria (blue-green algae) and green algae. The intact algae of marine snow in the deep sea are derived from two sources: the smallest forms are digestion-resistant cells that arrive from fecal pellets, possibly dividing while still within fecal material; other forms apparently descend from the euphotic-zone on marine snow. Additional biogenic materials on marine snow consist of abundant heterotrophic bacteria, large numbers of fecal pellets, olive-green "cells" and other breakdown products, and organic detritus from many sources. Protozoans are moderately abundant on marine snow, and are consuming the picoplankton-sized cells from these habitats. Marine snow houses a complex and highly concentrated detrital community that represents an important pelagic food source, and it is an agent for transporting surface-derived materials to depth at intermediate sinking rates (50-100 m/day).

1. Introduction

Direct observations by SCUBA divers (Johannes, 1967; Hamner *et al.*, 1975) and

1. Center for Coastal Marine Studies, University of California, Santa Cruz, CA 95064, U.S.A.

2. Department of Biological Sciences and Marine Science Institute, University of California, Santa Barbara, CA 93106, U.S.A.

from submersibles near the surface (Suzuki and Kato, 1953; Nishizawa *et al.*, 1954) indicate that amorphous, fragile particulates called "marine snow" or "macroscopic aggregates" are ubiquitous and abundant components of epipelagic waters. Visible flocs, ranging from 0.5 mm to many centimeters in diameter have been described from surface waters of the North Atlantic (Hamner *et al.*, 1975), the Gulf of California (Alldredge, 1976, 1979), neritic waters of the California Current (Trent *et al.*, 1978), and the tropical Pacific (Johannes, 1967). Field observations indicate that some macroscopic flocculent aggregates are produced by zooplankton: the feeding structures formed by larvaceans (Alldredge, 1972), pteropods (Gilmer, 1972), salps, veligers and polychaetes (Hamner *et al.*, 1975). Marine snow from surface waters harbors many organisms at concentrations greatly exceeding that in surrounding water, and the communities on particles differ in selective composition from those freely dispersed in the water (Silver *et al.*, 1978). Snow particles are organism- and nutrient-rich microhabitats, and may be sites of enhanced heterotrophic activity (Silver *et al.*, 1978; Shanks and Trent, 1979; Pomeroy and Diebel, 1980).

Marine snow is also abundant in the deep sea and is readily observed from submersibles (Beebe, 1934; Suzuki and Kato, 1953; Piccard and Dietz, 1961; Costin, 1970; Barham, 1979). However, collection and laboratory examination of intact macroscopic particulates has been restricted to samples collected from surface waters within the depth limitations of SCUBA. Intact marine snow from bathypelagic depths has not previously been collected or examined. One of us (A.A.) recently observed and successfully collected marine snow in the deep waters of the Southern California Borderland during three dives of the research submersible ALVIN. Herein we describe, enumerate, and compare the microorganisms and detrital particles associated with marine snow and in the surrounding water at bathypelagic depths. We discuss the possible sources of snow and associated microorganisms, the ecological significance of these aggregates to deep sea communities, and the importance of these systems to flux processes.

2. Methods

a. Field collection and observation. We include two types of aggregates in the term "marine snow," since both consist of visible, fragile associations of particles. We collected a flocculent aggregate (floc) from coastal waters west of San Diego, California on August 9, 1979 at 1000 m in East Cortez Basin (ALVIN Dive #950: 32°13'N, 118°18'W) and on August 11, 1979 at 1650 m in San Clemente Basin (Dive #952: 32°31'N, 118°03'W). Eight smaller, flake-like aggregates were obtained from 1000 m in San Clemente Basin on August 12, 1979 (Dive # 953: 32°26'W, 118°07'W). Bottom depths were 1750 m at San Clemente and 1878 m at East Cortez Basin, respectively. Marine snow was collected from ALVIN in a

horizontally mounted, transparent, plastic Van Dorn bottle (10 cm in diameter by 50 cm long) by maneuvering the submersible until the bottle enclosed an aggregate and then triggering the closing mechanism. We also collected water samples, which did not contain visible aggregates, in Van Dorn bottles at the same depth, time, and location. The Van Dorn bottles remained tightly sealed until the submersible surfaced, approximately 1 to 2 hours later. The aggregates were then gently pipetted from the bottles into small vials containing 2% glutaraldehyde in buffered, 0.45 μm millipore filtered seawater and stored in the dark at 4°C until laboratory examination (approximately 4 months later). Samples of 500 to 1000 ml seawater were also fixed in 2% glutaraldehyde. The collecting and handling techniques minimized both microbial growth within the bottles and contamination of the samples from surrounding water during submersible ascent.

We made qualitative observations of the relative distribution and abundance of marine snow with depth through the ALVIN ports during descent on Dive #950 in East Cortez Basin.

b. Microscopy. Marine snow samples were first examined in a 4 mm deep chamber on an inverted microscope, the linear dimension of the overall matrix measured, and the volume of the aggregate calculated. We obtained a ratio reflecting the extent of sample shrinkage due to preservation by comparing the original floc volume in the 1650 m San Clemente sample (before preservation at the time of sample collection) and the volume after fixation and storage. We used this ratio to calculate the original, field volume of the floc from 1000 m in East Cortez Basin, using dimensions measured in the laboratory. The 1000 m flakes from San Clemente Basin did not possess a mucous matrix and appeared to be of more rigid construction. Thus we consider that the flakes changed little in volume from the time of their collection and have used their preserved dimensions as the best estimate of their true volume. The volume measurements of each aggregate were used to standardize numerical comparisons between samples as numbers of organisms or particles per ml of original, unfixed volume.

Marine snow samples were vigorously dispersed (20 sec on a Vortex mixer) and measured subsamples removed for examination by light microscopy. Because of the small size of the flake aggregates, all 8 were pooled for microscopy and were analyzed subsequently as if they were one sample, since they were collected at the same depth and site. Water samples were shaken and 100 to 200 ml fractions removed for study. These aliquots from both marine snow and water were filtered onto 0.45 μm cellulose acetate filters (Gelman metrical) and the water mounts cleared with Zeis W15 immersion oil (Silver and Ringo, in prep.). The samples were then examined by high resolution phase and Nomarski optics and by transmitted light fluorescence microscopy, since autofluorescence of many specimens is maintained by this preparation. Organisms and detritus were counted and their

sizes measured during 3 to 6 replicate surveys of each filter preparation. Cyanobacteria (= bluegreen algae) were counted by fluorescence microscopy, observing their orange phycoerythrin autofluorescence using a mercury emission lamp, a 546 nm excitation filter and a 580 nm barrier filter (Wilde and Fliermans, 1979). Cyanobacteria were still intensely autofluorescent, and because of their small size ($< 1.5 \mu\text{m}$) could be readily distinguished from other possible phycoerythrin possessing forms (i.e., larger, eucaryotic cryptophytes). Samples were also examined with a standard blue excitation filter (BG 12) and 530 nm barrier filter for chlorophyll *a* fluorescence.

Because of the very small size of the abundant material on marine snow, we found both scanning and transmission electron microscopy (SEM and TEM, respectively) necessary to further characterize the aggregate communities. Samples were dehydrated through an alcohol series, air dried from Freon 113, gold coated, and examined on a JEOL JSM 2 SEM. TEM samples were placed overnight in a ruthenium red solution with sea water, rinsed with buffer, embedded in agar, cut in $1 \mu\text{m}$ cubes, and postfixed for 1 hour in 1% OsO_4 (method of Holland and Neelson, 1978). TEM samples were then dehydrated in an acetone series and embedded in M-Spur's Resin (Ringo *et al.*, 1979). Sections were cut with a diamond knife and viewed on a JEOL 100B microscope after staining in uranyl acetate and lead citrate. Because of the relatively large volume of the floc material, we were able to prepare aliquots for both TEM and SEM as well as for light microscopy. Due to the smaller size of the flake samples, we had only sufficient material for SEM and light microscopy. We did not have sufficient material from the water samples for TEM, but did examine some of these samples by SEM. We also prepared smaller quantities of floc material for TEM, using the 1650 m San Clemente sample, to determine whether specimen losses were occurring due to ejection of calcareous-walled cells during the sectioning process. We had noted that coccolithophorid protoplasts did not occur in the TEM sections, prepared as stated above, but that appropriate sized circular holes were present in the sections, indicating specimen loss after embedding. These additional preparations were decalcified for 1 hour in 2.5% (ethylenedinitrilo) tetraacetic acid (EDTA) in 2% glutaraldehyde solution following OsO_4 fixation. Following decalcification, the material was treated the same as that from the other samples.

c. Carbon calculation. We calculated the carbon content of the $> 2 \mu\text{m}$ organisms and waste products on marine snow from light microscopy data. We used the carbon to volume relationship presented in Mullin *et al.* (1966) for cells other than diatoms, including the olive-green bodies (previously known as "olive-green cells": Fournier, 1970) and grey bodies. For diatoms we used the carbon to volume regression of Strathman (1967). We calculated the carbon content of fecal pellets using the dry weight:density relationship of pellets reported in Small *et al.* (1979)

and the ratio from Johannes and Satomi (1966) for converting dry weight to carbon. Fecal fragments, or disrupted material of obvious fecal origin, was assumed to have approximately 1/2 the carbon per unit volume of intact pellets, based on qualitative comparison of pellets and fragments from light and electron microscopy.

For the $< 2 \mu\text{m}$ -sized cells, we calculated biomass using a new method described by Silver and Bruland (1981). Briefly, the numbers of a "reference" cell type, one readily recognized by both light and transmission electron microscopy, are determined by light microscopy. The reference cells are then sized either by light microscopy or TEM, and the total volume of these cells calculated for the sample. The abundance of other cells, ones too small to be recognized by light microscopy, can subsequently be calculated indirectly by comparing their abundance with that of the reference cell population in TEM sections; abundances of reference and other cell types are determined by cumulating cross sectional areas of specimens from numerous sections of the original sample. This method is ideal for small cells or detrital samples because it permits distinctions between intact cells, degrading cells, and other similarly-sized detrital particles. In our samples, we counted and measured only ultrastructurally intact, healthy-appearing cells, i.e., those that possessed intact walls and membranes and other appropriate internal structures. We used picoplankton sized ($< 2 \mu\text{m}$ diameter; Sieburth *et al.*, 1978) cyanobacteria as the reference population, counted these by autofluorescence (see above), and sized them from TEM sections. For our calculations, we determined the volume of picoplankton cells from numerous sections of each cell type. Volumes were converted to biomass from relationships in Watson *et al.* (1977) for bacteria, or that in Mullin *et al.* (1966) for eukaryotes. This TEM method for computing cell carbon is conservative, as used in the present study, because it assumes that all cyanobacteria seen in TEM section possessed phycoerythrin autofluorescence. [Johnson and Sieburth (1979) showed that some marine, picoplankton-sized cyanobacteria did not possess phycoerythrin.] If some cyanobacteria in our samples were not autofluorescent, then this ratio method underestimates the true abundance of other cells (by the ratio: fluorescent cyanobacteria/total cyanobacteria).

Although we were primarily interested in determining biomass of cells in the picoplankton-size fraction, we also present their numbers as an alternate measure of abundance. We calculated the cell numbers of heterotrophic bacteria and $< 2.0 \mu$ algae indirectly, since we could only distinguish these unequivocally by TEM. As explained above, we determined the cell volume for the total population of eucaryotic algae and heterotrophic bacteria in the picoplankton fraction by referencing their abundance to that of cyanobacteria; we then calculated numbers of algae and heterotrophic bacteria by dividing this total volume by the average volume of the respective cell type. The volume of the most common eucaryotic alga, a spherical *Chlorella*-like cell, was readily calculated using the cell diameter measured by TEM. The heterotrophic bacteria varied in size and shape, so we express abundance

for these as the equivalent numbers of coccoidal (spherical) cells that possess the average diameter for a heterotrophic bacterium measured in TEM section.

d. Sinking rates of marine snow. Sinking rates of near-surface marine snow have been calculated indirectly (Alldredge, 1979) and measured *in situ* by divers (Shanks and Trent, 1980); in both of these studies the sources or nature of most of the snow was unknown. Since some of the flocc material in our samples was obviously of larvacean origin, we collected larvacean houses to estimate the possible ages of this source of the deep-sea snow and to collaborate the findings of previous studies using a third, independent method. *Oikopleura dioica* in their houses were collected in shallow vertical tows in the Santa Barbara Channel. Intact, uncollapsed houses of these organisms were pipetted into 1000 ml graduated cylinders containing undisturbed, unfiltered sea water at 15° and 5°C. Houses were allowed to settle approximately 6 cm, and then the time each house took to sink 3.5 cm further was measured for 3 replicate trials at each temperature. House diameters were measured under a dissecting microscope and volumes estimated assuming the houses to be spheres.

3. Results

a. Field observations. During the dive in East Cortez Basin, macroscopic aggregates similar in gross appearance to those we have observed in surface waters were abundant throughout the water column to the bottom at 1878 m. Flakes, 0.5 mm to several mm in longest diameter were the most abundant visible aggregates observed. Preliminary observations of flakes passing through a 5.3 cm ring held outside the viewing port of the ALVIN suggest densities of at least one flake per liter. Larger, flocculent particles of marine snow, ranging from 3 to 4 mm to several cm in diameter, were rarer, although at least 5 to 6 flocs were visible from the port at any one time. Particularly high densities of marine snow occurred between 250 and 340 m and in 2 horizontal layers (10 to 20 m thick) at 850 m and 1500 m. The shape and internal filter structure of some of the visible flocs indicated that they were the remains of discarded larvacean houses (Alldredge, 1972; Barham, 1979). Numerous zooplankton, particularly larvaceans, medusae, siphonophores, pteropods, ctenophores and sergestid shrimp were also visible from the ports.

b. Origin of marine snow. The flocc samples from both basins contained many mucous sheets. These were generally fragmented, veil-like structures with no distinctive features when viewed by light microscopy and by either SEM or TEM. However, we also encountered net structures among the mucous materials on SEM (Fig. 1a-d). The net structures shown in Figure 1a,b were found in both flocc samples and are the internal feeding filters of larvacean houses (Flood, 1978). We do not

know the source of the mucus in Figure 1c, but the regularity of mesh and the pore size suggest the feeding filter of an organism that consumes picoplankton.

Flakes were examined both by light and SEM microscopy. They were the visible matrix of the 1000 m, San Clemente samples, but were also found in the two additional floc samples. The nature of most of the flakes is unknown, but most appeared comparatively rigid, with pitted or irregular surface detail on SEM (Fig. 1d), and occasionally they had associated picoplankton-sized cells on them.

c. Contents of marine snow and water samples.

Eucaryotes. Many organisms were associated with the marine snow. Relatively large forms, such as diatoms, coccolithophorids, and radiolarians, were the most conspicuous (Table 1). Many of these, however, were empty or showed considerable degradation of the internal protoplast. Moderate numbers of diatoms appeared to contain cellular material, as viewed on the light microscope, and TEM confirmed the presence of protoplasts in some specimens. Small pennate forms were generally in best condition and those found on marine snow appeared to contain cell contents more frequently than those from surrounding water, at least as judged by light microscopy. Coccoliths and coccolithophorids were also abundant in the material, but TEM examination indicated that the walls were empty and the protoplasts were missing. We calculated the abundance of intact coccospheres by SEM, and determined that these were nearly as abundant as intact frustules of diatoms (0.8 times as many coccospheres as diatoms, $N = 612$ cells counted by SEM in floc samples). Coccospheres were in excellent condition, showing no signs of disruption or etching by digestion or other solution process (Fig. 1e-g). The most abundant forms were cells under $10 \mu\text{m}$, including *Cyclococcolithus leptopora* (Murray and Blackman) Kampner, and *Emiliania huxleyi* (Lohmann) Hay and Mohler.

Additional cellular-like materials of very small size (usually $< 5 \mu\text{m}$) were noted in floc and water samples on the light microscope. These ranged from grey-green to deep green or olive and were counted with the "grey bodies" and "olive-green bodies," described below. Fluorescence microscopy indicated that some of these forms still retained chlorophyll fluorescence (Fig. 2a), though many appeared quite faded, possibly due both to storage time and leaching of pigments into the immersion oil. By TEM, we confirmed the presence of eucaryotic, coccoidal cells containing chloroplasts in this small size fraction in the floc samples. Common forms, or cells showing typical characteristics of the small, deep-sea algae, are shown in Figure 2b-d. The most frequently encountered group were the green algae, either picoplankton sized ($1.6 \mu\text{m}$ average diameter; $\text{SE} = 0.1 \mu\text{m}$, $n = 36$ observations) *Chlorella*-like cells, or cells assigned to the green algae because of their characteristic starch-like accumulations and general ultrastructure. Most of the abundant forms appeared to be in healthy condition, with the *Chlorella*-like cells even showing some evidence of recent division (Fig. 2c). These cells occurred commonly in

Table 1. Number of particles (\pm S.E.) per ml in marine snow and surrounding water.

| | No. particles/ml marine snow | | | No. particles/ml surrounding water | | |
|-------------------------------------|---------------------------------|-------------------------------|-------------------------------|---------------------------------------|------------------------|----------------------|
| | San Clemente 1,000—flakes | San Clemente 1,650m—floc | San Clemente 1,000m—floc | San Clemente 1,000m | San Clemente 1,650m | East Cortez 1,000 |
| cyanobacteria | 8.5(\pm 2.0) $\times 10^4$ | 2.6(\pm 0.4) $\times 10^4$ | 1.6(\pm 0.7) $\times 10^4$ | —** | 13.6(\pm 5.3) | 5.9(\pm 1.5) |
| grey bodies | 4.4(\pm 1.6) $\times 10^4$ | 8.4(\pm 2.0) $\times 10^4$ | 24(\pm 3) $\times 10^4$ | 67(\pm 15) | 81(\pm 20) | 106(\pm 27) |
| olive green bodies | 2.6(\pm 1.1) $\times 10^3$ | 7.1(\pm 2.1) $\times 10^3$ | 9.4(\pm 4.1) $\times 10^3$ | 4.3(\pm 1.5) | 4.9(\pm 1.9) | 11.6(\pm 4) |
| diatoms | 1.4(\pm 0.9) $\times 10^3$ | 5.0(\pm 1.8) $\times 10^3$ | 7.9(\pm 3.2) $\times 10^3$ | 2.2(\pm 1.4) | 1.5(\pm 0.8) | 1.1(\pm 0.3) |
| silicoflagellates and radiolaria | 0(\pm 0) | 5.4(\pm 2.5) $\times 10^3$ | 6.7(\pm 3.5) $\times 10^3$ | 0 | 0 | 0 |
| fecal pellets | 31(\pm 20) $\times 10^3$ | 2.3(\pm 0.6) $\times 10^3$ | 8.5(\pm 1.9) $\times 10^3$ | 3.5(\pm 2.1) | 3.6(\pm 0.9) | 13.3(\pm 4) |
| number of aliquots counted* | 4,6 | 3,5 | 4,4 | 3,0 | 3,5 | 4,5 |

* First number refers to replicates for phase optics count (all cells but cyanobacteria); second number refers to replicates counted by fluorescence microscopy (cyanobacteria).

** No count available, since sample was fixed in formaldehyde and thus autofluorescence of cyanobacteria extinguished.

Table 2. Carbon and numbers of picoplankton (0.2-2.0 μ m) sized cell on flocculent marine snow.

| | Avg diam (in TEM) sect | Cell vol X10 ³ μ m ³ / ml snow | | μ g C/ml | | Numbers/ml | |
|-----------------|---------------------------------|---|--------------------|------------------|--------------------|---------------------|---------------------|
| | | East | San | East | San | East | San |
| | | Cortez 1,000m | Clemente 1,650m | Cortez 1,000m | Clemente 1,650m | Cortez 1,000m | Clemente 1,650m |
| heterotrophic | | | | | | | |
| bacteria | 0.8 μ m | 33.7 | 206.8 | 4.4 | 26.9 | 0.9X10 ⁵ | 1.5X10 ⁵ |
| cyanobacteria | 0.9 μ m | 9.1 | 14.4 | 1.2 | 1.9 | 1.6X10 ⁴ | 2.6X10 ⁴ |
| coccoidal green | | | | | | | |
| eucaryote | 1.6 μ m | 16.6 | 38.6 | 4.3 | 10.0 | 0.8X10 ⁴ | 1.9X10 ⁴ |

fecal pellets, often amid obvious algal debris, and frequently in association with the cyanobacteria. The abundance and biomass of the *Chlorella*-like form is shown in Table 2.

A variety of additional cells and detrital remains of organisms were encountered in light microscopy surveys of marine snow and water samples. These other materials occurred much less frequently than the cell types just described, and thus we do not include counts of them here. Less abundant algal forms included euglenoids, dinoflagellates, diatom resting spores, and various cysts including those of chrysophytes (2 to 20 μ m). Additional materials were *Bodo*- and *Rhynchomonas*-like zooflagellates, choanoflagellates, amoebae, ciliates, fungal spores, nematocysts, fish scales, crustacean debris, terrigenous detritus (angiosperm pollen, lepidopteran scales), and naupliar larvae. TEM examination of the material showed that there were also moderate numbers of protozoans in the samples, many of which were < 5 μ m. These may have been difficult to recognize by light microscopy because of small size, distortion after fixation, and because of their general similarity to the numerous "grey bodies," described below. However, both forms which may have borne flagellae and others that were amoeboid, were noted intact in the sectioned material. Some of the specimens were seen to contain picoplankton cells, and some showed these in various stages of digestion (Fig. 2e,f).

Cyanobacteria. Green light excitation of the specimens revealed the presence of many phycoerythrin containing cells, approximately 1 μ m in size, which we enumerated as cyanobacteria. Fluorescent pairs of these cells were noted occasionally, especially in fecal pellets, and we interpret these couplets as possible products of recent division. Fluorescing cyanobacteria are shown in Figure 2a. Counts of the cyanobacteria are shown in Table 1. Cyanobacteria were strongly associated with particulates: even in the water sample, which lacked visible particulates, more than half the cells remained attached to very small particles, including fecal pellets, even after the vigorous shaking used in dispersing the sample. In the marine snow samples, slightly less than half the cyanobacteria remained inside membrane bound pellets after vigorous shaking to disperse the snow matrix.

TEM revealed the presence of abundant procaryotes that we interpret to be cyanobacteria (Fig. 3a,b), on the basis of their lamellar structure, their possession of polyhedral bodies (sites of CO₂ fixation), and the corresponding abundance of similarly-sized phycoerythrin containing unicells seen by fluorescence microscopy. Such cyanobacteria have not been recorded previously from the deep-sea, but ultrastructurally similar, orange-fluorescing forms are now known from surface waters of the ocean (Johnson and Sieburth, 1979: Type 1 cells). These have been designated *Synechococcus* or *chroococcoidal* cyanobacteria and occur in numbers between 10³-10⁵ cells/ml in warmer surface waters throughout the ocean (Waterbury *et al.*, 1979; Johnson and Sieburth, 1979). Orange-fluorescing, ultrastructurally identical forms also occur in discarded larvacean houses collected in surface waters from the Gulf of California, offshore waters of Santa Barbara, and Monterey Bay, California (Silver and Alldredge, unpub. obs.). Specimens in the deep-sea floc appeared ultrastructurally to belong to one type, with very few exceptions. The cyanobacteria were rod-shaped procaryotes averaging about 0.9 × 1.2 μm, average cross-sectional area 0.69 μm² (SE = 0.07 μm², n = 21 measurements). The cells appeared to be in healthy condition, because their cell wall and membranes were intact and because cells occasionally appeared to have divided recently (Fig. 3a). Moreover, almost all of the cells lacked the storage products (i.e., polyphosphate and cyanophycin deposits) that are usually abundant in senescent or stationary phase cells (Obukowicz and Kennedy, 1980). In sectioned material these cells were found frequently in intact fecal pellets, often in conjunction with the abundant picoplankton-sized eucaryotes, discussed below.

Heterotrophic Bacteria. TEM sections of the marine snow showed heterotrophic bacteria to be the most abundant cells in floc samples. As shown by TEM micrographs, their average cross-sectional area was 0.45 μm² (SE 0.12 μm², n = 82 measurements), corresponding to a 0.8 μm diameter cell. Most of the bacteria were typical gram negative procaryotes, and ranged in size from 0.2 μm specimens up to 40 μm spirochetes. The bacteria we enumerated were in good ultrastructural condition and were found both freely dispersed in the marine snow matrix and in fecal pellets. Wall fragments and other debris of possible bacterial origin were also abundant, particularly in fecal pellets. Biomass and numbers of intact bacteria are given in Table 2.

Grey and olive-green bodies. The most numerically abundant forms seen on the light microscope on marine snow and in the water were grey to light green spheroids, of nanoplankton dimension (< 20 μm), usually bearing no flagellae, and with irregular internal features. We call these "grey bodies." The next most numerous forms were a variety of heavily pigmented, green to brownish spheroids and ovals, of very compact and characteristic appearance, and we call these "olive-green bodies." These latter appear to be identical with the "olive-green cells" described

from deep sea samples by Hentschel (1936) and Fournier (1970) and others. Observations by TEM showed a range of forms that overlapped those of grey and olive-green bodies in size and these amorphous spheroids were also the most abundant forms in sectioned material. Whereas color distinguished these two classes of spheroids on the light microscope, we could not distinguish between the two classes by electron microscopy. In section, the conglomerates ranged from completely amorphous granular or fibrous masses to dense accumulations of the same materials with an occasional wall (probably bacterial) inside, to concretions with recognizable picoplankton-sized cells or numerous disrupted lamellae inside (Figs. 3c-f, 4a,b). These bodies almost always lacked an external bounding membrane or wall, although some possessed a heavier, cortical-like external layer (Fig. 3c). Most could be distinguished from the usual fecal pellet on the basis of greater compaction, more amorphous (less recognizable) internal material, smaller size, and lack of an external membrane. However, in a few cases the distinction between these concretions and obvious fecal products (sometimes of spherical dimension) was unclear both on TEM (Fig. 4b) and on the light microscope.

The numbers and the sizes of the grey and green bodies are given in Tables 1 and 3. Values of the standard error (SE) are shown only for counts and not for biomass in the tables in this paper. For forms other than the picoplankton cells, sizes were quite variable and the various categories were thus routinely sized as they were counted. For the grey and green bodies and the fecal pellets, replicate counts of the same sample for biomass differed to a moderate extent due to both size differences and to numbers of the various items counted: SE for replicates averages + 40% of the mean for biomass. Thus small differences between the biomass of grey and green bodies or fecal pellets are most likely not significant, and these values are presented primarily to show the general magnitude of the contribution of the various categories.

Fecal pellets. Fecal pellets were abundant in marine snow. Material of obvious fecal origin, and occasionally fragments of large pellets, were also an important constituent and were sized during routine counts. The numbers and sizes are shown in Tables 1 and 3 and the carbon contribution of both pellets and other material of fecal origin is shown in Table 4. Because we had to vigorously disrupt the marine snow to count the associated particles, we may have broken some fecal pellets. However, many pellets still retained intact membranes, as judged by both light and electron microscopy. The intact pellets in the samples belonged almost entirely to very small size categories (Table 3) and included elongate pellets, spherical forms, and oval or intermediate shapes. When viewed on SEM and in TEM section, fecal pellets and fecal fragments contained abundant calcareous debris (coccoliths), organic scales of algae, wall fragments of many cells, several classes of procaryotic and eucaryotic cells (discussed above), olive-green and grey bodies, and much

Table 3. Relative abundance of various size classes for detrital materials on flocculent marine snow.

| Size Class | Grey bodies | | | | Olive green bodies | | | | Fecal pellets | | | | |
|------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|------------------------|----|
| | % of total numbers | | % of total volume | | % of total numbers | | % of total volume | | % of total numbers | | % of total volume | | |
| | East Cortez 1,000m | San Clemente 1,650m | East Cortez 1,000m | San Clemente 1,650m | East Cortez 1,000m | San Clemente 1,650m | East Cortez 1,000m | San Clemente 1,650m | East Cortez 1,000m | San Clemente 1,650m | East Cortez 1,000m | San Clemente 1,650m | |
| 0-4.9 | 90 | 76 | 25 | 10 | 20 | 23 | 1 | 1 | } | 41 | 13 | 1 | 0 |
| 5.0-9.9 m | 7 | 30 | 18 | 37 | 48 | 50 | 16 | 19 | | 29 | 32 | 1 | 3 |
| 10-19 m | 3 | 4 | 57 | 53 | 32 | 27 | 83 | 80 | | 24 | 42 | 91 | 45 |
| 20-49 m | — | — | — | — | — | — | — | — | 6 | 11 | 8 | 25 | |
| 50-99 m | — | — | — | — | — | — | — | — | 0 | 2 | 0 | 27 | |
| 100-200 m | — | — | — | — | — | — | — | — | | | | | |

Table 4. Carbon per ml of material associated with marine snow and in water surrounding marine snow.

| | Carbon (μg)/ml marine snow | | | Carbon (μg)/ml surrounding water | | |
|-----------------------|--|-----------------------------------|----------------------------------|--|---------------------------|--------------------------|
| | San Clemente 1,000m flakes | San Clemente 1,650m floc | East Cortez 1,000m floc | San Clemente 1,000m | San Clemente 1,650m | East Cortez 1,000m |
| grey bodies | 0.9 | 2.3 | 3.6 | 0.7×10^{-3} | 1.5×10^{-3} | 1.2×10^{-3} |
| olive green bodies | 0.2 | 2.0 | 3.0 | 0.6×10^{-3} | 0.9×10^{-3} | 1.8×10^{-3} |
| diatoms | 0.4 | 0.1 | 0.3 | 0.0 | 0.1×10^{-3} | 0.3×10^{-3} |
| fecal pellets | 3.8 | 6.8 | 7.8 | 1.2×10^{-3} | 3.4×10^{-3} | 1.2×10^{-3} |
| fecal matter | 2.5 | 2.5 | 1.8 | 1.2×10^{-3} | 1.2×10^{-3} | 0.9×10^{-3} |
| Total | 7.8 | 13.7 | 16.5 | 2.5×10^{-3} | 7.1×10^{-3} | 4.2×10^{-3} |

amorphous organic matter (Fig. 4c). Although pellets of all sizes contained intact, healthy-appearing cells on occasion, the small pellets most consistently bore these cells.

d. Marine snow vs. water samples. The materials in marine snow and those free in the water column appeared to be quite similar. However, numbers and biomass of particles on both flakes and floc generally exceeded that in the surrounding water by at least three orders of magnitude (Table 1, 4). Moreover flakes and flocs contained approximately the same number of associated organisms, on the basis of numbers of associated particles per ml of marine snow, but the smaller dimensions of the flakes resulted in smaller absolute numbers of associated particles. Light microscopy examination indicated that cells associated with snow appeared to be less poorly disintegrated, most notably in the 1000 m floc sample, suggesting that the organisms on marine snow were more recently derived from the surface than those freely dispersed in the surrounding water. Since we did not have sufficient volumes of water to collect large numbers of cells for examination by TEM, we were unable to assess the ultrastructural condition of the dispersed cells or to calculate abundance of heterotrophic bacteria and pico-plankton-sized algae in the water samples.

e. Marine snow sinking rates. The laboratory measured sinking rates of mucus originating as discarded larvacean houses are shown in Table 5. Houses tested at 5°C were slightly larger than those tested at 15°C and thus had a slightly higher sinking rate. These differences, however, are not statistically significant. The larvacean houses from our deep sea samples were part of a larger floc sample, and thus the sinking rates presented in Table 5 are the minimum expected for the larger specimens of marine snow in the field.

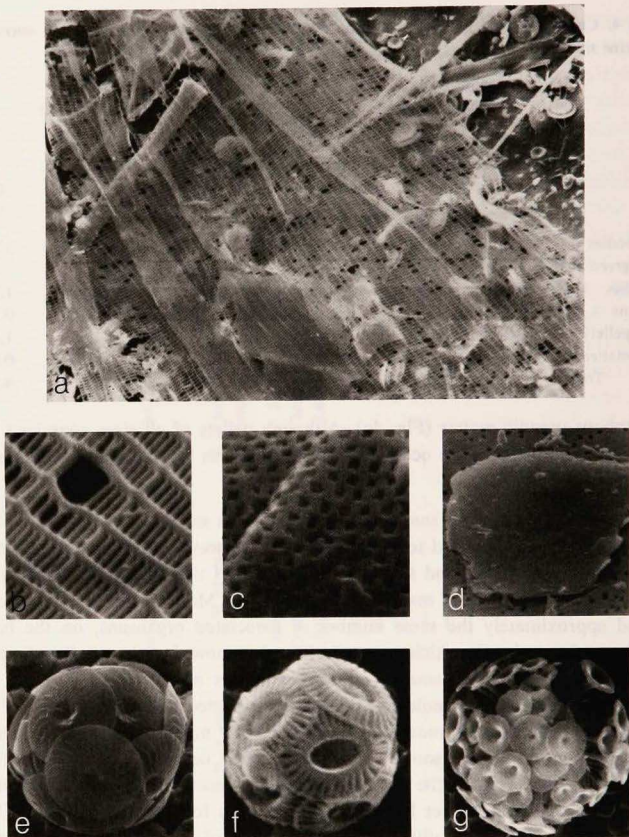


Figure 1. Bathypelagic aggregates and associated cells. Scanning electron micrographs. a) Filter mesh of marine snow from San Clemente Basin, 1650 m. Mesh is a portion of the feeding filter of a larvacean, and lies over phytoplankton debris. Magnification 1100 \times . b) Higher magnification view of 2a filter structure. Average mesh opening $0.06 \times 0.38 \mu\text{m}$. Magnification 17,000 \times . c) Unidentified mesh from 1650 m marine snow sample, San Clemente Basin. Mesh opening $0.15 \mu\text{m}$. Magnification 16,500 \times . d) Flake (clay particle) from 1000 m sample, San Clemente Basin, $12 \times 16 \mu\text{m}$. Magnification 2,500 \times . e) Coccolithophorid, *Cyclococcolithus leptopora*, 1650 m. San Clemente Basin, 1650 m. Specimen $10 \mu\text{m}$ diameter. Magnification 4,200 \times . f) Coccolithophorid, *Emiliani huxleyi*, 1650 m. San Clemente Basin sample. Specimen diameter $4 \mu\text{m}$. Magnification 9,300 \times . g) Coccolithophorid, *Discosphaera tubifera*, 1650 m sample, San Clemente Basin, specimen diameter $13 \mu\text{m}$. Magnification 3,000 \times .

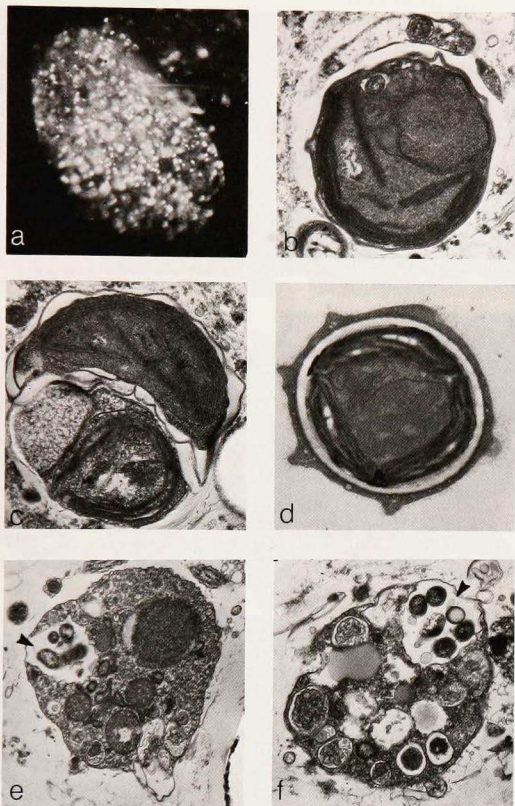


Figure 2. Cells associated with bathypelagic marine snow, 1650 m, San Clemente Basin. All except 3a are transmission electron micrographs. a) Fecal pellet with fluorescing phytoplankton cells: smallest cells are orange, larger ones are red auto-fluorescing. Green (546 nm) excitation filter, orange (580 nm) barrier filter. Pellet length 98 μm , light micrograph. Magnification 600 \times . b) Coccoidal alga, possibly *Chlorella*, inside fecal pellet. Cell diameter 1.3 μm . Magnification 37,100 \times . c) Two algal cells similar to specimen in 3b, in position suggesting recent cell division, inside fecal pellet. Couplet diameter 1.7 μm . Magnification 32,400 \times . d) Green algal cell. Protoplast diameter 2.7 μm , total diameter of walled cell, 3.3 μm . Magnification 15,200 \times . e) Protozoan cell, arrow pointing to vacuole enclosing bacteria. Protoplast width 2.9 μm . Magnification 16,200 \times . f) Protozoan cell, arrow pointing to vacuole with numerous bacteria inside, additional vacuoles showing advanced stages of digestion. Cell 2.4 \times 2.8 μm . Magnification 20,000 \times .

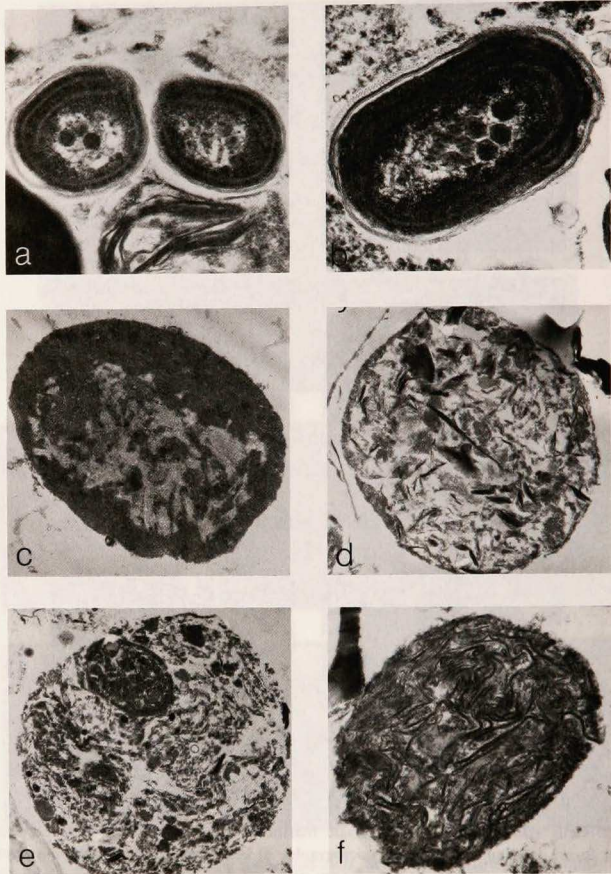


Figure 3. Cells and particles from marine snow. All from 1650 m sample, San Clemente Basin except 4a, from 1000 m sample in East Cortez Basin. Transmission electron micrographs. a) Cyanobacterial pair, possibly after cell division, inside fecal pellet. Cell diameter $0.8 \mu\text{m}$. Magnification $32,400\times$. b) Cyanobacterial cell, showing lamellae and polyhedral bodies, specimen inside fecal pellet. Cell $0.7 \times 1.2 \mu\text{m}$, magnification $47,900\times$. c) Olive-green or grey body. Size $2.2 \times 2.9 \mu\text{m}$. Magnification $20,000\times$. d) Olive-green or grey body. Size $3.7 \times 4.1 \mu\text{m}$. Magnification $13,400\times$. e) Olive-green or grey body enclosing another grey or green body. Diameter $6.7 \mu\text{m}$, magnification $8,100\times$. f) Olive-green or grey body with multiple lamellae inside. $1.4 \times 1.7 \mu\text{m}$. Magnification $32,100\times$.

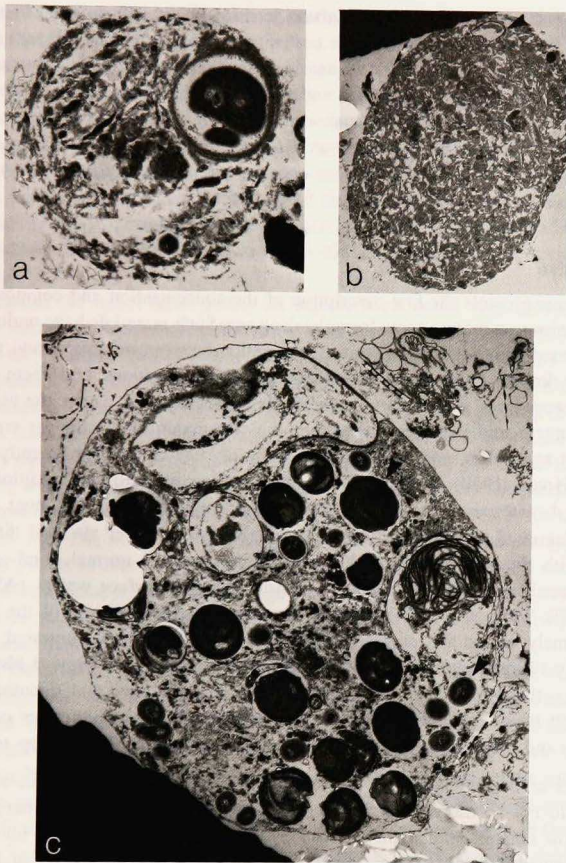


Figure 4. Waste products from marine snow sample, 1650 m, San Clemente Basin. Transmission electron micrographs. a) Olive-green or grey body, containing partially degraded algal cell. Body $6.1 \mu\text{m}$. Magnification $9,000\times$. b) Olive-green or grey body, or fecal pellet, enclosing cell wall (arrow) and small, intact bacterial cell (lower left). Specimen $7.4 \times 13.7 \mu\text{m}$. Magnification $4,300\times$. c) Fecal-pellet with enclosed *Chlorella*-like cells (example: upper arrow) and cyanobacteria (example: lower arrow). Additional, intact bacterial cells and degrading cell fragments in section. (Circular, white portions result from loss of material during sectioning of pellet.) Pellet dimensions $10.4 \times 12.6 \mu\text{m}$. Magnification $8,200\times$.

Table 5. Sinking rates (\pm SE) of larvacean houses (*Oikopleura dioica*) determined in the laboratory at two temperatures.

| Temp. (C°) | Specific gravity of seawater | Mean house diameter(mm) | Mean house volume(mm ³) | Sinking rate (m/day) | n |
|------------|------------------------------|-------------------------|-------------------------------------|----------------------|----|
| 16 | 1.0248 | 1.4(\pm 0.1) | 1.72(\pm 0.19) | 57.0(\pm 3.5) | 18 |
| 5 | 1.0270 | 1.5(\pm 0.1) | 1.87(\pm 0.29) | 64.6(\pm 5.8) | 9 |

4. Discussion

This paper presents the first description of the concentration and composition of microorganisms on marine snow from the deep sea. Such materials have undoubtedly been collected before, but the extent of association between organisms and particles cannot be known from standard bottle or pump collections and from normal handling procedures, which disrupt the fragile aggregates and scatter the associated microorganisms and particles throughout the water samples. Sediment traps also can collect aggregates, and similar materials have been recognized recently in trap samples (Honjo, 1980). However, aggregates may continue to gain additional cells and detrital materials after entering traps, or alternatively, may fragment, making the true degree of association between marine snow and other material difficult to assess. With the submersible ALVIN, we have extended normal hand-collecting methods used previously in studies of marine snow in surface waters (Alldredge, 1976, 1979; Trent *et al.*, 1978) to the deep sea. Because our use of the ALVIN was extremely limited and our sampling methods were previously untested, we collected only 10 aggregates at 3 locations. However, by using an array of histological procedures, this preliminary study yielded detailed qualitative and quantitative results which may significantly alter our perceptions of the communities of microorganisms and the nature and roles of enriched microhabitats in the deep sea.

a. Sources of marine snow and associated materials. The origin of most marine snow could not be determined from either the submersible or preliminary inspection by low magnification light microscopy. Examination by electron microscopy, however, showed that larvacean houses constituted part of the matrix of both floc samples and that additional unidentifiable structures were also present. The herbivorous larvaceans are characteristically found in the euphotic zone, but Barham (1979) reported large numbers of giant larvaceans inside their houses in the lower euphotic and upper mesopelagic zones (to 375 m depths) and thus houses can originate below the euphotic zone. Since the marine snow at depth contained numerous alga cells, such as the pennate diatoms, that often were not associated with fecal pellets, we have tentatively assumed these specimens of marine snow to have originated from the euphotic zone.

A comparison of the sinking rates of marine snow, fecal pellets, and algal cells suggests that the matrix of marine snow was a few weeks old, the associated fecal pellets were days to months in age, and many of the associated algae and other euphotic zone forms were in the aphotic zone for weeks or months. Likely sinking rates for amorphous marine snow are between 50-100 m/day (Alldredge, 1979; Shanks and Trent, 1980), and for larvacean houses are about 50 m/day (this paper). The age of the 1000 m and 1650 m marine snow matrix, if derived from the ocean surface, then, would be a few weeks: 20-23 days for marine snow sinking rates of 50 m/day, or 10-16 days for rates of 100 m/day. (If the houses derive from specimens such as those seen by Barham at 50-400 m, the ages are reduced by only 1-8 days.)

The age of the intact fecal pellets on marine snow could vary greatly. Sinking rates for individual intact fecal pellet in our samples are mostly less than 50 m/day, and for the dominant pellet size classes (5-50 μm) (see Table 3) are under 15 m/day (sinking rate calculated from regression of Small *et al.*, 1979). If the pellets were produced by zooplankton on marine snow or scavenged in deep water, they could be fresh (a few days old). If the pellets were scavenged in surface water by the more rapidly sinking snow, they would be approximately the age of the marine snow, i.e., a few weeks. If the pellets sank from surface waters independently, and just encountered marine snow at depth, they would be older, perhaps several months in age. The presence of intact pellet membranes, which were common on the small pellets in our samples, once was thought to indicate pellet youth. Now, some pellets at least a few weeks in age have been shown to possess undegraded membranes and certainly pellets released in colder waters below the mixed layer can maintain membranes over even longer periods (Small *et al.*, 1979).

Algae found as single cells on marine snow, and particularly those that do not survive digestion and thus cannot accumulate from fecal sources, must become associated with marine snow in the euphotic zone, because of their low sinking rates. Algal cells of the sizes that characterize our samples sink a few m/day or less (Smayda, 1971; Bienfang, 1980). The algae in our sample thus may be the same age as marine snow, or several weeks. The cells in small pellets could be no younger than several weeks, if they were packaged into these pellets in the euphotic zone, i.e., association with marine snow would give the most rapid sinking rates. Alternatively, the algae could already have been surviving or growing heterotrophically in the deep sea and consumed at depth. The age of such pellets and their enclosed algae could vary greatly. In both cases, the algae have spent considerable periods in the aphotic zone, and their intact condition attests to the likelihood that they are heterotrophs in the deep sea and that many are digestion-resistant cells.

b. Role in flux processes. Fecal pellets are presently regarded as the major agent controlling the flux of biogenic materials through the water column (McCave, 1975;

Bishop *et al.*, 1977; Turner and Ferrante, 1979). Marine snow also may play a role by accelerating the descent of waste materials that previously were thought to have sinking rates too low to contribute much to the overall flux. The predominant waste particles on marine snow are small, including fecal pellets, fragmented fecal materials, and the grey and olive-green bodies. Sinking rates of all classes appear to be considerably lower than that of marine snow, suggesting that the sticky marine snow has scavenged the wastes from the water column during descent, or that some of these were formed by organisms on the marine snow. Paffenhoffer and Knowles (1979) have noted that much greater quantities of small pellets are produced than the larger, better known ones considered in most models of particulate flux. They suggest that the very low sinking rates of pellets produced by small organisms, especially larval stages, will not allow these to penetrate to the deep sea, but that these serve as food resources for the euphotic zone. The olive-green bodies are ubiquitous and abundant cell forms of the deep sea (Fournier, 1970), and if they are waste products—which we discuss below—they are another important input of degrading organic material to the marine snow system. Presence of all of these waste products on marine snow indicates that their sinking rates are enhanced. Since the mass flux of particles is proportional to the product of the sinking rate and the mass, such an association indicates that the marine snow significantly increases the downward flux of these abundant, but otherwise slowly sinking materials. The dominance of waste products of such small size on marine snow shown in the present study and a previous one (Silver *et al.*, 1978) indicates that these wastes should, perhaps, be reconsidered as participants in the downward transport of materials, because of their association with marine snow.

c. Deep sea algae. Although the existence of algae in the aphotic zone has been noted previously (Wood, 1956; Bernard, 1963, 1967; Hamilton *et al.*, 1968; Fournier, 1970; Smayda, 1971; Malone *et al.*, 1973; Wiebe *et al.*, 1974), the abundance of healthy-appearing, ultrastructurally-intact algae, both procaryotic and eucaryotic, was one of the most unexpected of our laboratory observations on bathypelagic marine snow. The numerically dominant forms were ones that occurred abundantly in fecal pellets, were small (under 5 μm), and thus difficult to recognize by light microscopy, and had conspicuously thickened or mucilage covered (or both) cells walls. [We are presently characterizing the most abundant of these, or the *Chlorella*-like cell, and will describe this elsewhere (Silver and Alldredge, in prep.)] Diatoms were an important exception: intact specimens were generally 5-50 μm and occurred relatively infrequently in fecal material. These observations suggest that healthy-appearing algae reach the deep sea via two routes. First, the deep sea is inoculated with digestion-resistant algae from the feces of herbivores; these algae have structural features (i.e., wall morphology) consistent with digestion resistance, and are relatively poorly known, undoubtedly due to their

lack of mineralized walls or scales, and to their minute size. The green algae and cyanobacteria are represented within this group. The second class of deep sea algae encompass a larger range of sizes, and consist of forms that most likely sink into the deep sea on marine snow or non-fecal detritus, since they are not conspicuously digestion resistant. Many diatoms may belong to this second class of algae.

Although there are numerous allusions to the existence of digestion resistance in the literature on marine phytoplankton, the overall importance of such forms in the sea has apparently not been documented previously. Hargraves and French (1975) suggested that resting spores may be digestion-resistant stages in the life history of diatoms. Pomeroy and Deibel (1980) note the presence of abundant, autofluorescing phytoplankton in fecal pellets of salps and doliolids, and Silver (unpub. obs.) has noted live pennate diatoms gliding from disrupted, freshly collected salp pellets. Recently Urérre and Knauer (1981) noted the presence of many phytoplankton cells (some with contents, as judged by light microscopy; Urérre, pers. commun.) in fecal pellets collected from the deep sea and comment on the potential importance of fecal microcosms as deep-sea food sources. In the present study, we show that ultrastructurally intact autotrophs are an important fraction of the total biomass on deep-sea marine snow, even within the smallest (picoplankton) size fraction, and that the overall biomass of algal forms here greatly exceeds that of the bacteria.

In contrast to the situation for marine planktonic communities, Porter (1973) has shown the importance of digestion resistance in freshwater phytoplankton assemblages, suggesting such algae may be the numerical dominants when grazing pressure is intense. Porter (1974) showed that some freshwater species, including green algae, not only survive digestion, but actually absorb nutrients while passing through the herbivore gut, dividing soon after release in the feces. We have preliminary evidence for division after gut passage, from fluorescing couplets of cyanobacteria and from the presence of both dividing cyanobacteria and picoplankton sized eucaryotic algae in fecal pellets from bathypelagic marine snow. We have also begun to accumulate data from other samples that show digestion resistance in algal forms ultrastructurally identical to the ones discussed in this paper. In particular, we have noted intact eucaryotic cells identical to the *Chlorella*-like cells and the cyanobacteria in fecal material from salps and pteropods obtained from surface waters of the California Current (Silver and Bruland, 1981). We have also seen evidence for cyanobacterial growth in natural, mixed fecal pellets from the Monterey Bay area: freshly collected fecal pellets show significantly greater proportions of paired cells of fluorescing cyanobacteria than both water samples and samples of marine snow (excluding fecal pellets in the snow), and the frequency of paired couplets increases with incubation of the fecal pellets (Silver, unpub. observation).

Although previous studies have shown that algae can be cultured from deep-sea

innocula (Hamilton *et al.*, 1968; Malone *et al.*, 1973), and our own observations show the presence of many healthy-appearing cells here, the ability of algae to survive heterotrophically in the dark, and certainly to grow in darkness, has been much debated in the literature. Heterotrophic growth capability has been demonstrated in some algae, and dark survival in many more, but convincing arguments are rarely presented to relate these results to algal distributions or the nutritional regime of the water column (Droop, 1974; but see Vincent and Goldman, 1980; Wiebe *et al.*, 1974). We suggest that previous studies do not adequately test for heterotrophy by using species that actually occur in the deep sea, or by simulating conditions of the marine snow or fecal pellet microenvironments. In the first place, the abundant forms of the deep ocean are a rather unique assemblage of survivors, and are not generally representative of the better known surface phytoplankton, except for some diatoms. Secondly, conditions representative of the high nutrient environments characteristic of marine snow or fecal pellets (Silver *et al.*, 1978; Shanks and Trent, 1979; Silver and Bruland, 1981) have not been provided in the laboratory.

d. Detrital communities of marine snow in the deep sea. Pomeroy and Johannes (1968) first suggested that aggregates could be metabolic centers in the deep sea. Our results indicate that the deep-sea community associated with marine snow represents a microbial food web, with internal energy reserves. First, the abundant bacteria utilize dissolved organic material (DOM), much of which may be derived from degradation of materials in the detritus. Second, the algae do not appear to be phagotrophs (i.e., do not contain vacuoles containing particulates), and thus they most likely are using DOM, if alive. Third, protozoans, or eucaryotic unicells lacking chloroplasts frequently did contain vacuoles with particulates, including both structural photoautotrophs and bacteria commonly present in this habitat. These protozoans were often small ($< 5 \mu\text{m}$), and their relatively frequent occurrences in our samples suggests they are important consumers of the first trophic level here, or the DOM utilizers. The importance of such small protozoa in communities of the euphotic zone has become evident in recent years (Sieburth *et al.*, 1978; Haas and Webb, 1979; Silver *et al.*, 1980), and such organisms may be major consumers in the deep-sea detrital systems as well. Deep ocean communities, however, are normally envisioned as encompassing larger organisms, either living plankton and nekton in a "ladder of migration," or detrital-feeding metazoa, which utilize non-living material raining from overlying layers. The results of our study suggest that food chains in the deep sea may consist of non-living detrital sources with bacterial and algal forms as primary converters of the non-living material, and that these fall prey to μm -sized consumers as well as to large particle feeders, just as do the nanoplankton autotrophs and bacterial primary producers of the surface ocean.

Although the community that inhabits deep-sea marine snow consists primarily of μm -sized organisms, the detrital matrix has dimensions of mm to cm (or perhaps

Table 6. Total content of floc particles.

| | Total numbers | | Total carbon μg C | |
|--------------------------------------|----------------------------|---------------------------|----------------------|--------------------|
| | San Clemente | East Cortez | 1,650m | 1,000m |
| | 1,650m (20ml floc) | 1,000m (0.25ml floc) | 1,650m (20 ml) | 1,000m (0.25ml) |
| total picoplankton | 2.0X10 ⁵ | 1.2X10 ⁵ | 0.8 | 0.0 |
| grey bodies | 169(±36)X10 ⁴ | 6.4(±0.8)X10 ⁴ | 46.4 | 0.9 |
| olive green bodies | 14.1(±4.3)X10 ⁴ | 2.3(±1.0)X10 ⁴ | 39.2 | 0.8 |
| diatoms* | 10.0(±3.7)X10 ⁴ | 2.0(±0.8)X10 ⁴ | 1.9 | 0.1 |
| silicoflagellates and radiolaria* | 10.8(±5.0)X10 ³ | 1.7(±0.9)X10 ³ | 0.0 | 0.0 |
| fecal pellets | 4.7(±1.1)X10 ⁴ | 2.5(±0.4)X10 ³ | 47.7 | 3.4 |
| fecal matter** | — | — | 2.3 | 2.2 |
| Total | | | 138.3 | 7.4 |

* Carbon content is calculated by assuming that only those forms that were clearly seen on the light microscope to contain protoplasm actually contributed carbon. Thus the total numbers represent all frustules seen, but the carbon values correct for empty frustules. (TEM micrographs indicate many frustules that were probably considered "empty" by light microscopes, still contained fragments of protoplasm, and the carbon values here, therefore, are conservative.)

** Counts of fecal matter are not given because the material is amorphous and was measured as equivalent spherical volumes, which are not readily converted to counts. Carbon content was calculated on the basis of dimensions of the fecal matter.

m in some cases). Thus marine snow can serve as a food item to organisms that require large particles and also to small browsers that remove individual items from the matrix (see Alldredge, 1972; Silver *et al.*, 1978). Larger consumers may include fish, such as Myctophids, which contain marine snow-like materials (B. Robinson, pers. commun.) and tunicates, which contain large quantities of the common constituents of marine snow (Fournier, 1973). Browsers may include copepods, which also contain common constituents of marine snow (Harding, 1974). The consumption of marine snow at depth should be accompanied by the formation of fresh fecal pellets in deep water. Such secondary repackaging, or the formation of fresh pellets containing reworked deep-sea detritus, has been noted in several previous studies (Honjo, 1978; Urérre and Knauer, 1981), and marine snow could well prove to be a likely food source for the detritivores producing these fecal products at depth.

The food value of marine snow is difficult to evaluate. The high concentrations of organisms and fecal particles on marine snow indicate that the carbon content of this system (Table 6) is at least 3 orders of magnitude higher than the average value for deep water (3-10 μg Poc/1; Menzel, 1974). That is, the carbon content of 1 ml of marine snow is equivalent to that of one liter of seawater. Moreover, this carbon estimate for snow is conservative, since we did not include the value of the matrix itself nor the many amorphous particles associated with it: Alldredge

(1976) demonstrated μg quantities of carbon in larvacean houses and Gordon (1970) showed that flakes from deep water (a part of marine snow) are proteinaceous. However, we have noted that the most characteristic constituents of marine snow are digestion resistant forms, grey and green bodies (see below), and fecal pellets, all materials refractory to digestion. Thus, although the carbon value of these systems may be high, the nature of the materials in marine snow indicates a relatively small proportion of the carbon may actually be available for assimilation by detritivores. Alternatively, the presence of such carbon-rich systems in the deep sea may have resulted in the evolution of especially efficient digestive systems (perhaps by incorporating a microbial flora) in detritivores. In the euphotic zone with its populations dominated by more readily digested prey, such adaptations might not be cost-effective; in the more food-limited environment of the deep-sea, with the majority of potential food items in refractory materials, more efficient assimilatory mechanisms would be of considerable selective value.

e. Grey and olive-green bodies. One of the most enigmatic and characteristic particles known from the deep sea are the olive-green "cells." We suggest that most of these are waste products. The pigmented "cells" have been noted by many other workers, who described them as olive-green cells (Hentschel, 1936; Fournier, 1970), yellow-green cells (Schiller, 1931), or yellow cells (Fryxell *et al.*, 1979). These forms are generally absent or infrequent in surface samples, increase to a mid-depth maximum at a few hundred m, and then gradually decrease in numbers, averaging 10^2 to $10^4/1$, depending on techniques used for collection (Hentschel, 1936; Hamilton *et al.*, 1968; Fournier, 1971; Fryxell *et al.*, 1979). An examination of the original descriptions of these pigmented "cells," plus our own observations suggests that two different types of materials have been included: true photosynthetic cells, and the more enigmatic, amorphous concretions. We discuss these two types next.

The photosynthetic cells probably include members of a variety of algal taxa. These have not been readily recognized because of their small size, the often poor condition of specimens after retrieval from the deep-sea, and the relatively low-resolution optics that resulted from the use of the inverted microscope or previous filter-clearing methods. In our specimens, the eucaryotic algae, which were generally $< 5 \mu\text{m}$, frequently contained chloroplasts that occupied a large proportion of the cell volume, the chloroplasts were not readily distinguishable as separate organelles except by TEM or by careful study with Nomarski optics, and thus the cells could easily be considered to be featureless, colored spheres. The deep-sea cyanobacteria are an especially difficult group, because of their very small size (usually $< 1.5 \mu\text{m}$ in our samples); in fact similar forms have only been recognized very recently in surface waters, although they are exceedingly abundant there also (Johnson and Sieburth, 1979; Waterbury *et al.*, 1979).

The second type of pigmented "cell," or the amorphous bodies, constitute the

largest fraction in deep-sea samples (this study; Fournier, 1970). Because of the presence of pigment and lack of internal structure, these forms have frequently been assigned to the bluegreen algae (= cyanobacteria) (Fournier, 1970). The lack of cell membranes and walls, together with the lack of cytoplasmic-nucleoplasmic differentiation typical of bacteria (Fig. 3c-f, 4a,b, this paper; Type I cell: Fournier, 1970), however, indicates these cannot be procaryotes, and the lack of all internal structure shows these surely are not normal eucaryotes. Recently, Silver and Bruland (1981) found large numbers of these same forms in surface herbivores and suggested these are breakdown products of algae, resulting from gut passage through metazoans. The wide variety of forms seen in the deep-sea material here suggest that a number of additional processes may also be responsible for producing the pigmented cells.

Ultrastructural examination of the amorphous bodies showed a range of forms from minute, 1 μm concentrations, to somewhat larger bodies occasionally containing intact cells or cell walls, to 20 μm bodies that may have been highly compacted fecal pellets. At least 4 different processes could produce the grey and pigmented bodies seen in the present study. First, these could be autolyzed cells, i.e., ones that had died and whose internal organelles had undergone the normal enzymitic breakdown following cell death. Anderson (1975a,b) suggested the pigmented "cells" were diatom products that were in advanced stages of senescence (but probably still alive); however even in such advanced stages in living cells, cell walls and membranes are still intact and some internal structure is maintained (Palisano and Walne, 1972; Gomez *et al.*, 1974), features not present in the deep-sea specimens. Second, the bodies could be the remnants of single cells that have passed through the gut of a metazoan, since identical forms occur abundantly in feces (Silver and Bruland, 1981). Thirdly, the pigmented "cells" could be the discharged vacuole contents of protozoan grazers. Wastes from protozoans lack a membrane (Allen, 1978), and such unicellular forms were present on marine snow. Fourth, these could be the highly condensed fecal pellets of small metazoa, particularly crustacean larvae (see Paffenhoffer and Knowles, 1979), which are known to occur at these depths (Ur erre and Knauer, 1981). Since all 4 of these waste categories are produced by digestion of cellular materials, the end products may be similar in appearance and thus difficult to distinguish. Moreover, ultrastructural descriptions of all but the second type of waste bodies are unavailable from marine systems, and thus the origin of the pigmented "cells" from such additional sources await evaluation.

5. Conclusions

1. Marine snow was conspicuous at all depths to the bottom during the dives in the Southern California Borderland. Concentrated layers of 10 to nearly 100 m

vertical thickness occurred at several depths. Larvacean houses constituted some of the flocculent aggregates, but the source of most of the other marine snow was not evident from the submersible.

2. The aggregates consisted most frequently of "flakes," whose origin is unknown. Flocculent, or mucous-like aggregates were larger but less common. Mucous sheets were evident in this second type of aggregates, and larvacean feeding filters were found in both samples of these aggregates. Additional filter-like structures were also encountered in these, but the origin of these other structures is presently unknown.

3. Sinking rates of abandoned, field-collected larvacean houses, a likely source of the deep-water aggregates, were approximately 50-100 m/day. If the houses in the bathypelagic marine snow samples originated from the euphotic zone, these sinking rates predicate house ages to be several weeks.

4. The contents of the aggregates consisted of much fecal detritus, empty skeletons and wall materials of algae and protozoa, and numerous healthy-appearing bacteria, algae, and protozoa.

5. Sizes of intact fecal pellets on the marine snow were quite small, averaging less than 50 μm in length. Sinking rates of such pellets indicate that they settle more slowly than does marine snow. Thus the small pellets were either scavenged by the marine snow during descent or were produced on marine snow. In either case, the sinking rate of these pellets, and thus their mass flux, is increased by their association with marine snow.

6. The most abundant intact cells on marine snow were the heterotrophic bacteria. These number at least $10^5/\text{ml}$ but their biomass is a minor fraction of the total on the aggregate, due to their small size. However, their biomass accounts for 44% and 69% of the picoplankton total for the shallower and deeper floc samples, respectively. The remainder of the living biomass in this size category belongs to forms that are structurally photoautotrophs.

7. The most abundant structural photoautotrophs in the deep sea are coccoidal cyanobacteria, which number approximately $10^4/\text{ml}$, and a small coccoidal eucaryote, probably a green alga, with similar abundance. Both cell types occur frequently in fecal material, appear structurally intact inside the fecal pellets, and show occasional evidence of division there. These forms appear to be inoculated into the deep sea via the feces of herbivores.

8. Diatoms and coccolithophorids were abundant larger algal forms on marine snow, both numbering 10^3 - 10^4 cells/ml. However, most of the coccolithophorids are empty, though the coccospheres were intact and unetched. Moderate numbers

of the diatom frustules, especially small pennates, contained intact protoplasts, as observed both by light microscopy and transmission electron microscopy. The diatoms contribute the major biomass to the bathypelagic snow samples, because of the comparatively large size of the cells. Most of the diatoms were not in fecal material and probably reached depth directly on the marine snow.

9. The intact ultrastructural condition of many of the algae in marine snow suggests that they are metabolically active. Their volumetric importance in this habitat suggests, if alive, they may be major heterotrophs in the upper bathypelagic community. The role of these forms in deep sea communities awaits evaluation.

10. Olive-green bodies (= "cells") and similar, but unpigmented, grey bodies were volumetrically important constituents of marine snow. The origin of these remains unclear, but we suggest most of these are breakdown products resulting from a number of processes. Some may be remnants of algae digested by herbivores, or cells that have undergone autolysis. Others may be egested vacuolar residues of protozoans or fecal pellets of small metazoans, as evidenced by their small sizes, lack of external walls or cell membranes, internal disorganization, and occasional presence of wall fragments or complete picoplankton-sized cells internally.

11. Protozoans were evident in many of the TEM sections and some of these contained picoplankton in various stages of digestion. The abundance of these forms suggest that protozoans may be important intermediaries in detrital food chains of the deep sea.

12. The carbon concentration of marine snow exceeds that in surrounding water by at least 3 orders of magnitude: we estimate, conservatively, that 1 ml of marine snow contains 8-16 μg C. Such particulates, especially the larger ones (such as the 20 ml aggregate), constitute sources of heterogeneity in measurement of carbon and associated biogenic materials in deep sea samples. Such particulates also offer potentially large food reserves to consumers that can utilize the detrital and cellular materials associated with marine snow.

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