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Major role of bacteria in biogeochemical fluxes in the ocean's interior

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Spatial and temporal patterns in the flux of sinking organic matter are central to the understanding of elemental dynamics and food-web energetics in the global ocean^{1–3}. Heterotrophic bacteria have been shown to play a part in the decomposition of large, rapidly sinking organic particles within and below the euphotic zone^{4–8}. These previous studies suggest that decomposition by attached bacteria can explain only a trivial fraction of the observed decrease in the flux of organic matter with increasing depth. We report here that free-living bacteria, rather than the particle-feeding zooplankton, are the principal mediators of particle decomposition in the central north Pacific gyre and the eutrophic Santa Monica basin. We suggest that bacterial growth in the mesopelagial gives rise to the large-scale production of fine (0.3–0.6 μm), non-sinking particles at the expense of large, rapidly sinking particles. Our results have implications for models of biogeochemical dynamics of organic particles and surface-reactive materials such as radionuclides in the ocean's interior^{3,9}.

During the PRPOOS (Plankton Rate Processes in Oligotrophic Oceans) cruise in the north Pacific gyre (28°45.5' N, 118°47.6' W) in August–September 1985, we used water-bottle sampling to obtain depth profiles. Similar profiles were obtained from the eutrophic Santa Monica basin (33°45.5' N, 118°47.6' W) in May 1986. We took two 0.8-km-deep profiles in the basin and eight profiles in the gyre (of which four were 1-km deep). We used seawater from the same bottle for determining the bacterial abundance, bacterial organic carbon (BOC), bacterial secondary production (BSP; by the thymidine incorporation method¹⁰), particulate organic carbon (POC), and related parameters. We took measurements in both the euphotic and aphotic zones, but here we discuss only the layer from just below the euphotic zone to the bottom of the profiles (gyre, 110–1,000 m; basin, 39–800 m; water depth was >4,000 m in the gyre and 920 m in the basin).

BOC in the gyre formed a surprisingly large fraction of the POC; the mean value of the BOC/POC ratio was 0.43 (see Fig. 1). These estimates are in fact conservative as the POC values

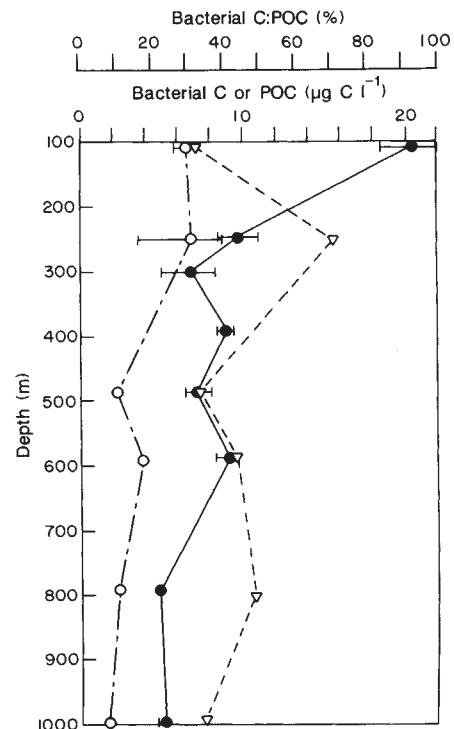


Fig. 1 Depth profiles of POC (closed circles), and BOC (open circles), and BOC:POC in the gyre (open triangles). Bars represent the range for duplicate samples in the case of POC, and in BOC profiles they show 1 s.d. BOC was calculated from cell counts and cell volume measurements by epifluorescence microscopy^{25,26} and by using 20 fg C cell⁻¹ for <0.07 μm^3 bacteria¹¹. POC was measured by filtering seawater samples on GF/F filters (effective retention = 0.7 μm) followed by analysis by a CHN (carbon, hydrogen, nitrogen) analyser²⁷. A correction was made for loss of bacteria through GF/F filters¹¹.

were corrected for maximum loss of bacteria through the GF/F (glass-fibre) filters during sample filtration for POC measurements¹¹. Our values of POC are close to the upper limit of an extensive data set from a station in the central Pacific¹², whereas our values of the abundance of cells are lower than those measured earlier at the same station¹³, and are well within the range for other open-ocean environments⁶. POC and bacterial abundances in the gyre were previously measured at 500 and 900 m¹³, from which we calculate a BOC/POC ratio of ~0.7 (after correcting POC values for loss of bacteria through GF/F filters). High BOC/POC ratios are probably a common feature that has simply gone unnoticed before now. Even in the euphotic zone of the gyre the ratio was 0.4 ± 0.15 ($n = 15$). The corresponding value in the basin was 0.43 ± 0.19 ($n = 17$). Such high ratios have implications for the size spectrum of POC and biogeochemical cycling pathways in the oceans.

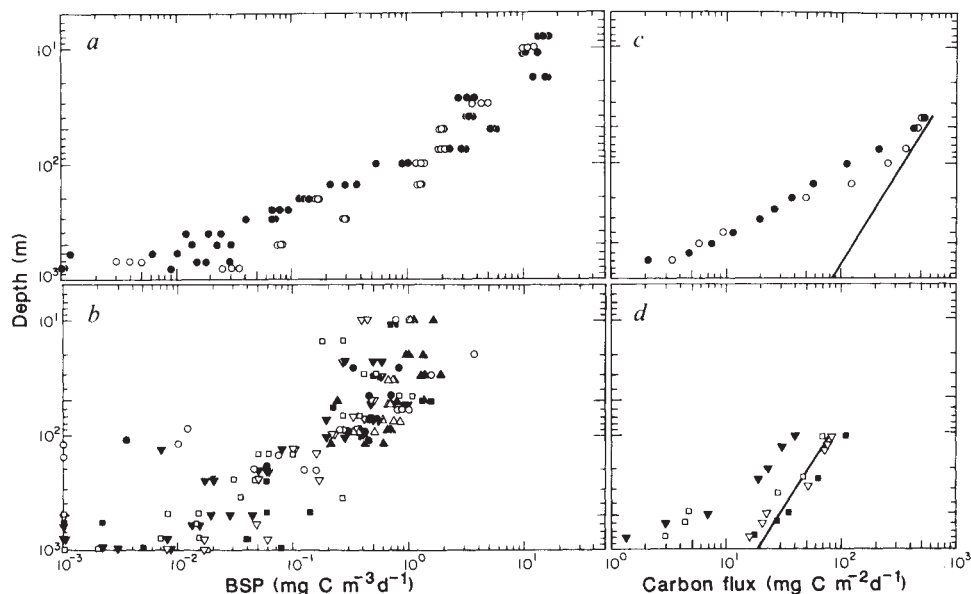
Epifluorescence microscopy showed that <5% of the bacteria were attached to particles, and 98% of the cells passed through 1 μm Nuclepore filters¹⁴. Free-living bacteria were probably not released via disintegration of fragile particles during sampling because particle-attached bacteria are generally large (0.3–1.0 μm^3)^{6,15}, whereas in our samples cells as large as 0.1 μm^3 were rare. Size fractionation after incubation with [³H]thymidine generally showed >95% of the incorporation in bacteria passing through the 1 μm filters. Recently it has been shown that marine aggregates harbour dense bacterial populations⁶, but these populations are few in number and contain a trivial fraction (<0.5%) of the bacterial assemblage. In view of these facts we believe that >95% of the mesopelagic bacteria in our profiles were free living, and were responsible for most of the bacterial production.

We measured bacterial production rates in the aphotic zones

Fig. 2 *a, b*, Depth profiles of bacterial secondary production (BSP). *a*, Two profiles (open and filled circles) in the Santa Monica Basin (33°45.5' N, 118°47.6' W), taken 2 days apart in May 1986. *b*, Eight depth profiles (of which four are 1-km deep) in the central north Pacific gyre (28° N 155° W), represented by different symbols, taken during August-September 1985. All replicate sample measurements are shown. *c, d*, Depth versus F_B ($=2 \int_{z_B}^z \text{BSP } dz$, where z_B = depth at bottom of the profile and z = depth of integration); data points in *c* (gyre) and *d* (basin) are derived from BSP values in *a* & *b* respectively. Solid lines in *c* and *d*: depth versus sinking carbon flux calculated on the basis of local primary production and the sinking-flux model of Betzer *et al.*¹⁶. Symbols represent different profiles.

Methods. Seawater samples were collected with cleaned Go-Flo bottles or Niskin bottles on CTD (conductivity-temperature-depth) rosette in depth-profiles to 1 km (gyre) and with Niskin bottles to 0.8 km (basin).

BSP was measured by the thymidine (TdR) incorporation method¹⁰ at *in situ* temperature and surface pressure²⁸. BSP was calculated from TdR incorporation rates by using a conservative conversion factor of 1.18×10^{18} cells per mole TdR incorporated in DNA²⁹. [³H] in DNA as a percentage of [³H] in cold trichloroacetic acid precipitate in euphotic zone was 65.7%, decreasing with depth to 29% at 450 m and below; the decrease from below the euphotic zone to 450 m was described by regression equation: (% [³H] in DNA = $60.924 - 0.0709 \times \text{depth (m)}$). The precision of BSP measurements in the aphotic zone was on average $22.7 \pm 14.2\%$ (mean ± 1 s.d.; $n = 23$). Primary production was measured by the ¹⁴C method in the gyre by Marra and Heineman³⁰ and in the basin by Eppley.



of the two study areas to address the following questions. (1) Are bacteria quantitatively important in the use of particulate organic matter (POM) flux? (2) Assuming steady-state conditions (growth = loss), how rapidly is the bacterial assemblage turned over due to various causes, including scavenging by rapidly sinking particles and by predation? (3) Does bacterial production reflect differences in the magnitude of surface primary production (PP) (456 and 1,254 mg C m⁻² day⁻¹ in the gyre and basin, respectively; Table 1)? Aphotic-zone integrated BSP was 39 mg C m⁻² d⁻¹ in the gyre and much higher (253 mg C m⁻² day⁻¹) in the basin (Table 1). Clearly, the magnitude of BSP reflected the sinking flux out of the euphotic zone. Bacterial abundance in the aphotic zones of the two areas was comparable (data not shown); thus the 6-fold difference in BSP reflects higher specific growth rates (and assemblage turnover rates) in the basin. Although sinking flux (estimated from PP and Betzer's model¹⁶) at 800 m in the basin and 110 m in the gyre were comparable, BSP at 800 m in the basin was ~10 times lower than BSP at 110 m in the gyre (Fig. 2). Thus, BSP not only reflects the magnitude of C flux but also reflects properties that affect its bacterial use; factors such as packaging, size spectrum, and the chemical composition of sinking organic matter are probable modifiers of the depth variation of bacterial decomposition of sinking flux. For instance, high abundance of surface phytoplankton in the basin may support the production of large, compact, rapidly sinking fecal pellets¹⁷ not significantly disintegrated and solubilized in the lower mesopelagic zone, although they may contain nutrients for bacteria.

We estimated C use by bacteria conservatively on the basis of BSP by assuming 50% carbon assimilation efficiency¹⁸. Integrated C flux into bacteria (F_B) for the aphotic zone in the gyre and the basin was 78 and 507 mg C m⁻² day⁻¹, respectively (Table 1; F_B may be somewhat overestimated if some BSP was released as dissolved organic matter (DOM) and re-used by bacteria¹⁹). As discussed, F_B mainly represents C flux into free-living bacteria. We now compare F_B with PP, new production (NP)²⁰, and sinking flux (F)¹⁶ to consider F_B in the context of sinking-flux biodynamics. Bacteria in the aphotic zone used sinking flux equivalent to 17% (gyre) and 40% (basin) of the

surface PP ($F_B/PP = 0.17$ and 0.40, respectively; Table 1). As $F < PP$, these values are also minimum estimates for F_B/F , that is the fraction of sinking flux used by bacteria. To estimate F_B/F more realistically we calculated F by Eppley and Peterson's new-production model²⁰ and by Betzer's carbon-flux model¹⁶. F_B/F calculated from the Eppley and Peterson model was 0.41 (gyre) and 0.81 (basin) whereas Betzer's model gave a value of 0.98 (gyre) and 0.80 (basin). Thus, F_B/F calculated from two conceptually and methodologically different models for determining F leads us to conclude that bacteria, rather than particle-consuming animals, were the main users of sinking C flux. Importantly, this result requires that a large part of the sinking organic matter must become DOM and thereby becomes accessible to the free-living bacteria. Consequently, this process represents a large-scale production of fine (0.3–0.6 μm diameter), non-sinking POC (that is, free-living bacterial cells) at the expense of large, rapidly sinking particles. As F_B explains a large fraction of F , it could be useful in making independent, minimum estimates of F . F_B determination is easy, rapid and

Table 1 Carbon flux parameters for the gyre and the basin

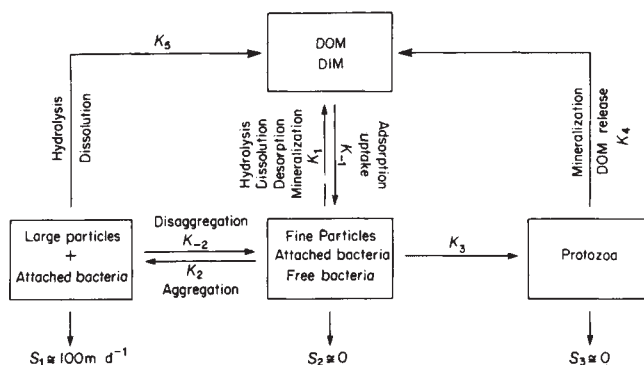
	Gyre	Basin
PP (mg C m ⁻² d ⁻¹)	456*	1254†
Euphotic depth (m)	110*	39
F (mg C m ⁻² d ⁻¹)	79	634
F_B (mg C m ⁻² d ⁻¹)	78 ± 34 (110–1,000 m)	507 ± 22 (39–800 m)
F_B/PP	0.17 ± 0.07	0.40 ± 0.02
F_B/NP	0.41 ± 0.18	0.81 ± 0.04
F_B/F	0.98 ± 0.42 (110–1,000 m)	0.80 ± 0.03 (39–800 m)

PP, primary production; F , flux of sinking organic matter entering the aphotic zone, calculated as in legend to Fig. 2; F_B , Integrated $2 \times$ BSP from the top of the aphotic zone to the bottom of the profile (depth-intervals in parentheses); NP, new production, calculated on the basis of PP, as in Eppley and Peterson²⁰.

* Ref. 30.

† Eppley, R. W. *et al.* (unpublished manuscript).

Fig. 3 Schematic diagram for pathways of sinking particulate organic matter (POM) flux, modified from the model of Bacon *et al.*³. DOM and DIM represent dissolved organic matter and dissolved inorganic matter, respectively. K_1 – K_5 denote the rate constants for various flux pathways. S_1 – S_3 denote the sinking rates of various particle classes. See text for details.



inexpensive. It could supplement the sediment-trap measurements of the magnitude and spatial and temporal (including depth) variation of F , and could allow for extensive coverage.

What mechanisms could explain the large-scale POM solubilization as revealed by the high F_B/F values? Attached bacteria could compete with particle-consumers by rapidly solubilizing POM via hyper-production of exohydrolases⁸ (this makes POM inaccessible to the organisms that eat particles and releasing into the microenvironment both the colonizer's progeny and most DOM produced by hydrolysis. Counter-intuitively, although particle-attached bacteria grow in size, they generally do not multiply faster than the free-living ones; hence, their *direct* role in particle mineralization remains small (that is they play the critical role of particle solubilization, yet the DOM thus produced is mainly used by the free-living bacteria). This is consistent with the observation that <5% of thymidine incorporation is due to particle-attached bacteria. Karl *et al.* (personal communication) found that microbes (including bacteria) attached to sinking particles collected in sediment traps play a minor role in the decomposition of sinking POM; other biological or physicochemical processes must be mainly responsible for particle mineralization. According to our hypothesis, the colonizing bacteria become enlarged to permit rapid synthesis of exohydrolases. Enzyme hydrolysis may also contribute to particle disaggregation and fragmentation. This would explain how the POM of large particles might be solubilized by the attached bacteria and be used mainly by free-living bacteria. Also, bacteria and metazoan digestive enzymes passed into the fecal pellets may continue the hydrolysis as the fecal pellets descend through the water column; the resulting DOM could be used by both attached and free-living bacteria⁸. Although the mechanistic details of DOM production are not yet understood, our results indicate that sinking organic matter mainly follows the route: POM → (fragmentation) → DOM → free-living bacteria (Fig. 3).

Based on depth-averaged BSP and BOC, the turnover times of BOC (K_2 and K_3 ; Fig. 3) below the euphotic zone were 16 days (basin) and 65 days (gyre). BOC could be scavenged by sinking particles (K_2) or mineralized *in situ* (K_3), for instance by protozoan predation. Protozoa (2–5 μm), morphologically similar to the bacterivorous microflagellates in epipelagic²¹ and mesopelagic^{22–23} zones, were present (10^6 l^{-1}) just below the euphotic zone, decreasing to 10^4 l^{-1} at 1 km. We estimate that ~40% of BSP could be consumed by microflagellates, assuming they cleared $20 \mu\text{l}$ seawater $\text{cell}^{-1} \text{ h}^{-1}$ (ref. 21). Although direct demonstration and quantification of bacterivory is awaited, our observations are consistent with the presence of a mesopelagic microbial loop potentially capable of mineralizing part of the BSP.

Our results provide a biological explanation for the observations and the model of Bacon *et al.*³ regarding particle and radionuclide fluxes (Fig. 3, modified from ref. 3 to incorporate a role for bacteria). These authors postulate that there is an unchanging pool of fine, non-sinking particles responsible for most radionuclide adsorption. They further envisage a con-

tinuous exchange of material between large and fine particles via disaggregation-aggregation as the particle sinks. Bacteria, being a significant fraction of POC, could comprise the unchanging pool of fine, non-sinking particles responsible for radionuclide adsorption (we estimate that ~90% of all organic particulate surface in our samples was due to bacteria); they can stick to suspended and sinking particles, resulting in the observed slow, seasonally varying vertical flux of fine particles (that is, bacterial biomass) and associated materials²⁴. Our model (Fig. 3) emphasizes particle solubilization and bacterial production and complements Bacon's model by providing a mechanism for sustaining the presence of fine, non-sinking particles. It also provides a framework for explaining the recently observed patterns of sinking flux of Chernobyl nuclides⁹; surface-reactive nuclides would largely adsorb onto free bacteria which would then be swept down by large particles such as marine snow and fecal pellets. Thus, free bacteria, by virtue of being the dominant biotic surface and metabolic component in the mesopelagic, act as principal mediators of exchange equilibrium between dissolved and particulate phases involving both the sinking organic matter and associated surface-reactive materials. We conclude that the distributional patterns and growth dynamics of free bacteria could strongly influence the spatial and temporal patterns of biogeochemical cycling of materials in the ocean's interior.

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