31°C. Butterflies were introduced individually into a 45 by 45 by 45 cm cage containing eight equal-sized inflorescences of the two test species in water vials arranged in a 4 by 4 alternating array. Sample sizes were 42 for test 1 and 37 for test 2.
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# Bacterioplankton: A Sink for Carbon in a Coastal Marine Plankton Community

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**Recent determinations of high production rates (up to 30 percent of primary production in surface waters) implicate free-living marine bacterioplankton as a link in a "microbial loop" that supplements phytoplankton as food for herbivores. An enclosed water column of 300 cubic meters was used to test the microbial loop hypothesis by following the fate of carbon-14–labeled bacterioplankton for over 50 days. Only 2 percent of the label initially fixed from carbon-14–labeled glucose by bacteria was present in larger organisms after 13 days, at which time about 20 percent of the total label added remained in the particulate fraction. Most of the label appeared to pass directly from particles smaller than 1 micrometer (heterotrophic bacterioplankton and some bacteriovores) to respired labeled carbon dioxide or to regenerated dissolved organic carbon-14. Secondary (and, by implication, primary) production by organisms smaller than 1 micrometer may not be an important food source in marine food chains. Bacterioplankton can be a sink for carbon in planktonic food webs and may serve principally as agents of nutrient regeneration rather than as food.**

UNTIL RECENTLY, BACTERIA WERE viewed as relatively minor components of marine plankton communities (*1*). That view is now being revised as a result of new discoveries by marine microbiologists. Free-living heterotrophic bacterioplankton less than 1 μm in diameter may constitute up to 20 percent of the carbon biomass in marine coastal waters (*2–4*). With gross growth efficiencies of over 50 percent (*5*) and rapid growth rates often surpassing two divisions per day (*6*), bacterioplankton are potentially important biomass producers (*2, 7*). This discovery has prompted the hypothesis that bacterioplankton could be an important source of nutrition at the base of plankton food webs, supplementing phytoplankton in the diets of microzooplankton (*3, 8*). However, researchers have concluded that heterotrophic microflagellates 2 to 10 μm long are the principal predators of bacterioplankton because larger grazers are not capable of efficient removal of micrometer-sized prey (*9, 10*). It is believed that these small bacteriovores are grazed in turn only by ciliates or the smallest juvenile stages of other planktonic forms. Thus the large bacterial production may enter the classical marine metazoan food chain only via the protozoa (*2, 8*) and not directly.

Bacterioplankton and their predators form the hypothetical "microbial loop" that returns to the main food chain energy lost as dissolved organic matter (DOM) (*2, 3, 8*). Although bacteria appear to scavenge DOM released by phytoplankton (*2, 5, 6*) and herbivores (*2, 11*) with great efficiency, the extent to which bacterial production is a salvage pathway supplementing primary production as food for herbivores remains uncertain. This speculation about the trophic structure of microbial food webs has been termed the link versus sink question (*5, 12*). Are bacteria a source of carbon for higher organisms, or are they mainly the terminal group in a detrital food chain?

We report the results of a large-scale experiment designed to examine the fate of bacterial production in a representative coastal marine plankton community. Most studies of production and grazing by marine and freshwater plankton have been performed in containers at most a few liters in volume (*13*). Such experiments are easy to replicate, but sampling, surface-volume effects, and containment lead to serious artifacts that affect the various components of

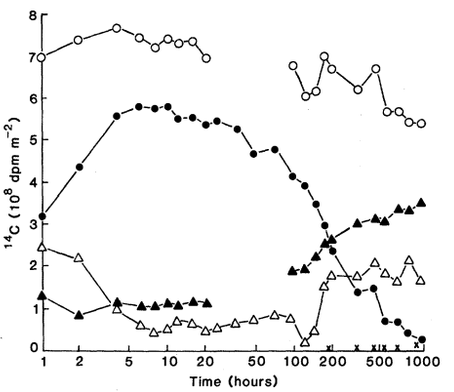


Fig. 1. Partitioning of labeled carbon initially fixed by bacterioplankton from [$^{14}$C]glucose inoculated throughout the enclosed 15-m water column. The dissolved label was initially removed from solution by bacterioplankton; it later reappeared in solution as dissolved organic and inorganic carbon. Total water column content of $^{14}$C label was estimated by integrating data from six depths (2.5, 5, 7.5, 10, 12.5, and 15 m) and by pumping out a collecting cone at the bottom of the enclosure. Symbols: (○), total label; (●), $^{14}$C-labeled POC; (△), $^{14}$C-labeled DOC; (▲), $^{14}CO_2$; and (x), sediment. Breaks in lines indicate missing samples. The experiment began at noon on 12 May 1983.

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the plankton differentially (*14*). Carbon flow through an intact plankton community was monitored in an enclosed 300-$m^3$ water column at Loch Ewe, Scotland (*15*). Experiments on trophic relations in ecosystems conducted at this scale address the complexity of trophic exchanges among most of the relevant elements of the plankton community (*13, 15*). We present evidence indicating that the bacterioplankton were a sink for carbon and not a link to higher order consumers via a microbial loop.

The experiment was conducted in a 15-m-deep water column enclosed in a 5-m-diameter cylinder of reinforced polyethylene. The plankton community within this "bag" was an assemblage of bacteria, protozoans, diatoms, copepods, and gelatinous zooplankton typical of the cold (8° to 10°C), weakly stratified Scottish sea lochs in mid- to late spring (*15*). The entire water column in the bag was inoculated with 6 mCi of [U-$^{14}$C]glucose. The labeled glucose served as a tracer of the flux of bacterial carbon through the food web. It was assumed that the glucose would be taken up extensively, if not exclusively, by the bacterioplankton (*16*). The distribution of the tracer in three operationally defined pools of carbon [dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and particulate organic carbon (POC)] was used to construct a mass balance for the $^{14}$C label during the 55-day experiment (*17*). In addition, labeled particulate matter was concentrated and separated into six size fractions (*17*) to enable the size distribution of labeled carbon initially assimilated by bacteria to be followed over the first 13 days. The loss of label from the water column due to sedimentation was estimated periodically by pumping settled material from a cone at the bottom of the bag (*18*).

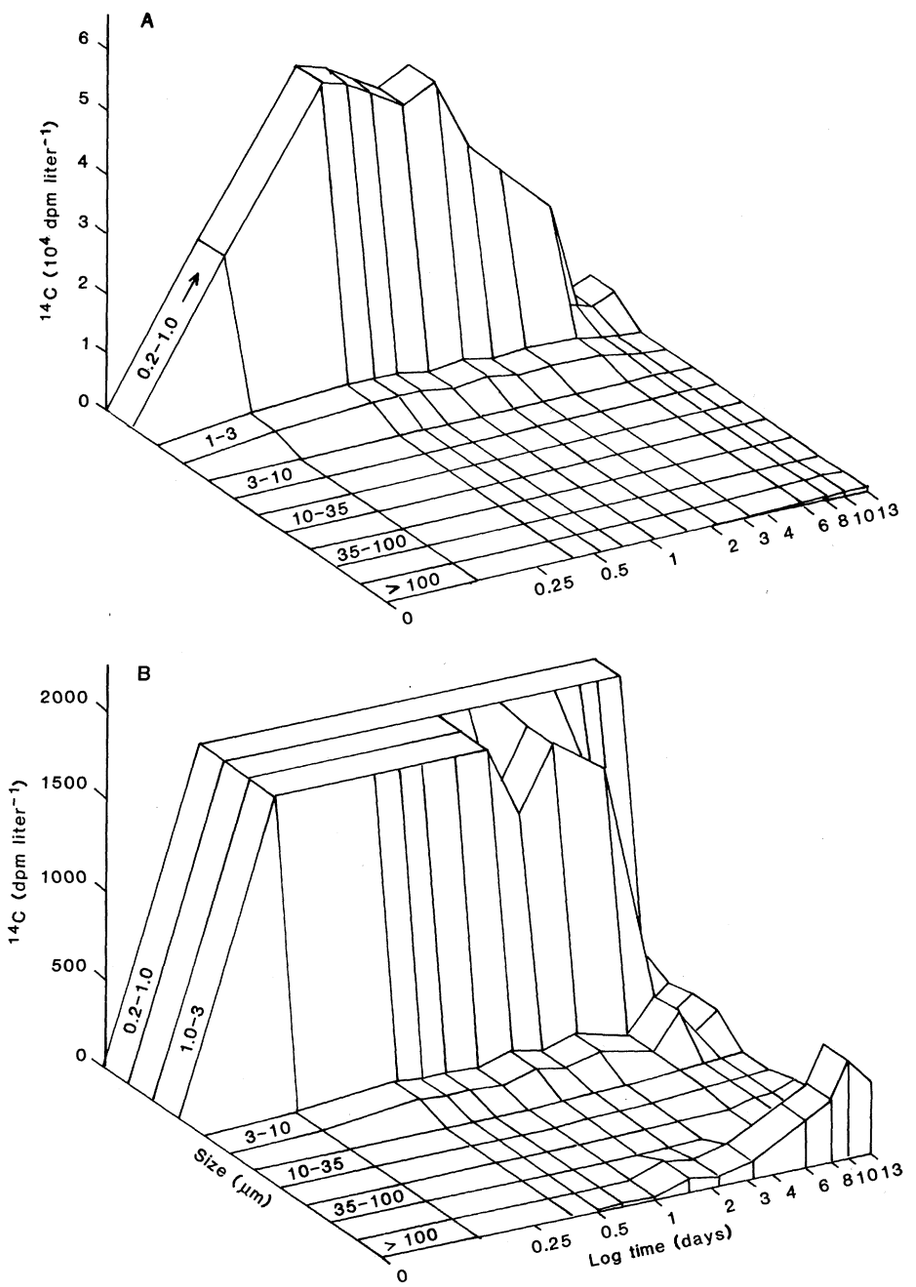


Fig. 2. (A and B) Redistribution of labeled carbon within the particulate pool at 5 m over the first 13 days of the experiment. (B) Rescaled version of the same data as in (A), showing only a very small, slow passage of label from the <1-μm bacterioplankton to larger organisms. With the whole-system data shown in Fig. 1, these results suggest that carbon removed from solution by the bacterioplankton did not pass to larger organisms, and instead was respired as $CO_2$ or released as DOC.

Partitioning of the tracer in the plankton with time appeared to proceed in two stages. There was an initial 4- to 6-hour phase in which over 90 percent of the labeled glucose was rapidly removed from solution (Fig. 1). As the design of the experiment required, the glucose was metabolized by particles (presumably free bacteria) passing through 1-μm filters (Fig. 2 and Table 1). About 4 percent of the isotope was in the >1-μm size fraction after 3 to 10 hours (Table 1). This initial phase was followed by a longer one (55 days) in which the label was redistributed among the dissolved, and to a lesser extent, particulate pools in the water column (Fig. 1 and Table 1). During this period (from about 6 hours to 55 days) there was a slow and progressive loss of tracer from the total suspended particulate material (rate of about 7 percent per day, or about 10 percent of the initial uptake rate) (Figs. 1 and 2). Most of the label was lost directly from the <1-μm fraction of the POC as less than 5 percent of the label passed into size fractions larger than 1 μm (Fig. 2 and Table 1). Over the 55-day period 3 percent of the added label appeared in the sediment that settled out of the water column (Fig. 1) (*18*). Thus we recovered as suspended or settled POC, DOC, and DIC 80 percent of the label originally added to the system (Fig. 1). The missing label could be due to exchange of respired $^{14}CO_2$ with the atmosphere. If this is the case, then by the end of our experiment the major proportion of glucose carbon was fixed by bacteria and passed directly to $^{14}CO_2$ or to reformed $^{14}$C-labeled DOC. These results suggest that, in our experiment, bacteria were important principally as regenerators rather than as food for larger consumers.

To determine trophic exchanges of labeled carbon in the particulate pool, we measured the amount of radioactivity accumulated in different size classes of particulate matter in samples taken from a single depth within the enclosure (*17*). Our princi-

Table 1. Distribution of $^{14}C$ label initially added to water column as [U-$^{14}C$]glucose. Data are expressed as percentages of the total amount of label recovered from a depth of 5 m 1 hour after addition. This is the depth from which large-volume (24-liter) samples were taken for size fractionation studies (*17*). ND, not determined.

| Time (days) | POC size fraction (μm) | | | | DOC | DIC |
|---|---|---|---|---|---|---|
| | <1 | >1 | >10 | >100 | | |
| 0.13 | 45 | 3 | 0.05 | 0.01 | 15 | 13 |
| 0.42 | 67 | 4 | 0.04 | 0.02 | 05 | 14 |
| 0.54 | 65 | 4 | 0.04 | 0.02 | 10 | 14 |
| 0.71 | 62 | 4 | 0.06 | 0.03 | 08 | 15 |
| 1.04 | 57 | 3 | 0.05 | 0.02 | 06 | ND |
| 1.54 | 61 | 4 | 0.17 | 0.13 | 10 | 30 |
| 2.21 | 46 | 2 | 0.15 | 0.09 | 16 | 36 |
| 3.29 | 39 | 3 | 0.23 | 0.16 | 09 | 30 |
| 6.08 | 28 | 3 | 0.52 | 0.40 | ND | 25 |
| 8.21 | 10 | 4 | 0.95 | 0.54 | 20 | 29 |
| 10.00 | 2 | 3 | 1 | 0.74 | ND | ND |
| 13.04 | 3 | 4 | 0.84 | 0.51 | 18 | 28 |
| 22.00 | ND | ND | ND | ND | 14 | 22 |
| 33.00 | ND | ND | ND | ND | 15 | 30 |
| 55.00 | ND | ND | ND | ND | 12 | 30 |

pal finding was unexpected: only a small amount of tracer initially fixed by the free-living bacterioplankton was subsequently detected in size classes larger than 1 μm (Fig. 2 and Table 1). After the initial phase of rapid incorporation by bacteria, some passage of the label into larger particles was seen (Fig. 2B), but at no point did we observe in the total POC larger than 1 μm more than 4 percent of the label initially added to the system (Table 1).

There was a slow accumulation of label in larger particles at the same time as a larger net loss of label from the total particulate pool (Figs. 1 and 2B). As in an earlier study (*19*), we found no evidence for the transfer of labeled carbon through the 10- to 30- and 30- to 100-μm fractions to the >100-μm fraction (Fig. 2B). Although it is possible that the transfer through these fractions was so fast that it was missed by our sampling routine, this seems unlikely. Simple modeling shows this is possible only if the intermediate-sized organisms have growth rates many times greater than those of their prey—an improbable situation. We cannot resolve from our data whether the >100-μm fraction obtained its label by direct grazing on <10-μm particles, by slow utilization of the labeled glucose or subsequently regenerated $^{14}C$-labeled DOC, or via the uptake of dissolved label by attached bacteria.

There is evidence that free-living bacterioplankton are removed quantitatively by protozoan grazers, some of which pass through 1-μm filters (*10, 20*). It is conceivable, therefore, that we actually had two trophic levels in our operationally defined "bacterial" fraction and that the reduction of label in this fraction may be attributed to ingestion and respiration of the bacterial carbon by very small eukaryotes (*20*). But even if this were the case, the considerations cited above (*9*) suggest that larger zooplankton could not efficiently harvest these very small bacteriovores.

Our results do not support the idea of a microbial loop returning carbon lost as DOC from larger organisms back through the bacterioplankton to the larger zooplankton. Although we performed only a single experiment at one location, we believe that the results have great importance for our understanding of the structure and function of marine planktonic ecosystems. In some oceanic areas, up to 80 percent of primary production is by cyanobacteria less than 1 μm in diameter (*21*). It has been reported that 10 to 60 percent of the primary production passes directly through the bacterioplankton, with about half of that being fixed into bacterial cells (*5–7*). Our results suggest that the carbon assimilated by plankton smaller than 1 μm may not pass into conventional food chains leading to metazoan secondary production. Thus significant fractions of the total carbon fixed by organisms smaller than 1 μm could be lost from the trophic system. This loss may result from respiration of bacterial carbon by small bacteriovores, and not just from the bacteria themselves. Although the bacterioplankton may be unimportant as sources of carbon for larger organisms, our findings point to their importance in the regeneration of inorganic nutrients (*6–8*). The functional significance of microbial plankton, which compete with more intensively studied phytoplankton and zooplankton for energy and nutrients and scavenge their wastes, remains to be clarified. The extent to which microbes serve as links or sinks may influence the export of particles from the euphotic zone. Further ecosystem-scale experiments like ours may be a powerful tool for answering these questions.

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17. Radioactivity in the DOC, DIC, and POC pools was determined on separate subsamples by filtration and acidification to separate or remove labeled POC and DIC (as $CO_2$) from the labeled DOC. The concentration of $^{14}CO_2$ was estimated from the difference between the filtered and acidified samples. These data from six depths were integrated to estimate the total label in the water column in each compartment. For detailed analyses of radioactivity incorporated in the particles, we collected 24 liters of water from a depth of 5 m in the bag on approximately a logarithmic time scale (Fig. 2). The sample water was passed through a plexiglass reverse-flow concentrator containing in series (and in order of water passage) 100- and 35-μm Nitex screens and 10- and 3-μm Nuclepore polycarbonate membranes 150 mm in diameter. This procedure gave concentrates of the original particle population into size classes of >100, 100 to 35, 35 to 10, and 10 to 3 μm. Particles smaller than 3 μm were not concentrated. Particle-free water was passed through the concentrator to remove particles smaller than 3 μm and dissolved radioactivity from the >3-μm size fractions. These size fractions were filtered onto appropriately sized Nuclepore filters in triplicate, digested in ethanolamine and methoxyethanol, and assayed by liquid scintillation counting. Concentration factors up to 24:1 enabled us to estimate accurately small amounts of label in large size fractions (<10 dpm liter$^{-1}$). These size classes nominally represent the following crude groupings of heterotrophic organisms: 0.2 to 1 μm, free-living bacterioplankton and very small eukaryotes; 1 to 3 μm, microflagellates and attached bacteria; 3 to 10 μm, protozoa; 10 to 35 μm, larger ciliates and small microcrustaceans; 35 to 100 μm, larger microzooplankton; and >100 μm, larger zooplankton.
18. Sediment appearing in the cone at the bottom of the bag was collected by pump. Aliquots of 10 to 100 ml were filtered, digested, and assayed by liquid scintillation counting. Previous studies conducted at Loch Ewe suggested that total sedimentation in the bag may be underestimated. With fluorescent beads used as a tracer for settling particles, a mean recovery of 14 percent with large variation has been noted (I. Baird, personal communication). We report the observed radioactivity recovered in the sediment without correction. However, if our data are corrected for 14 percent recovery of sediment, we can account for over 90 percent of the added label. But this relatively high apparent flux of $^{14}C$-labeled POC to the bottom is difficult to reconcile with our observation that little of the added label passed through the larger size fractions which would be likely to produce sinking particles. An alternative explanation is that the label (dissolved or in particles smaller than 1 μm) was adsorbed onto refractory flocculent particles. If this were the case, our conclusion about bacterioplankton as a sink would be strengthened.
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