Use of Monodispersed, Fluorescently Labeled Bacteria to Estimate In Situ Protozoan Bacterivory†

BARRY F. SHERR,* EVELYN B. SHERR, AND ROBERT D. FALLON‡

University of Georgia Marine Institute, Sapelo Island, Georgia 31327

We have developed a procedure for preparing monodispersed, fluorescently labeled bacteria (FLB), which may be used to measure virtually instantaneous rates of protozoan bacterivory in natural waters. FLB can be prepared both from natural bacterioplankton assemblages and from clonal isolates and can be stored in frozen suspension or freeze-dried without apparent loss of fluorescence intensity. They are not toxic to protozoa and can be metabolized to support bacterivorous protozoan growth rates equal to those on the same strain of unstained, viable bacteria. In experiments comparing uptake of FLB with uptake of fluorescent latex microspheres by protozoan assemblages in a salt marsh tidal creek, we found that both pelagic oligotrichous ciliates and phagotrophic flagellates ingested FLB with a frequency 4- to 10-fold greater than they ingested the microspheres. Consequently, it appears that the use of latex microspheres leads to underestimation of protozoan bacterivory and that the FLB technique is superior for estimating instantaneous rates of in situ protozoan grazing on bacterioplankton.

As a result of the recent interest in microbial food webs in aquatic ecosystems, various techniques have been used to estimate in situ protozoan grazing on bacteria; no one method has been identified as optimal. The first techniques used to directly estimate rates of bacterivory in natural waters involved assessing changes in bacterial standing stocks during incubations of 12 to 24 h, with experimental manipulations to reduce or eliminate protozoan grazing (14, 17, 21, 24, 25, 30). Subsequently, several methods were developed which require less sample manipulation and shorter incubation times, generally less that 4 h; these include the *Escherichia coli* minicell technique of Wilkner et al. (29), the [³H]thymidine radiotracer approach of Lessard and Swift (19), and the uptake of bacterial-sized fluorescent latex microspheres by in situ protozoa (3, 6, 20). Of these methods, the microsphere technique has seemed to be the most promising, since the experimental protocol is simple, the incubation time is short (10 to 60 min), and there is no problem of separating bacteria from protozoa as in the radiotracer method.

In this paper, we describe a procedure for preparing monodispersed, fluorescently labeled bacteria (FLB), which may be used to determine nearly instantaneous rates of in situ protozoan bacterivory by using the same experimental protocol as that for the fluorescent-microsphere technique. Our preliminary results comparing uptake of FLB and microspheres by estuarine phagotrophic ciliates and flagellates suggest that many protozoa preferentially ingest bacteria. Therefore, estimates of rates of bacterial consumption based on protozoan ingestion of microspheres are likely to be too low.

MATERIALS AND METHODS

Preparation of FLB. Monospecific bacterial cultures were isolated from the water column of a freshwater pond on Sapelo Island, Ga., and from the Duplin River, a tidal

embayment adjacent to Sapelo, and grown on yeast extract media. Natural assemblages of estuarine bacterioplankton (in situ abundance, 2.5×10^6 ml^{-1}) were concentrated from 4 liters of water filtered through a hollow-fiber filter (no. H1MP01-43; pore size, 0.8 μm; Amicon Corp., Lexington, Mass.) with a nominal molecular size cutoff of 0.1 μm and a flow rate (without back pressure) of 1,000 ml min^{-1}. The final 30-ml volume contained 3.2×10^8 cells ml^{-1}.

FLB were prepared as diagrammed in Fig. 1. The bacterial isolates were harvested in mid- to late logarithmic phase by centrifugation in a J-21M centrifuge (Beckman Instruments, Inc., Fullerton, Calif.) with a JA-14 rotor (Beckman) at 12,000 rpm (22,000 × g) for 12 min. The bacterial pellets were suspended in 10 ml of a 0.05 M Na$_2$HPO$_4$–0.85% NaCl solution (adjusted to pH 9), and large clumps of bacterial cells were removed by filtration through a filter (pore size, 3 μm; Nuclepore Corp., Pleasanton, Calif.). The natural bacterioplankton assemblage in the 30-ml concentrate was also pelleted by centrifugation and suspended in 10 ml of the buffered saline solution, Final cell concentrations ranged from 10^8 to 10^{10} cells ml^{-1}. Two milligrams of 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF) (Sigma Chemical Co., St. Louis, Mo.) was added, and the cell suspensions were incubated in a water bath at 60°C for 2 h. The incubation was followed by three washes with the phosphate-buffered saline and suspension in a 0.02 M tetrasodium PP$_i$–0.85% NaCl solution, and the cell suspension was sonicated with a model W 185 Sonifier Cell Disrupter with tapered microtip (Heat-Systems-Ultrasonics, Inc.) at a 30-W power level for four 1-s bursts to disperse any remaining bacterial clumps (28). Aliquots (2 ml) were frozen at −20°C in 10-ml plastic scintillation vials. Some of these frozen aliquots of FLB were freeze-dried, stored at room temperature, and subsequently thawed, rehydrated with distilled water, and sonicated as described above to redisperse the bacteria.

Enumeration of bacteria and protozoa. FLB, natural bacterioplankton, and protozoa were enumerated by using a Zeiss Universal Epifluorescence Microscope with a 75-W xenon lamp. FLB were filtered onto unstained 0.2-μm Nuclepore filters and enumerated at ×1,250 with Zeiss filter
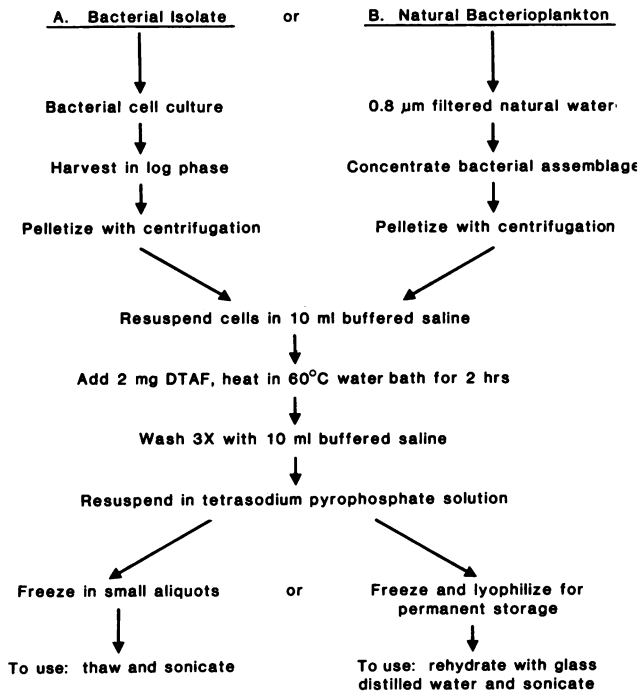

FIG. 1. Schematic diagram of the procedure for preparing and storing FLB.

set 47 77 09 (BP 450–490 excitation filter, FT 510 beam splitter, and LP 520 barrier filter). With this filter set, the FLB fluoresced a bright apple green. Natural bacterioplankton were enumerated by acridine orange (AO) direct counts (AODC) (16) on irgalan-black-stained 0.2-μm Nuclepore filters by using the same filter set as for the FLB. Phagotrophic protozoa were enumerated by being stained with 4′, 6′-diamidino-2-phenylindole (DAPI) (22), filtered onto a 0.8-μm Nuclepore-black filter, and examined at ×160 (ciliates) or ×1,250 (flagellates) with Zeiss filter set 47 77 02 (G 365 excitation filter, FT 395 beam splitter, and LP 420 barrier filter).

Experimental protocols. All experiments were carried out with 400-ml WhirlPak bags that had been presoaked in 10% (vol/vol) HCl and rinsed thoroughly in deionized water; previous experience has shown that this treatment improves reproducibility of results obtained with different bags (25).

Growth of ciliates and flagellates on FLB. To assess the nutritional quality and potential toxicity of the FLB, we conducted population growth experiments with a pond water bacterial isolate, designated FW-1 (a gram-negative short rod with an average cell size of 0.10 μm³, or a 0.6-μm effective spherical diameter), *Uronema marina* (a ubiquitous marine bacterivorous ciliate), and a mixed culture of bacterivorous flagellates.

We isolated *U. marina* from the Duplin River and maintained the ciliate on wheat grain culture. A population of *U. marina* was grown to stationary phase in 400-ml WhirlPak bags containing 200 ml of 0.2 μm-filtered estuarine water and 10⁷ unstained, viable cells of FW-1 ml⁻¹ as a food source. The ciliates were allowed to reduce the bacterial concentration to 10⁴ cells ml⁻¹, and then 1.0-ml portions of the ciliate culture were transferred to four WhirlPak bags, each containing 100 ml of 0.2-μm filtered estuarine water. The initial concentration of ciliates within each bag was approximately

80 cells ml⁻¹. FLB were added to two of the bags to a final concentration of 2 × 10⁷ cells ml⁻¹. An equal concentration of unstained, viable cells of the freshwater isolate was added to the two other bags, and all four bags were incubated at 25°C in the dark. Subsamples (10 ml) were taken from each bag at time zero and periodically thereafter for 24 h.

The flagellate growth experiment was carried out in the same manner. A mixed culture of bacterivorous flagellates, obtained by incubating screened estuarine water (10-μm screen) with 1 mg of nutrient broth (added to promote bacterial growth) liter ⁻¹ for 48 h, was grown up on 10⁷ cells of unstained FW-1 ml⁻¹ When the flagellates had increased to 10⁴ ml⁻¹, 0.5-ml portions of the flagellate culture were added to duplicate WhirlPak bags containing 50 ml of 0.2-μm filtered estuarine water and 1.3 × 10⁷ cells of stained (FLB) or unstained FW-1 ml⁻¹. The flagellate density was about 900 cells ml⁻¹ at time zero. Subsamples (5 ml) were taken from each bag initially and at periodic intervals for a total of 30 h. Samples for both ciliate and flagellate growth experiments were killed with tetraborate-buffered Formalin (final concentration, 2%) and enumerated within 3 days of completion of the experiment.

To estimate gross growth efficiencies of ciliates and flagellates on FLB, FLB were enumerated and protozoan biomass was determined at the start of logistic growth and at the end of the experiments. Numbers of FLB were converted to carbon biomass by using an average bacterial cell biovolume of 0.10 μm³ and a carbon conversion factor of 0.22 pg of C μm ⁻³ (4). Biomasses of protozoa were estimated by determining the linear dimensions of each enumerated cell, calculating their volumes by using the equation for a sphere or a prolate spheroid, and multiplying by 0.08 pg of C μm⁻³ (26).

Protozoan uptake of FLB and of fluorescent microspheres. We compared the uptake of FLB prepared from FW-1 with the uptake of fluorescent latex microspheres (average diameter, 0.51 μm; Polysciences, Inc., Warrington, Pa.) by phagotrophic protozoa in estuarine water. Subsamples (20 to 100 ml) of freshly collected, unscreened seawater were added to WhirlPak bags. Before an experiment, the natural bacterioplankton abundance was determined by AODC. In an initial experiment, we added FLB or microspheres at 10% of the natural bacterial abundance to tidal creek water and to a subsample of the culture of mixed bacterivorous flagellates and took samples of the water every 30 min for 90 min. Since the results of this experiment indicated that equilibrium between protozoan ingestion and digestion-egestion of bacteria or microspheres was reached before 30 min, a subsequent shorter-term experiment was conducted. For estimates of ciliate bacterivory, FLB and microspheres were added to separate bags at time zero at a concentration 5% of the natural bacterial abundance, and 5-ml subsamples were taken immediately and at 5-min intervals for 20 min. For estimates of flagellate bacterivory, FLB and microspheres were added to separate bags at a concentration of 30% of the natural bacterial abundance, and 5-ml subsamples were taken at 10-min intervals for 40 min. Small samples were also taken at time zero for the determination of actual abundance of FLB or microspheres. The time series subsamples were immediately fixed with borate-buffered Formalin (final concentration, 2%), and samples were inspected by epifluorescence microscopy within 3 days.

The rate of ingestion of FLB or microspheres by ciliates and flagellates was determined by first scanning the time series samples under DAPI fluorescence (at ×160 for ciliates and ×1,250 for flagellates) and, when a protozoan was
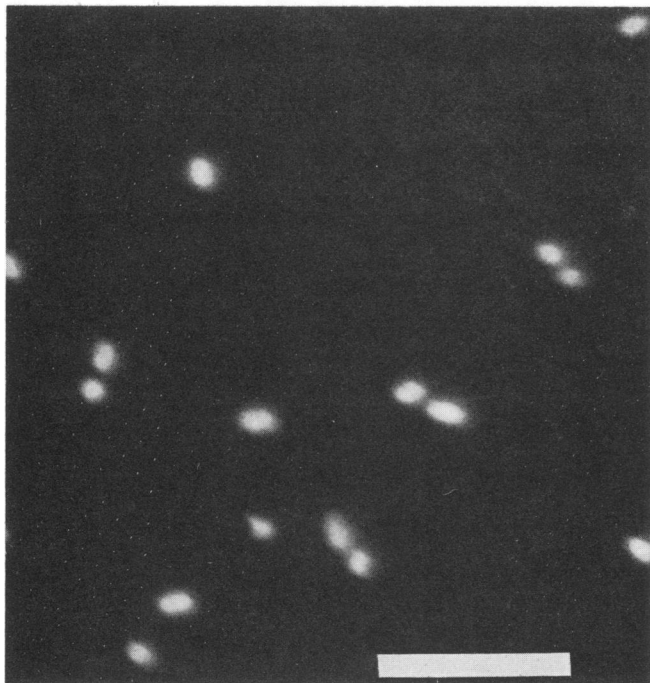
FIG. 2. Cells of a freshwater bacterium (FW-1) stained with DTAF as described in the text. Bar, 5 μm.

located, switching filter sets to determine the number of FLB or microspheres within the cell (at ×1,250 for both ciliates and flagellates). A total of 60 to 100 ciliates or flagellates were examined for each time point subsample, and the average number of particles cell $^{-1}$ (FLB or microspheres) was determined for the population at each time point. Although the protozoa were not taxonomically identified, we were able to distinguish two major groups of ciliates in our samples: oligotrichs (class Spirotrichea, subclass Choreotrichia) and scuticociliates (class Oligohymenophorea, subclass Hymenostomatida) (18). We calculated separate particle uptake rates for oligotrichs, scuticociliates, and flagellates from the linear portion of the curve of average number of particles cell $^{-1}$ with time by using simple regression analysis (Statpak Onevreg program; Northwest Analytical Inc., Portland, Oreg.). From the uptake rates of FLB and the absolute concentration of FLB in the experimental treatments, we were then able to calculate per-cell clearance rates as described by Fenchel (9).

RESULTS

Concentrating natural assemblages of bacterioplankton. Ultrafiltration with the hollow-fiber filter proved to be an excellent way to concentrate suspended bacteria from large volumes of estuarine water. Using a new cartridge, we obtained a 100-fold concentration of cells, reducing 4 liters to 30 ml in 30 min. Comparison of the number of bacterioplankton between the in situ and concentrated suspensions indicated a recovery of 96% with no observable cell clumping.

Preparation and storage of the FLB. Staining of all bacterial preparations was successful when the outlined protocol was followed. Staining of clonal isolates resulted in an FLB population of uniform size, morphology, and staining intensity (Fig. 2). In contrast, natural populations showed a wider range in all three characteristics. Clumping of bacterial
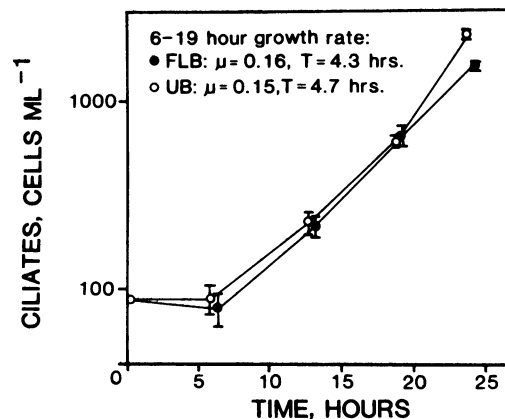

FIG. 3. Time course of the growth of *U. marina* fed either FLB or unstained bacteria of the same clonal strain (FW-1).

concentrates was eliminated by suspending FLB in a 0.02 M solution of tetrasodium PP$_i$, followed by short bursts of sonication. Refrigerated FLB suspensions stored overnight showed no tendency to clump. Minor clumping did occur after FLB suspensions were stored frozen or freeze-dried and subsequently thawed or rehydrated; however, the FLB could easily be redispersed via sonification. The stained bacteria retained their initial bright fluorescence when kept at room temperature either in the pp$_i$, solution or in estuarine water for up to 3 days and when stored frozen or lyophilized for months.

Protozoan growth experiments. The population growth rates (μ) of both the *U. marina* and mixed flagellate cultures were not different with FLB or unstained, viable bacteria of the same strain (FW-1) as a food source (Fig. 3 and 4). Although food vacuoles full of FLB could be seen in the protozoa throughout the incubation (Fig. 5), the cytoplasm of the ciliates and flagellates never became stained with DTAF. During the experiments, both the ciliates and flagellates grew for about four generations with population doubling times of 4.3 to 4.7 h. We calculated average gross growth efficiencies of 47 and 36% for ciliates and flagellates, respectively, growing on FLB.
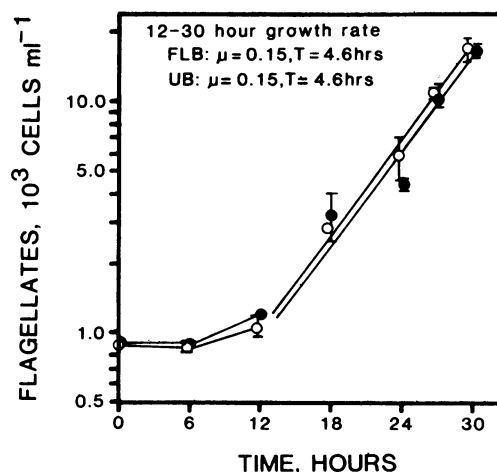

FIG. 4. Time course of the growth of mixed bacterivorous microflagellates fed either FLB (●) or unstained bacteria (○) of the same clonal strain (FW-1).
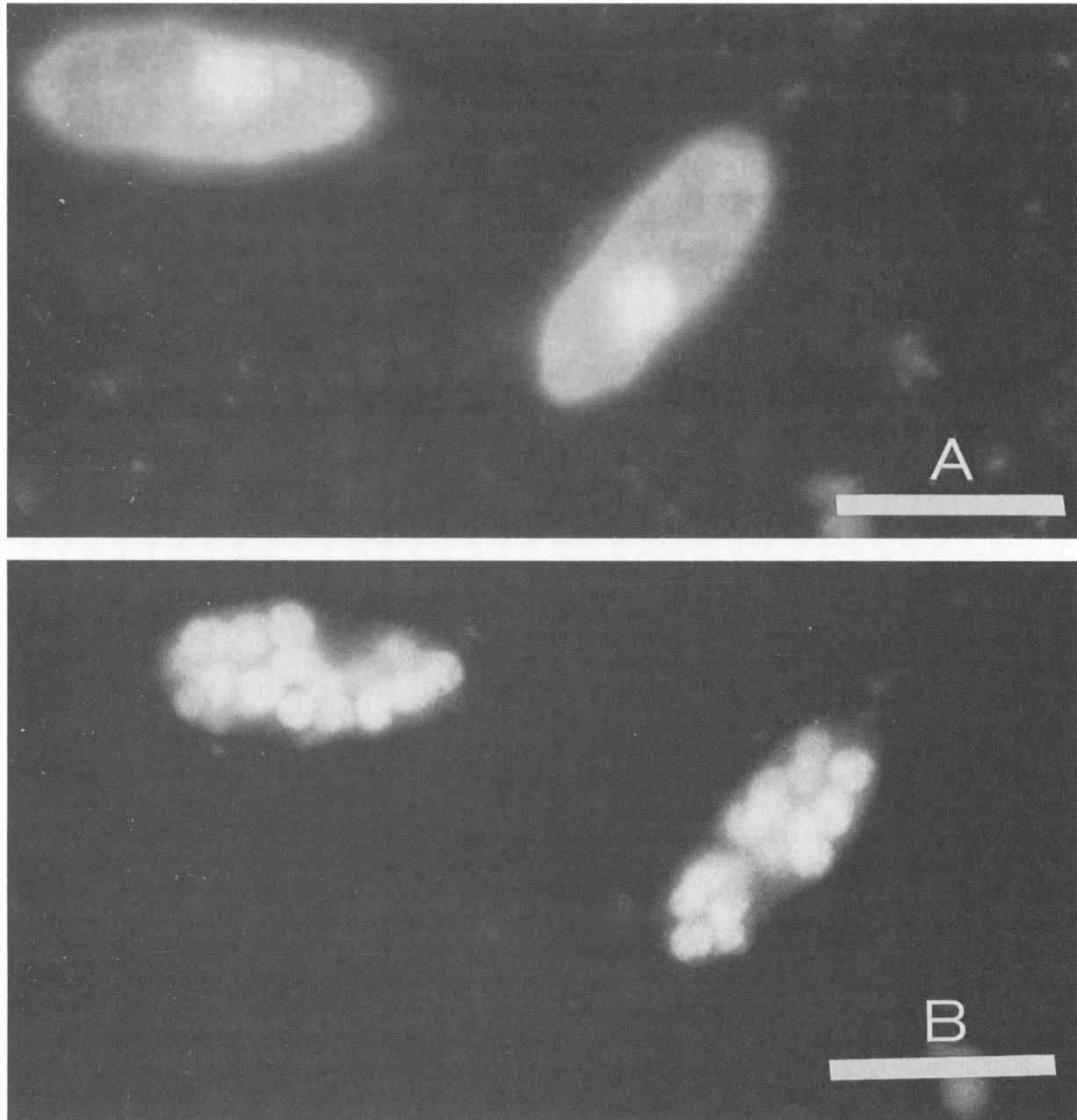
FIG. 5. Two *U. marina* after 24 h of ciliate growth on FLB. (A) Cells visualized with DAPI fluorescence; (B) spherical food vacuoles within the cells, each individual vacuole containing 10 to 20 FLB, visualized with the AO epifluorescence filter set. Note that the ciliate cytoplasm is unstained. Bars, 10 μm.

Protozoan uptake of FLB versus microspheres. In the preliminary experiment with tidal creek water and with the mixed bacterivorous flagellate culture, we did not sample frequently enough to establish a good initial uptake rate. Instead, we found that from 30 to 90 min, the average number of FLB or microspheres per ciliate or flagellate remained fairly constant. The results of this experiment suggested a preference for FLB over microspheres of 4:1 for the ciliates, which were mostly 8- to 20-μm oligotrichs, and of 7:1 to 10:1 for the flagellates (Table 1).

In the second experiment with a natural assemblage of tidal creek protozoa, we sampled every 5 to 10 min to obtain short-term uptake rates of FLB and of microspheres. We found two different uptake patterns for the populations of scuticociliates and small oligotrichs in the creek water. The scuticociliates (Fig. 6A) showed linear uptake of both FLB and microspheres for 20 min, while the oligotrichs took up FLB at a rapid rate for 10 min, after which the average number of FLB per cell leveled off (Fig. 6B). A 10-μm oligotrich with numerous ingested FLB is shown in Fig. 7. The flagellates ingested FLB at a steady rate for 30 min (Fig. 8). Both the oligotrichs and flagellates had much lower uptake rates for the fluorescent microspheres, with FLB/microsphere uptake ratios of 10:1 for the ciliates and

6:1 for the flagellates (Fig. 6B and 8). The scuticociliates had identical uptake rates for both FLB and microspheres (Fig. 6A). We calculated average clearance rates from the rate of uptake of FLB of 140 nl cell^{-1} h^{-1} for the scuticociliates, 260 nl cell $^{-1}$ h^{-1} for the oligotrichs, and 3.2 nl cell^{-1} h^{-1} for the flagellates. In our tidal creek water sample, we enumerated approximately 80 scuticociliates ml^{-1}, 100 oligotrichs ml^{-1}, and 2,000 aplastidic flagellates ml^{-1}. From these population abundances and per-cell clearance rates, we estimated that the ciliates and flagellates could clear 90 and 15% of a unit volume of water per day, respectively.

DISCUSSION

For preparing FLB, the stain DTAF appears to be superior to other fluorochromes (AO, fluorescein isothiocyanate, and DAPI), with which we had previously tried to prepare FLB. The excitation and emission wavelengths for DTAF are 492 and 513 nm, respectively, which are almost identical to those of the more frequently used protein-specific stain fluorescein isothiocyanate. Although fluorescein isothiocyanate-stained bacteria initially produced equally bright fluorescence, the fluorescence intensity tended to fade rapidly (within 30 s) in blue light compared with DTAF, which did not exhibit serious bleaching for up to 5 min. In addition, suspensions of fluorescein isothiocyanate-stained cells kept for several hours at room temperature in the dark showed a marked reduction in fluorescence intensity as a result of continual bleeding of the dye from the cells. Bleeding of the stain from bacterial cells was also a major problem with AO and DAPI. In contrast, DTAF-stained cells retained bright fluorescence, with no bleeding of the stain, for 3 days at room temperature and for months when stored frozed or freeze-dried.

Although we have not yet attempted to prepare FLB from a wide range of bacterial isolates or natural assemblages, any bacteria that can be grown in culture or concentrated from dilute suspension may in theory be successfully subjected to the staining-storage procedure described above. However, many strains may tend to form clumps when concentrated from senescent cultures. This is thought to be a result of divalent cation-mediated glycocalyx bridging (28). The problem may be minimized for species isolates by harvesting the growing population of cells during the log phase. If some degree of clumping still occurs, the concentrate can be briefly sonicated as described in Materials and Methods without danger of cell lysis. We have found that after being stained the FLB can be monodispersed if the bacteria are sonicated in a 0.02 M solution of tetrasodium PP$_i$; PP$_i$ is a chelating agent which probably acts to sequester calcium and magnesium ions, which are thought to be involved in extracellular polymeric bridging reactions (28).

The FLB method appears to be a straightforward, fairly simple approach to determining rates of bacterivory by in
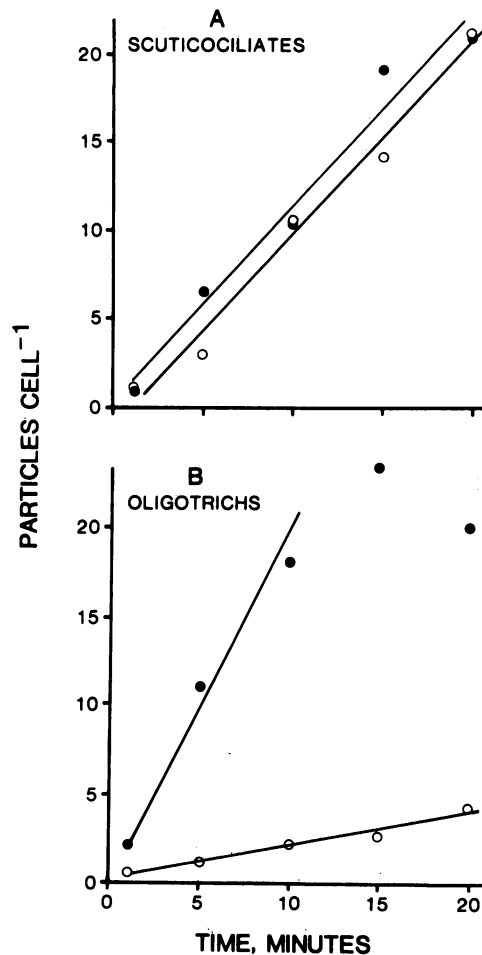

FIG. 6. Short-term uptake of FLB (●) and fluorescent microspheres (○) by estuarine scuticociliates (A) and oligotrichous ciliates (B).

situ populations of phagotrophic protozoa. The advantage of this technique is that bacteria are used as a food source to measure virtually instantaneous protozoan ingestion rates. Presumably, these rates should be the most accurate direct estimates yet available. However, additional testing of this approach and comparison with other techniques are indicated. For example, we do not know to what extent in situ protozoa discriminate between different strains or sizes of bacteria. The bacterial isolate we used for the preliminary assays reported here was from fresh water and had an average size of 0.10 μm^3, about twice the average biovolume of estuarine bacteria (26). To date, we have completed only one experiment comparing the uptake of FLB prepared from strain FW-1 and natural bacterioplankton by phagotrophic flagellates. The results showed no significant difference between ingestion of FW-1 and natural bacterioplankton for a bacterivorous flagellate culture, but an in situ assemblage of estuarine flagellates ingested FW-1 twice as rapidly as they did the natural bacterioplankton (data not presented).

Results of the protozoan growth experiments showed that FLB are not toxic to protozoa and in fact can be metabolized to support rapid population growth of ciliates and flagellates (Fig. 3 and 4). Because FLB are digested, assays of protozoan bacterivory that involve the use of FLB must be of very

TABLE 1. Preliminary results comparing protozoan uptake of FLB and fluorescent microspheres

Protozoa	Mean no. per cell ± 1 SD of[a]:	
	FLB	Microspheres
Tidal creek ciliates (mostly oligotrichs)	10.5 ± 1.2	2.7 ± 1.2
Tidal creek flagellates	0.77 ± 0.23	0.10 ± 0.04
Mixed bacterivorous flagellates	0.87 ± 0.37	0.07 ± 0.08

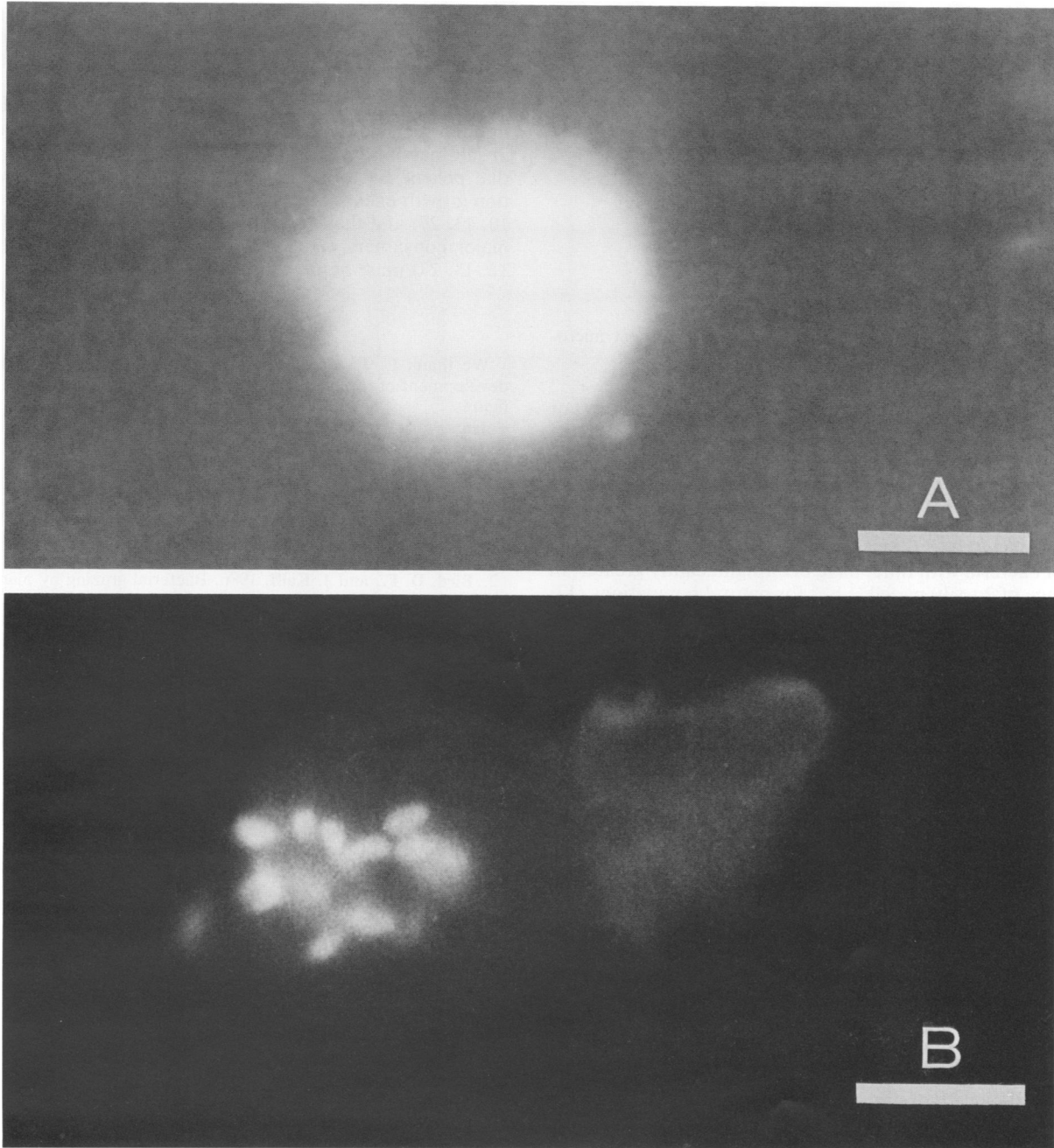[a] Number after equilibrium between rates of ingestion and egestion-digestion had been attained.

FIG. 7. Oligotrichous ciliate (diameter, 10 μm) from natural protozoan assemblage grazing FLB. (A) Cell visualized with DAPI fluorescence; (B) food vacuole containing 17 FLB visualized with the AO epifluorescence filter set. Bar, 5 μm.

short duration; after 10 to 30 min, the rate of ingestion of FLB apparently begins to equilibrate with the rate of digestion of FLB (Fig. 6 and 8). It should be possible to estimate some aspects of protozoan feeding physiology, e.g., rates of food vacuole formation and rates of digestion of bacteria, by using FLB.

Comparison of protozoan uptake of FLB and fluorescent microspheres showed that while *U. marina* took up both types of particle at the same rate, oligotrichous ciliates and flagellates did discriminate against microspheres in favor of bacteria (Table 1; Fig. 6 and 8). Our results are similar to those of M. Pace (Abstr. Annu. Meet. Am. Soc. Limnol. Oceanogr. 1986, p. 96), who found in a series of chemostat experiments that although a scuticociliate, *Cyclidium* sp., showed no perference for latex microspheres or bacteria, some cultured flagellate species did ingest bacteria more rapidly than they ingested microspheres. Recent information that phagotrophic protozoa may be chemosensory to their prey (5; M. J. Sibbald, L. J. Albright, and P. R. Sibbald, Mar. Ecol. Prog. Ser., in press) suggests that protozoa are capable of distinguishing between food and inert particles.

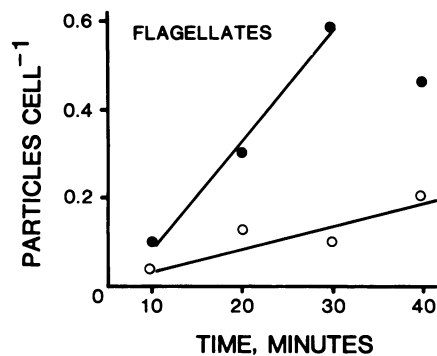If, as we have shown here, in situ protozoa ingest micro-

FIG. 8. Short-term uptake of FLB (●) and fluorescent microspheres (○) by estuarine microflagellates.

spheres more slowly than they ingest bacteria, then assays of bacterivory involving the use of bacterial-sized microspheres should be underestimates. Published data indicate that this is so. Clearance rates determined by using microspheres for population of aplastidic flagellates are on the order of 0.4 to 1.2 nl cell^{-1} h^{-1} (6, 20). Indirect estimates of flagellate clearance rates, determined from the disappearance of bacteria with time, have been significantly greater, in the range of 2 to 30 nl cell^{-1} h^{-1} for natural populations (17, 25) and as high as 79 to 336 nl cell $^{-1}$ h^{-1} for cultured flagellate species grazing 10^5 to 10^6 bacteria ml^{-1} (7, 11). From the uptake of FLB, we calculated that a natural assemblage of flagellates in tidal creek water had an average clearance rate of 3.2 nl cell^{-1} h^{-1}; this value is higher than the clearance rates determined for other flagellate by using microspheres and is within the range of 2 to 10 nl cell^{-1} h^{-1} that was estimated for phagotrophic nanoplankton on the basis of selective inhibitor experiments also carried out with tidal creek water (25). Our rates of flagellate bacterivory may be artificially low, however, owing to ejection of ingested material (i. e., microspheres or stained bacteria) by phagotrophic flagellates upon preservation with formalin, a phenomenon recently described by Sieracki et al. (M. E. Sieracki, L. W. Haas, D. A. Caron, and E. J. Lessard, submitted for publication).

There is evidence that some microalgae in fresh and marine waters will ingest bacterial-sized particles (2, 8; K. Porter, Hydrobiologia, in press). Much of this evidence is based on the uptake of fluorescent microspheres by pigmented flagellates and other phytoplankton (2; Porter, in press), although Estep et al. (8) presented electron micrographs of bacteria within food vacuoles of oceanic, plastidic chrysomonads. We looked for uptake of microspheres or FLB by phytoplankton but only rarely found apparent ingestion of either type of particle by pigmented flagellates or by the autotrophic ciliate *Mesodinium* sp., which were abundant in tidal creek water. McManus and Fuhrman (20) also reported that pigmented nanoplankton showed no significant ingestion of microspheres in their coastal water samples.

A major finding of our initial bacterivory experiments with FLB is that small pelagic ciliates, both oligotrichs and scuticociliates, can be voracious consumers of bacterioplankton. In a separate series of experiments, we have measured grazing rates for several estuarine ciliates which were 10 to 100 times higher than previously estimated rates of ciliate bacterivory (25a). It has only recently been appreciated that small aloricate ciliates are abundant in the sea and are potentially important bacterivores (15, 23, 26). We estimated

from the FLB uptake rates in the tidal creek water sample that the natural ciliate population could clear 90% of the water column of bacteria in a day, while the assemblage of colorless flagellates could clear only 15% of the water column in a day. This finding supports our previous suggestion that pelagic ciliates could be the dominant bacterivores in salt marsh tidal creeks (26). The generally accepted ideas that pelagic bacterivorous ciliates cannot maintain population growth except at microsites of high bacterial density (9, 10, 13, 27) and that aplastidic flagellates are universally the major consumers of bacterioplankton in marine waters (1, 12, 13, 27) must be reevaluated.

ACKNOWLEDGMENTS

LITERATURE CITED

1. Azam, F., T. Fenchel, J. G. Field, J. S. Grey, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10:257–263.
2. Bird, D. F., and J. Kalff. 1986. Bacterial grazing by planktonic lake algae. Science 231:493–494.
3. Borsheim, K. Y. 1984. Clearance rates of bacteria-sized particles by freshwater ciliates, measured with mono-disperse fluorescent latex beads. Oecologia 63:286–288.
4. Bratbak, G., and I. Dundas. 1984. Bacterial dry matter content and biomass estimations. Appl. Environ. Microbiol. 48:755–757.
5. Capriulo, G. M., J. Tavernas, and K. Gold. 1986. Ciliate feeding: effect of food presence or absence on occurrence of striae in tintinnids. Mar. Ecol. Prog. Ser. 30:145–158.
6. Cynar, F. J., and J. M. Sieburth. 1986. Unambiguous detection and improved quantification of phagotrophy in apochlorotic nanoflagel lates using fluorescent microspheres and concomitant phase contrast and epifluorescence microscopy. Mar. Ecol. Prog. Ser. 32:61–70.
7. Davis, P. G., and J. M. Sieburth. 1984. Estuarine and oceanic microflagellate predation of actively growing bacteria: estimation by frequency of dividing-divided bacteria. Mar. Ecol. Prog. Ser. 19:237–246.
8. Estep, K. W., P. G. Davis, M. D. Keller, and J. M. Sieburth. 1986. How important are oceanic algal nanoflagellates in bacterivory? Limnol. Oceanogr. 31:646–650.
9. Fenchel, T. 1980. Suspension feeding in ciliated protozoa: functional response and particle size selection. Microb. Ecol. 6:1–11.
10. Fenchel, T. 1980. Relation between particle size selection and clearance in suspension-feeding ciliates. Limnol. Oceanogr. 25:733–738.
11. Fenchel, T. 1982. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. Mar. Ecol. Prog. Ser. 8:225–231.
12. Fenchel, T. 1982. Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar. Ecol. Prog. Ser. 9:35–42.
13. Fenchel, T. 1984. Suspended marine bacteria as a food source, p. 301–316. In M. J. R. Fasham (ed.), Flows of energy and materials in marine ecosystems. Plenum Publishing Corp., New York.
14. Fuhrman, J. A., and G. B. McManus. 1984. Do bacteria-sized marine eukaryotes consume significant bacterial production? Science 224:1257–1260.
15. Gast, V. 1985. Bacteria as a food source for microzooplankton in the Schlei Fjord and Baltic Sea with special reference to ciliates. Mar. Ecol. Prog. Ser. 22:107–120.
16. Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33:1225–1228.

17. Landry, M. R., L. W. Haas, and V. L. Fagerness. 1984. Dynamics of microbial plankton communities: experiments in Kaneohe Bay, Hawaii. Mar. Ecol. Prog. Ser. 16:127–133.

18. Lee, J. J., S. H. Hutner, and E. C. Bovee. 1985. An illustrated guide to the protozoa. Society of Protozoologists, Lawrence, Kans.

19. Lessard, E. J., and E. Swift. 1985. Species-specific grazing rates of heterotrophic dinoflagellates in oceanic waters, measured with a dual-label radioisotope technique. Mar. Biol. (Berlin) 87:289–296.

20. McManus, G. B., and J. A. Fuhrman. 1986. Bacterivory in seawater studies with the use of inert fluorescent particles. Limnol. Oceanogr. 31:420–426.

21. Newell, S. Y., B. F. Sherr, E. B. Sherr, and R. D. Fallon. 1983. Bacterial response to presence of eukaryote inhibitors in water from a coastal marine environment. Mar. Environ. Res. 10:147–157.

22. Porter, K. G., and Y. S. Feig. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25:943–948.

23. Rivier, A., D. C. Brownlee, R. W. Sheldon, and F. Rassoulzadegan. 1985. Growth of microzooplankton: a comparative study of bacterivorous zooflagellates and ciliates. Mar. Microb. Food Webs 1:36–51.

24. Sanders, R. W., and K. G. Porter. 1986. Use of metabolic inhibitors to estimate protozooplankton grazing and bacterial production in a monomictic eutrophic lake with an anaerobic hypolimnion. Appl. Environ. Microbiol. 52:101–107.

25. Sherr, B. F., E. B. Sherr, T. A. Andrew, R. D. Fallon, and S. Y. Newell. 1986. Trophic interactions between heterotrophic protozoa and bacterioplankton in estuarine water analysed with selective metabolic inhibitors. Mar. Ecol. Prog. Ser. 32: 169–179.

25a.Sherr, E. B., and B. F. Sherr. 1987. High rates of consumption of bacteria by pelagic ciliates. Nature (London) 325:710–711.

26. Sherr, E. B., B. F. Sherr, R. D. Fallon, and S. Y. Newell. 1986. Small aloricate ciliates as a major component of the marine heterotrophic nanoplankton. Limnol. Oceanogr. 31:177–183.

27. Sieburth, J. M. 1984. Protozoan bacterivory in pelagic marine waters, p. 405–444. In J. E. Hobbie and P. J. L. Williams (ed.), Heterotrophic activity in the sea. Plenum Publishing Corp., New York.

28. Velji, M. I., and L. J. Albright. 1986. The dispersion of adhered marine bacteria by pyrophosphate and ultrasound prior to direct counting, p. 249–259. In International Colloquium on Marine Bacteriology. Second Centre National de la Recherche Scientifique IFREMER, Actes de Colloques, 3, Brest, France.

29. Wikner, J., A. Andersson, S. Normark, and A. Hagstrom. 1986. Use of genetically marked minicells as a probe in measurement of predation on bacteria in aquatic environments. Appl. Environ. Microbiol. 52:4–8.

30. Wright, R. T., and R. B. Coffin. 1984. Measuring microzooplankton grazing on planktonic marine bacteria by its impact on bacterial production. Microb. Ecol. 10:137–149.