Heterotrophic Bacteria and Bacterivorous Protozoa in Oceanic Macroaggregates

Abstract. *Oceanic macroaggregates (marine snow and* Rhizosolenia *mats) sampled from the Sargasso Sea are associated with bacterial and protozoan populations up to four orders of magnitude greater than those present in samples from the surrounding water. Filamentous, curved, and spiral bacteria constituted a higher proportion of the bacteria associated with the particles than were found among bacteria in the surrounding water. Protozoan populations were dominated numerically by heterotrophic microflagellates, but ciliates and amoebas were also observed. Macroaggregates are highly enriched heterotrophic microenvironments in the oceans and may be significant for the cycling of particulate organic matter in planktonic food chains.*

Macroscopic aggregates appear to be ubiquitous in the planktonic environment, where they provide enriched microenvironments of nutrients and organic carbon for both autotrophic and heterotrophic processes in the oligotrophic ocean (*1–3*). They may be nutritionally valuable for zooplankton and nekton grazing on them (*4, 5*) and are important in the flux of organic carbon to the deep sea (*3, 6, 7*). Despite qualitative visual observations of high concentrations of bacteria and protozoa on macroscopic aggregates (*8*), the potential importance of heterotrophic microorganisms in these microenvironments has received little attention (*3, 9*). We now report the results of a preliminary examination of marine snow (detrital aggregates of heterogeneous origin) and *Rhizosolenia* mats (*1, 10*) collected in the southern Sargasso Sea by divers. Bacteria and bacterivorous protozoa in these microcommunities occurred in densities 10 to 10^4 times that of the same populations in the surrounding water.

Samples of marine snow and *Rhizosolenia* mats were collected by divers from the surface to 25 m depth at three locations in the mid-Atlantic (Table 1) on R.V. *Atlantis* cruise 109-III (August 1981). *Rhizosolenia* mats are composed of two cohabiting species of this diatom genus (*1*) that form discrete, cohesive patches, approximately 2 to 5 cm in diameter, in the water. Marine snow may originate from various sources (*4, 11–13*). We have identified two sources of labile organic material that may give rise to marine snow in the oligotrophic ocean. The first are the mucus feeding webs made by thecosomatous pteropods to capture microzooplankton and phytoplankton (*14*). We observed numerous spherical or oval mucus bubbles, 1 to 4 cm in diameter, some of which had the pteropod *Creseis virgula* hanging beneath them. Where abandoned by the pteropods, these webs constitute a mass of organic carbon already inoculated with microorganisms. The second potential source of marine snow is suggested by our experience in collecting ctenophores (*15*). When disturbed, these animals slough sheetlike veils of mucus into the water. We do not yet know if this is a normal cleansing procedure, as in corals, or a response to stress, but the quantity of mucus released suggests that this material could be an important source of marine snow.

Sterile 20-ml plastic syringes were used to sample *Rhizosolenia* mats and marine snow so as to minimize contamination and reduce the volume of surrounding water while allowing collection of weakly attached microbial populations. Sample volumes of diatom mats and marine snow ranged from 0.4 to 5.8 ml. We could identify approximately half of the marine snow aggregates as abandoned pteropod mucus webs by their shape and texture; the rest of the aggregates were unidentifiable.

Table 1 gives the results of direct epifluorescence counts of bacteria, total nanoplankton (heterotrophic and photosynthetic flagellates) (*16*), and *Trichodesmium* spp. filaments and *Rhizosolenia* spp. frustules, and most probable number (MPN) cultural estimates for protozoa on marine snow and *Rhizosolenia* mat samples. Bacteria were counted by staining with 4′,6-diamidino-2-phenylindole (*17*) and total nanoplankton by staining with acridine orange (*16*). We estimate that in the initial collection, the aggregates were diluted 1.5 to 6 times with surrounding water and that population densities of microorganisms for aggregate samples are therefore slightly underestimated. Aggregate volume is probably a poorer measure of the actual size of the microbial habitat than is total surface area of the aggregate. Unfortunately, the convoluted structure of the aggregates makes the latter parameter impossible to measure.

Total bacterial densities in the aggregate samples exceeded densities of free bacteria by two to more than 100 times (without correcting for aggregate dilution by surrounding water), averaging an 18-fold enrichment over bacterioplankton concentration (*18*). Aggregate and surrounding water samples also differed in the distribution of the morphological types of bacteria. Noncoccoid forms (rods, spirals, filaments, and curved rods) constituted 54 percent of the bacteria in the aggregate samples, but only 21

Table 1. Direct microscopic counts (epifluorescence) and most probable number (MPN) cultural estimates of the microorganisms present on macroaggregates sampled at three stations in the southern Sargasso Sea (24°30′N, 55°07′W; 24°30′N, 62°02′W; 24°30′N, 68°33′W). Controls (surrounding water) were collected at 1 m and at 35 m in acid-rinsed (1*N* HCl) Niskin bottles. UN indicates that no organisms were observed at the lowest dilution.

Number of samples	Parameter	Direct epifluorescence (counts per milliliter)						MPN estimates (per milliliter)		
		Bacteria ($\times 10^6$)			Nanoplankton ($\times 10^3$)	*Trichodesmium* filaments	*Rhizosolenia*	Flagellates	Amoebas	Ciliates
		Total	Cocci	Noncocci						
					Marine snow					
16	Range	0.66–32.79	0.32–18.28	0.30–14.51	4.1–127.8	97.1–13,760	UN–107.9	3–2,400	UN–23	UN–23
	Average	5.64	2.59	3.05	21.3	1,792.2	15.8	742.6	2.6	2.9
					Rhizosolenia *mats*					
2	Range	2.12–19.30	0.97–12.00	1.15–7.30	28.3–285.7	UN–5.4	992.9–11,386	240–6,200	UN	UN
	Average	10.71	6.48	4.22	157.0	2.7	6,414.4	3,220	UN	UN
					Controls					
5	Range	0.29–0.32	0.24–0.25	0.05–0.08	0.42–1.07	UN	UN	0.02–0.61	UN–0.31	UN
	Average	0.31	0.24	0.06	0.76	UN	UN	0.23	0.01	UN

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percent of the bacterioplankton samples. The noncoccoid cells in the aggregates included many extremely long filamentous and spirochete-like bacteria never observed in the surrounding water (Fig. 1, A to C). Therefore, numerical differences between bacterioplankton populations and bacterial populations on aggregates actually underestimated differences in bacterial biomass. As judged from bacterial volume estimates in aggregate and control samples, bacterial biomass would be, on average, 43 times greater than control for marine snow and 66 times for *Rhizosolenia* mats (*19*).

Previous estimates of bacterial concentrations on particles (including marine snow) have been relatively low—equal to or less than typical concentrations of bacteria from the water (*3, 9, 20, 21*). These low estimates may be due to a number of factors, including the age of the aggregates (that is, the stage of microbial colonization) or the amount of labile organic material initially present in the aggregate, but in some cases may be due to inaccuracies of the enumeration techniques (*22*).

Direct counts of total nanoplankton (2- to 20-μm cells) in the aggregates ranged from 4.1×10^3 to 285.7×10^3 cells per milliliter, exceeding the population in the surrounding water by five to more than 370 times. These populations contained both photosynthetic forms and heterotrophic protozoa, but samples were not examined quickly enough to determine the relative numbers of these subgroups (*23*). Larger phytoplankters consisted almost entirely of species of *Trichodesmium* or *Rhizosolenia*. *Trichodesmium* was present in high densities in all marine snow samples, whereas *Rhizosolenia* was observed in only two marine snow samples.

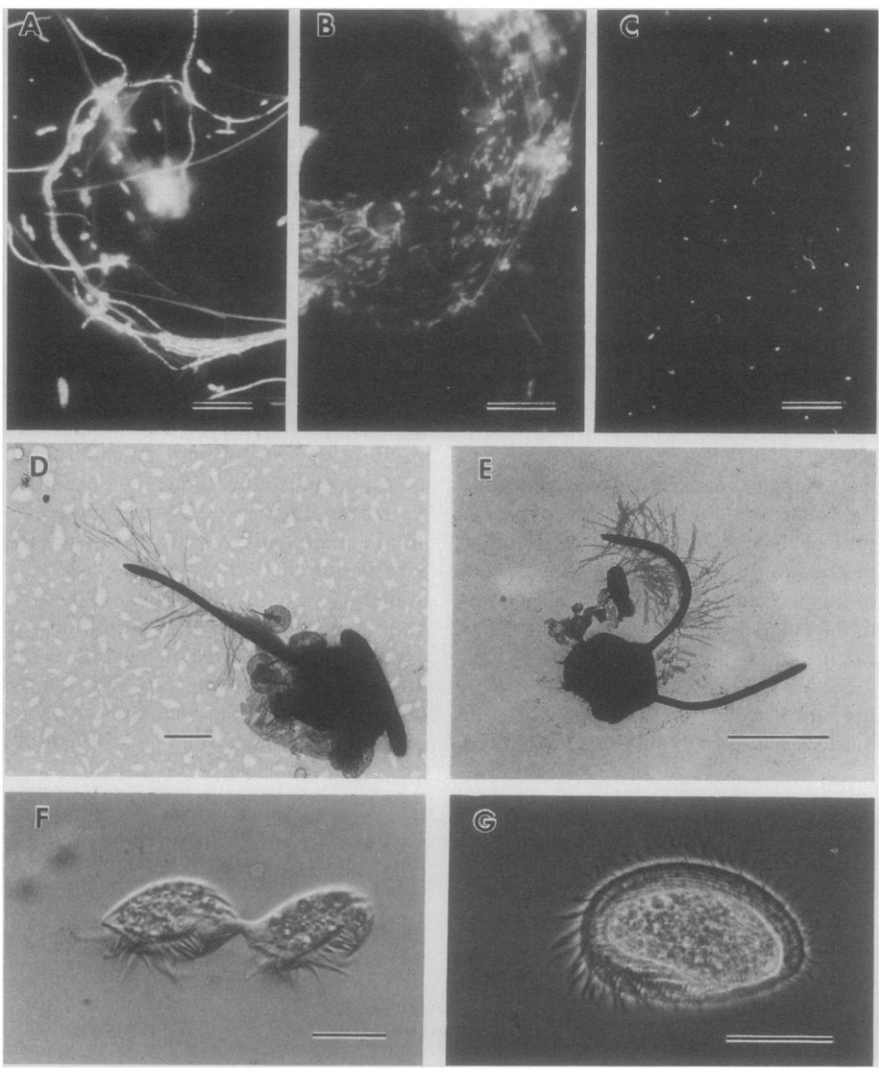

Fig. 1. Bacteria and protozoa associated with marine snow. (A to C) Epifluorescence photomicrographs of diamidinophenylindole-stained bacteria associated with marine snow (A and B) and free bacteria in the surrounding water (C). Scale bars, 10 μm. (D to G) Representative protozoa cultured from marine snow. Electron micrographs of ammonium molybdate–stained specimens of the flagellate *Paraphysomonas imperforata* (D) and an unidentified biflagellate (E) cultured from mucus of the ctenophore *Leucothea multicornis*. Scale bars, 1 μm. The ciliates *Euplotes* sp. (dividing form, differential interference microscopy) (F) and *Peritromus* sp. (phase-contrast microscopy) (G) cultured from marine snow. Scale bars, 50 μm.

Enumeration of protozoa was done by the MPN cultural technique (*24*). Bacterivorous flagellates were the most prevalent of the cultured protozoa. Approximately 50 percent of these were bodonids, but other small (less than 5 μm) mono- and biflagellates such as *Paraphysomonas imperforata* (Fig. 1, D and E) were present. Estimated flagellate density was highly variable (3 to 70,000 cells per milliliter), but was demonstrable in all samples. Numbers of heterotrophic flagellates from the macroaggregates exceeded the average MPN estimates of protozoa from the surrounding water by 1 to 4 orders of magnitude. Amoebas, primarily of the family Paramoebidae, and bacterivorous ciliates were less common, ranging from 3 to 23 cells per milliliter when present (*25*). Amoebas and ciliates were not always found in the culture with the lowest dilution (representing 0.1 ml of the initial aggregate sample). However, their numbers may still be greater than the reported values for bacterivorous ciliates and amoebas from free water samples (*24, 26*). Direct epifluorescence counts of total nanoplankton exceeded the cultural estimates of protozoa in all cases, presumably because of the presence of photosynthetic forms and of heterotrophic flagellates that did not grow in culture.

The different aggregate samples varied considerably in counts of bacteria, nanoplankton, and larger phytoplankton. This may be due partly to the degree of dilution of aggregates during collection, but differences in the microbial populations on the aggregates would also be expected because of differences in the composition and colonization histories of the aggregates.

Our preliminary investigation of the microbiological populations associated with marine snow and *Rhizosolenia* mats indicates that these aggregates can provide a microenvironment of elevated heterotrophic microbial activity. These populations may serve directly as a food source for surface-dwelling and deep-sea organisms that feed on or in the aggregates. Bacteria and protozoa may be more nutritionally important than the digestion-resistant phototrophs that also characterize marine snow (*3, 27*). Microbial activity may increase the overall nutritional value of the aggregates to grazers by reducing the carbon-to-nitro-

gen ratio or by converting relatively refractor organic compounds into digestible material or recyclable nutrients (*2*). Finally, high bacterial concentrations in macroaggregates may explain the occurrence of bacterivorous ciliates in an environment where bacterioplankton concentrations may be too low to support ciliate growth (*28*). Some species observed in this study are more typical of a benthic than a planktonic environment [for example, *Peritromus* (Heterotrichida) and *Euplotes* (Hypotrichida) (Fig. 1, F and G)]. Macroaggregates in the open ocean constitute microenvironments which, like the sea-air interface (*29*), are sites of elevated heterotrophic microbial activity in an otherwise oligotrophic environment.

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18. Bacterioplankton concentration averaged 3.06 $\times 10^5$ ml^{-1} (standard deviation, 0.13; $N = 5$). Samples were taken by Niskin bottles (5 liters) at depths of 1 m and 35 m.
19. Estimates were based on an average size of noncoccoid bacteria of 0.3 μm diameter and 1.0 μm length for aggregate samples, and 0.3 μm diameter and 0.6 μm length for controls. Volume was calculated as for a cylinder. Cocci in both aggregate and controls averaged 0.3 μm diameter and volume was calculated as for a sphere.
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22. Fixation and embedding for transmission electron microscopy (*3*) could result in the loss of bacteria weakly associated with the aggregates. Enumeration of colony-forming units (*9*) cannot distinguish bacteria attached to a single particle and also omits bacteria that will not grow on the culture media used.
23. Samples were examined approximately 12 weeks after sampling—sufficient time for some degradation of chlorophyll pigments.
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25. We cannot establish the absolute nature of bacterial predation for the cultured ciliates since they may have eaten heterotrophic flagellates growing in the cultures. However, subsequent clonal culture has established the adequacy of bacteria as a food source for some of the common species.
26. Averages for ten stations transecting the North Atlantic at 24°30′N latitude on R.V. *Atlantis* cruise 109-III were less than three cells per liter for amoebas and bacterivorous ciliates.
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30. Supported by NSF grants OCE80-24441 and OCE78-26388 and by the Woods Hole Oceanographic Institution education program. Ship support was provided by NSF grant OCE80-18514. We thank E. B. Small for ciliate identification. Contribution No. 5234 from the Woods Hole Oceanographic Institution.

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Aphasia and Speech Organization in Children

Abstract. *A long-standing controversy concerns whether lateralized cerebral specialization for speech and language is present at the time of language origins (developmental invariance) or whether it gradually develops from initial bilaterality (developmental progression). This controversy is complicated by conflicting reports of the incidence of childhood aphasia. The discrepancies are largely due to one early study. When methods for estimating speech organization distributions are applied to later studies, the developmental invariance position is supported.*

The rarity of speech disturbance following unilateral right-hemisphere brain damage provides conclusive evidence that the vast majority of right-handed adults are left-hemisphere dominant for speech. Evidence concerning the speech organization of children is much less clear, as some studies have reported a higher incidence of aphasia after right-sided lesions in children than in adults (*1*). On the basis of these reports, it is commonly believed that children are born with hemispheric equipotentiality for language and that lateralization occurs as the child matures (*2, 3*). If so, the lateralization seems to be complete by 5 years of age (*3*). There is considerable variability between studies, however, in the reported incidence of aphasia following right lesions. Not all of these studies support the developmental maturation position (*4*). Furthermore, electrophysiological, neuroanatomical, and behavioral data, which are at least as important as aphasia data for judging speech laterality in young children, do not support the developmental maturation position (*5*). Thus, several authors have adopted the developmental invariance position that speech organization in children is similar to that in adults and does not change with age (*4–6*). The relative validity of the developmental maturation and developmental invariance positions seems to depend solely on the relative magnitudes of the proportions of bilaterally organized children and adults.

Methods have recently been proposed for estimating from aphasia incidence the proportions of right- and left-handed adults with right speech, *P(RS)*, left speech, *P(LS)*, and bilateral speech, *P(BS)* (*7, 8*). When applied to adult data these methods suggested a unilateral model for right-handers with an estimated 99 percent and 1 percent having left and right speech, respectively (*8*). We have now applied these methods to childhood aphasia data from the literature. Our overall results support the developmental invariance position.

In a recent review of the literature on childhood aphasia, Satz and Bullard-Bates (*5*) used a method developed by Satz (*7*) to investigate the validity of the developmental maturation and invariance positions. Here we used different exclusionary rules and improved statistical methods (*8*) to assess this issue. The following criteria were required for the inclusion of subjects in our data analyses: (i) Some speech was reported before the onset of the lesion, regardless of how minimal (in some cases this was the expression of several words only). (ii) The patient was under 16 years of age at the time of lesion onset. (iii) Evidence was available that the lesion effects were unilateral. In each study reviewed, the unilaterality of lesions was confirmed by at least one of the following: autopsy, clinical testing, radiographs, arteriograph, pneumoencephalogram, or electroencephalogram. (iv) The presence or absence of aphasia had been assessed after unilateral injury. Aphasic symptoms included any one of the following: loss of speech accompanied by hemiplegia or hemiparesis, paraphasia, or problems in comprehension, expression, reading, writing, or naming. Subjects that were reported with only dysarthria or with only mutism were classified as nonaphasic. (v) Lesion onset occurred

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