Differential survival of bacteria ingested by zooplankton from a stratified eutrophic lake

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Abstract

Field studies were performed in stratified Lake Oglethorpe to isolate and identify culturable, free-living bacterioplankton from lake water and those associated within zooplankton. Heterotrophic colony counts of whole water were lower than acridine orange epifluorescent direct counts by 3–4 orders of magnitude, whereas colony counts were increased by an order of magnitude when the lake water was fractionated for zooplankton, then treated and sonicated to release bacteria from within zooplankton. Cultured bacterial species were segregated by sampling depth among the epilimnion, metalimnion, and hypolimnion and the zooplankton fractions. Laboratory feeding experiments with Daphnia ambigua and mixed suspensions of these lake bacteria demonstrated that zooplankton-associated bacteria survived induced gut retention in D. ambigua for 23 h longer than free-living bacteria. Feeding rate experiments showed no difference between ingestion or clearance rates of the mixed cultured bacteria, but bacterial inhibition studies showed that survivors inhibited nonsurvivors. These studies suggest that some bacterioplankton can survive digestion and interact in guts of zooplankton.

Bacterioplankton communities have seasonal, spatial, and between-system varia-

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There are strong positive correlations between bacterial production and both primary production (Cole et al. 1988) and grazing (Sanders et al. 1989). Grazing is believed to be such an important factor that it is assumed to be operating even when there is no direct evidence of bacterial ingestion or death (Pace 1988). Grazing has been proposed to influence bacterial abundance and production both directly through consumption of bacteria and indirectly through grazing on phytoplankton with subsequent release of organic substrate (Pedró-Alió and Brock 1983; Porter 1984; Güde 1988). Sieburth and Davis (1982) proposed that heterotrophic protozoan grazing may stimulate bacterial growth either by recycling organic C and other nutrient and energy sources or by holding bacterial numbers below carrying capacity and thus preventing substrate limitation. In the absence of substrate limitation, cells are maintained in log growth phase, and turnover and production may be enhanced. Due to the limiting concentrations of nutrients in many aquatic ecosystems, significant differences in recycling rates of one or more nutrient resources could regulate the production of species capable of utilizing these resources. This regulation in recycling of substrate by grazers may result in seasonal and spatial variations in bacterial production within systems and differences along the trophic gradient from oligotrophy to eutrophy (Porter et al. 1988).

The inherent resistance of some bacterial species to digestion is a factor that has been ignored in analyzing the effect of grazing on bacterial populations. Grazing rates measured by short-term uptake of labeled bacteria or tracer particles determine ingestion rates, and mortality is assumed to follow. Grazing may not always result in cell death, however, and could actually enhance bacterial growth through survival in the enriched “microenvironment” of a grazer’s digestive system. Edwardsiella tarda and Aeromonas salmonicida, common bacterial pathogens of freshwater fish, exhibit enhanced growth rates when cocultured with Tetrahymena pyriformis (King and Shotts 1988). Chlorine isolation methods used to isolate viable intracellular bacteria from protozoa showed that intracellular survival and uptake of nutrients by these bacteria were the probable mechanisms supporting the increased growth.

In this study, we used chlorination followed by neutralization of the chlorine as a technique to eliminate free-living bacteria from lake water containing crustaceans and protozooplankton. Ingested bacteria were then released from the grazers by sonication and plated for enumeration and identification (King et al. 1988). These methods were used to test three hypotheses: that numerous bacteria in aquatic ecosystems are ingested by zooplankton, but not all bacteria experience mortality due to ingestion; that surviving species may have enhanced growth due to the time spent in nutrient-rich microzones of zooplankton; and that enhanced growth may facilitate enhanced isolation of the bacteria on nonselective agar medium.

These hypotheses were tested through field studies involving the isolation and identification of bacteria from whole water and from various treated and sonicated zooplankton size fractions. Laboratory experiments to discriminate between selective ingestion and differential survivorship (resistance to digestion) were conducted and implications for regulating bacterioplankton noted.

**Materials and methods**

**Field site**—Lake Oglethorpe is a shallow \( z_{\text{max}} = 8 \) m, 30-ha, eutrophic, man-made lake in northeastern Georgia that has been studied extensively during the past 10 yr with reference to bacterial abundance, production, and grazing by macro- and microzooplankton (Porter et al. 1983; Sanders et al. 1989). Samples for this study were collected on 25 September and 8 October 1987 at the end of summer stratification. The September sampling was a qualitative field study to test the isolation methods for bacterial culture and zooplankton size fractionation. The October study was performed quantitatively to enumerate and identify the bacteria and zooplankton.
Before sampling, oxygen and temperature were measured at 0.25-m intervals with a YSI model 57 dissolved oxygen and temperature meter (Yellow Springs Instrument Co.) to determine the extent of the well-mixed upper waters (epilimnion), the anaerobic lower waters (hypolimnion), and the transition zone (metalimnion). Samples were collected at depths representative of the epilimnion, and the top and bottom of the metalimnion on the two dates because previous studies showed seasonal and vertical differences in abundances and activities of microorganisms in these different layers of water (McDonough et al. 1986; Sanders et al. 1989).

Field survival studies—Whole lake water was collected aseptically at each depth for bacterioplankton with a sterile bag sampler (model 1030, General Oceanics Inc.). These samples were spread plated onto trypticase glucose extract (TGE) agar (Difco Lab.) and incubated at three temperatures (20°C, 25°C, and 37°C) for direct culture of aerobic or facultative anaerobic bacteria from whole lake water. Culturable bacteria were enumerated with standard pour-plate methods for colony-forming units (CFU) (Am. Public Health Assoc. 1985), and total bacterial abundance at each depth was determined by the epifluorescent direct count method (Porter and Feig 1980).

Whole water samples for macro- and microzooplankton were collected from each depth with a 5-liter Niskin sampler (General Oceanics Inc.). Sequential filtration for size separation of macro- and microzooplankton was performed (Fig. 1). Macrozooplankton were concentrated on a 63-μm Nitex screen (Tetco Inc.), rinsed three times, and resuspended in 0.22-μm filter-sterilized (Millipore) lake water. Subsamples of the >63-μm fraction were fixed in 2% sucrose-saturated Formalin solution for enumerating and identifying crustaceans, rotifers, and protists. The rest of the concentrated macrozooplankton fraction (>63-μmμ) was narcotized with 0.22-μm filter-sterilized, carbonated water for 5 min to prevent egestion of gut contents. The macrozooplankton were then placed into filter-sterilized (particle-free) lake water for ~15–20 min before further treatment. Time for removal of the original lake-water sample until further treatment and, hence, the time at which retention of gut contents was induced by anesthesia and placement in particle-free water was ~30–45 min. The filter-sterilized lake water and carbonated water were cultured on TGE agar and incubated at 25°C for 48 h to test for sterility.

Two microzooplankton fractions were prepared by gentle, sequential filtration of the 63-μm filtrate through 20-μm Nitex netting (to collect ciliates, rotifers, and algae) and over 1-μm filters (Nuclepore Corp.) to collect nanoflagellates. These size fractions were gently resuspended in 0.22-μm-filtered sterile lake water. Separate subsamples from these two fractions were fixed in 1% Lugol’s iodide for enumeration and identification of the microzooplankton and nanoflagellates.

Plankton from each of the fractionated samples were chlorinated in 10 mg liter⁻¹ NaOCl for 5 min (Am. Public Health Assoc. 1985). Then chlorine was neutralized for 5 min by adding sterile 1% sodium thiosulfate. Samples were sonicated at 50 W for 15 s with a Branson sonifier-cell disrupter 200 (Branson Sonic Power Co.) to disrupt all animals and protist cells and release internal bacteria (King and Shotts 1988; King et al. 1988). Samples were cultured and enumerated for aerobic and facultative anaerobic bacteria on TGE as described above. Total CFU ml⁻¹ for size fractions were calculated to account for concentration and represent the CFU protected inside organisms per milliliter of whole lake water.

Controls were performed at one depth (5.25 m) to show that chlorine levels were sufficient to eliminate all bacteria in the water outside the zooplankton. Subsamples of zooplankton fractions were sonicated first as described above, treated with 10 mg liter⁻¹ of free chlorine residual, neutralized, and cultured on TGE. These plates were scored for colony growth after incubation at 25°C for 48 h. Additional subsamples were fixed before and after chlorine treatment and examined by light microscopy to test for chlorine lysis of the organisms (Fig. 1).

Bacterial isolates cultured from whole lake water and treated zooplankton fractions were grouped by colonial and cellular mor-
Fig. 1. Flow diagram showing fractionation and treatment of sampled lake water for the isolation and enumeration of bacterioplankton from lake water and those associated with zooplankton. Enumerations of heterotrophic colony counts (CFU) from sampled water were made from treatments of (left to right): sonicated whole water; size-fractionated whole water with subsequent chlorination, neutralization, and sonication; sonicated, chlorinated whole water; and untreated whole water.

Grouping was confirmed by standard biochemical identification methods (Holt and Krieg 1984; Sneath 1986; Austin 1988), and bacteria were subsequently identified to genus.

Laboratory survival studies—Preliminary experiments showed that most of the bacterivorous zooplankton that remained intact during both size fractionation and chlo-
Bacterioplankton survival

rination were isolated from the >63-μm macrozooplankton fraction and included the cladoceran Daphnia ambigua. The zooplankton in this fraction had been removed directly from the water column, anesthetized, and placed into particle-free water for ~30-45 min before treatment (chlorination, neutralization, and sonication). This procedure resulted in gut retention of ingested particles by Daphnia.

In a series of laboratory experiments, we examined the hypothesis that bacterial species might have survived ingestion and digestion within some of the macrozooplankton by determining the number of CFU from D. ambigua guts over residence time in the gut, thus providing an explanation for the differences in culturable species composition that were observed in the field studies (see results, Tables 1 and 2). The bacterial feeding inoculum consisted of a combination of one representative bacterial species cultured only from whole lake-water samples and thus presumed digestible, Staphylococcus sp. 2 (see results, Table 2), and two rod-shaped Corynebacterium species cultured separately from both whole lake water and the >63-μm treated zooplankton fraction of whole lake water (see results, Table 1) and hypothesized to be indigestible. These bacteria also represented two morphologically different cell types (cocci and rods) which could affect feeding rates by Daphnia in these experiments. Staphylococcus sp. 2 was also fed to D. ambigua to examine its survivorship in the absence of other bacteria and to test the possibility of interaction among bacterial species while in the gut.

Bacterial strains used in these experiments were grown separately on TGE agar at 25°C for 24 h, aseptically washed off the plates with sterile, aged, filtered (0.22 μm) tapwater (SAFT), and pipetted into centrifuge tubes. All cultures were pelleted by centrifugation (3,015 × g) for 10 min at 4°C. The supernatant was discarded, and bacterial pellets were washed three times in SAFT as above. Bacteria were resuspended in SAFT and adjusted to appropriate concentrations with a cell concentration-turbidity relationship determined with a Spectronic 210 UV spectrophotometer at a wavelength of 660 nm (Bausch and Lomb). Serial 10-fold dilutions were done in SAFT to obtain desired bacterial concentrations of 10⁶ CFU ml⁻¹ before the feeding experiments.

The cladoceran D. ambigua was chosen as a macrozooplankton representative from the field samples because it is known to ingest natural-sized bacteria and microspheres at least as small as 0.5 μm (Porter 1984; Sanders et al. 1989), and these animals have a seasonal population peak in October (Orcutt and Porter 1984; Leeper pers. comm.). Daphnia ambigua isolated from Lake Oglethorpe during the field studies was cultured (20°C) on the alga Chlamydomonas reinhardtii in open tanks containing aged filtered tapwater (SAFT).

Daphnia ambigua adults were isolated from the culture tank via a pipette, and transferred to a sterile beaker of SAFT. The animals were allowed to swim out of the pipette tip, thereby minimizing the amount of culture water transferred to the beakers. The animals were incubated without added food at 25°C for 24 h to allow digestion of any ingested algae or bacteria and to achieve gut clearance before adding the test strains of bacteria.

A subsample of D. ambigua was removed after the 24-h “starvation” period to test for culturable bacteria remaining in the animals and to ensure that there were no contaminant bacteria inside the starved animals during the feeding experiments. Five animals were removed and narcotized in 0.22-μm-filtered, carbonated water for 5 min. They were placed in a chlorine treatment solution (4.0 mg liter⁻¹ of free available chlorine, pH 7.0, 25°C) for 5 min, after which the solution was neutralized with 1% sodium thiosulfate. After sonication, the resulting suspension was plated on TGE agar as described above. Preliminary experiments performed to establish lethal doses of free chlorine residual for the three bacterial strains showed that all microorganisms were killed in 5 min by >2.0 mg liter⁻¹ free chlorine residual (data not shown).

The starved animals were transferred into a feeding chamber that consisted of a Plexiglas tube with 308-μm screening (Tetco Inc.) on the bottom. This chamber was mounted
Table 1. Description and estimated percentage of total bacterioplankton cultured on 8 October 1987 from whole lake water and treated lake water taken from the same depth.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Bacteria*</th>
<th>Estimated % CFU ml⁻¹</th>
<th>Whole water</th>
<th>Treated water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>(+) Corynebacterium sp. 2†</td>
<td>6.9</td>
<td>16.5‡</td>
<td></td>
</tr>
<tr>
<td>5.25</td>
<td>(+) Corynebacterium sp. 3</td>
<td>2.8</td>
<td>25.4‡</td>
<td></td>
</tr>
<tr>
<td>5.75</td>
<td>(+) Corynebacterium sp. 4</td>
<td>1.7</td>
<td>3.2‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-) Bacillus sp. 2</td>
<td>1.4</td>
<td>6.4§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-) Acinetobacter sp.</td>
<td>1.4</td>
<td>19.1§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-) Pseudomonas sp. 8</td>
<td>27.5</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

* (+) = Gram-positive; (-) = gram-negative.
† Total abundance of species with overlap between control (whole water) and treated fractions.
‡ Cultured from >63-μm fraction.
§ Cultured from 63–20-μm fraction.
|| Cultured from <20-μm fraction.

Vertically in a 500-ml beaker containing SAFI with the bottom of the chamber above the bottom of the beaker. The chamber was then inoculated with the two corynebacterial species and Staphylococcus or Staphylococcus alone to a final concentration of 3 × 10⁶ cells ml⁻¹ of each species and incubated for 2 h at 25°C. The feeding suspension was gently stirred from the bottom of the beaker with a magnetic stir bar (without causing an obvious vortex at the surface) to prevent settling of the bacteria. Stirring did not disturb the swimming patterns of *D. ambigua* in the feeding chamber.

The feeding chamber was aspirated after the 2 h of feeding to remove most of the SAFI and bacteria (while keeping the *Daphnia* in suspension) and then rinsed several times with fresh SAFI. A continuous flow of SAFI at a rate of 76 ml h⁻¹ washed the feeding chamber to remove the remaining uningested bacteria. Spread plates for CFU ml⁻¹ (TGE, 25°C) showed that < 10 CFU ml⁻¹ were present external to the *Daphnia* in suspension at any time the continuous-flow system was operating.

After a 2-h feeding period (at t = 0), 5–10 *Daphnia* were removed from the feeding chamber, narcotized, treated with free chlorine residuals (4 mg liter⁻¹ of NaOCl, pH 7.0, 25°C), neutralized, and sonicated as above to release ingested bacteria. Sonicated test suspensions were plated for enumeration of CFU of each bacterial species on TGE agar and incubated at 25°C for 24 h. This procedure was repeated at intervals representing t = 30, 60, 120 min, and 6, 19, and 24 h after removal of the bacterial food suspension.

Selective feeding studies—Feeding rates of *D. ambigua* were determined with cultured fluorescently labeled bacteria (FLB) of the two bacterial types (rods and cocci) used in the laboratory survival studies described above. It was assumed that there was no difference in *D. ambigua* feeding rates between viable, heat-killed and fluorescently labeled bacteria of the same species. All FLB feeding experiments were performed in duplicate.

The FLB were prepared from the *Corynebacterium* sp. 3 (Table 1) and *Staphylococcus* sp. 2 (Table 2) cultured in brain-heart infusion broth (Difco Laboratories) for 48 h at 25°C by the method of Sherr et al. (1987). After sonication, subsamples of each FLB suspension were examined via epifluorescence microscopy and found to contain very few clumps of bacteria. Each suspension of FLB was checked for bacterial growth or contamination by inoculation into brain-heart infusion broth before the experiments.

Animals were starved in SAFI as described in the survival studies above, and 50 randomly chosen adult animals were transferred for acclimation into each of two sterile 150-ml beakers (designated A and B) each containing a mixture of ~2.0 × 10⁶ cells ml⁻¹ of heat-killed, unstained *Corynebacterium* sp. and 2.0 × 10⁶ cells ml⁻¹ of heat-killed, unstained *Staphylococcus* sp. The animals in both beakers were allowed to acclimate for 30 min in these suspensions before the addition of the FLB of either species.

The FLB-feeding inoculum consisted of either the mixed inoculum of 1.0 × 10⁶ *Corynebacterium* FLB and 1.0 × 10⁶ of heat-
Table 2. Species of bacteria cultured separately from either whole water or treated zooplankton fractions (size-fractionated, chlorinated, neutralized, and sonicated) but not cultured from both. The percentage of CFU ml\(^{-1}\) was calculated including the species from Table 1 that were found in common from both whole lake water and treated lake water. The percentage CFU ml\(^{-1}\) combined for all depths in whole water from Tables 1 and 2 sum to 100, as do the percentage CFU ml\(^{-1}\) for treated fractions.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Whole water</th>
<th>Treated fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacteria*</td>
<td>% CFU ml(^{-1})</td>
</tr>
<tr>
<td>1.00</td>
<td>(-) Aeromonas sp.†</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>(-) Pseudomonas sp. 1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(-) Edwardsiella sp.</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(+) Bacillus sp.</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(-) Pseudomonas sp. 3</td>
<td>1.4</td>
</tr>
<tr>
<td>5.25</td>
<td>(-) Pseudomonas sp. 4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(+) Staphylococcus sp. 2</td>
<td>1.4</td>
</tr>
<tr>
<td>5.75</td>
<td>(-) Pseudomonas sp. 5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(-) Proteus sp. 2</td>
<td>1.3‡</td>
</tr>
<tr>
<td></td>
<td>(-) Pseudomonas sp. 6</td>
<td>0.6‖</td>
</tr>
</tbody>
</table>

* (+)—Gram-positive; (−)—gram-negative.
† Species numbers on identical genera represent different species or strains due to different biochemical profile numbers.
‡ Cultured from >63-μm fraction. Estimated total abundance from this fraction (Tables 1 and 2) was 6.1 × 10⁷ CFU ml\(^{-1}\).
§ Cultured from 63- to 20-μm fraction. Estimated total abundance from this fraction (Tables 1 and 2) was 4.8 × 10⁷ CFU ml\(^{-1}\).
‖ Cultured from <20-μm fraction. Estimated total abundance from this fraction (Tables 1 and 2) was 4.9 × 10⁷ CFU ml\(^{-1}\).

killed, unstained Staphylococcus sp. (beaker A) or the mixed inoculum of 1.0 × 10⁶ Staphylococcus FLB and 1.0 × 10⁶ of heat-killed, unstained Corynebacterium sp. (beaker B). These mixtures of the bacteria were similar to those in the laboratory survival experiments. This design facilitated accurate counting of only one FLB species in the presence of an equal number of the second species.

The animals were allowed to feed in the FLB suspensions for 15 min, collected on a sieve, heat killed in 60°C SAFT water, and rinsed 5 times with SAFT to remove non-ingested bacteria. Microscopic observations confirmed the removal of uningested bacteria. The animals from each beaker were then counted, placed in glass scintillation vials containing 1 ml of SAFT, and sonicated at 44 W for 20 s to release ingested bacteria. Subsamples of the resulting bacterial suspensions were collected on 0.2-μm Nuclepore filters and the FLB counted with epifluorescent microscopy. The level and duration of sonication used in the experiments did not significantly affect the numbers of either of the bacterial species in FLB form (data not shown).

FLB were measured visually for comparison of size (length in μm) and volume (μm³) via two-dimensional epifluorescent images (1,200×) of randomly chosen cells. Cocci volumes were calculated, assuming perfect spheres, via the equation: \(\text{vol} = \frac{4}{3}\pi r^3\) where \(r\) is \(\frac{1}{2}\) the measured diameter. The rods were calculated assuming cylinders with hemispherical ends via the equation: \(\text{vol} = \frac{\pi}{4} \times w^2(l - w/3)\) where \(l\) is length and \(w\) is width (Fuhrman 1981; Krambeck et al. 1981).

Size and volume measurements of each FLB type (cocci and rods) showed that mean length and volume of the FLB rods were significantly larger \((P < 0.01)\) than the FLB cocci. Mean length \((±SE)\) of Corynebacterium sp. 3 FLB was 2.29±0.06 μm, mean width was 0.55±0.07 μm, and mean volume was 0.50±0.06 μm³. The spherical cocci had a mean diameter of 0.65±0.06 μm and mean volume of 0.15±0.03 μm³.

Bacterial inhibition experiments—The stab-overlay (Pugsley 1985) and patch test (DeLorenzo and Aguilar 1984) methods were used to test for interbacterial inhibition among the strains ingested together by Daphnia during the laboratory survival experiments. Briefly, the stab-overlay method involved stabbing the test bacterium (which was chosen from isolated colonies of Corynebacterium sp. 3 and 4 and Staphylo-
coccus sp. 2) into nutrient agar, followed by incubation at 25°C for 24 h. The plates were inverted, and a 9-cm pad of Whatman No. 1 filter paper soaked with ~0.3 ml of chloroform was placed in the lid of the plates. The agar-containing portion of the plate was closed over the lid. After 30 min the pads were removed and the plates were overlaid with 3 ml of soft nutrient agar (50°C) containing 10^6 CFU ml^-1 of indicator bacteria grown previously in nutrient broth at 25°C for 24 h and washed 3 times in sterile distilled water before resuspension into the nutrient agar.

When either of the two colonies from the corynebacteria were stabbed, the *Staphylococcus* was used as the indicator strain in the nutrient agar; when colonies from the *Staphylococcus* were stabbed, one or the other corynebacteria was overlaid as the indicator strain. The plates were then inverted, incubated at 25°C for 24 h, and plaques were scored to test for inhibition.

The patch test was similar to the overlay test, except the indicator strain in soft agar was overlaid onto fresh nutrient agar before inoculation and incubation of the stab culture. The soft agar was allowed to harden, and the test bacterium was stabbed into this prepared agar. The plates were then inverted, incubated at 25°C for 24 h, and scored for plaques.

**Results**

**Field survival studies**—The well-mixed epilimnion extended from the surface to 4 m during the initial field experiment (25 September 1987). The rapid drop in temperature and oxygen with depth defined the metalimnion from 4 to 7 m, and the hypolimnion (below 7 m) was anoxic. Qualitative and quantitative differences were found on 25 September at all depths (Fig. 2) between colony types cultured from whole water and fractionated whole water that had been chlorinated, neutralized, and sonicated to release bacteria from within zooplankton. Sonication followed by chlorination effectively eliminated all viable bacteria and served as a control for the survival from chlorination of attached bacteria (Fig. 2, column 6). The abundance and diversity of colony types was greater in whole water sonicated to release bacteria from inside zooplankton compared with untreated whole water (Fig. 2, column 2 vs. 1). The >63-μm size fraction of chlorinated-neutralized-sonicated zooplankton (Fig. 2, column 3) contained the greatest abundance and diversity of colonies. This size fraction contained primarily crustaceans and rotifers.

By 8 October 1987, the top of the metalimnion had dropped to 5.25 m. Standard plate counts (CFU ml^-1) used to estimate densities of bacteria culturable on TGE agar were 3–4 orders of magnitude lower than total bacterial densities enumerated by epifluorescence direct counts on this date (Table 3). Standard plate counts were 4–16 times higher for bacteria from lake water sonified to release bacteria from within zooplankton compared to cultured, untreated, whole lake water (Table 3)—a finding similar to the pattern seen on the earlier sampling date (Fig. 2).

Size fractionation of the zooplankton at 5.25 m produced defined species separation (Table 4). The >63-μm fraction provided almost complete separation of the crustaceans and rotifers from protozoans, but the colonial mixotrophic alga *Dinobryon* was retained in the larger fraction. Bacterivorous macrozooplankton consisted of the cladoceran *D. ambigua*, and the rotifers *Keratella* and *Kelllicottia* at individual concentrations of between 0.4 and 1.3 ml^-1 of concentrate before and after chlorine treatment (Table 4). Individual cells of *Dinobryon* colonies were counted at densities of >900 and >400 ml^-1 in subsamples taken before and after chlorination. Both ciliate and flagellate abundances were about equal in each of the two size fractions of microzooplankton before chlorination (Table 4). Bacterivorous ciliate densities of different species ranged from 1 to 12 ml^-1 in the 20–63-μm fraction and 14–34 ml^-1 in the 1–20-μm fraction. Bacterivorous flagellates were more abundant than ciliates (200–300 ml^-1) before treatment with free chlorine residuals.

All macro- and microzooplankton were killed by treatment with a free chlorine residual of 10 mg liter^-1, but differences in susceptibility to chlorine lysis were observed. Crustacean and rotifer densities (No.
Fig. 2. Photograph of heterotrophic plate counts from the water column on 25 September 1987 showing differences in colony types and abundances with depth and treatment. Plates from whole water showed an increase in colony type and numbers after sonication (columns 1 and 2), while sonication followed by chlorination eliminated all bacterioplankton (column 6). Fractionation of the water at each depth followed by chlorination, neutralization, and sonication produced different colony types (columns 3, 4, and 5).

Free chlorine residuals (10 mg ml\(^{-1}\)) eliminated all bacteria outside of the zooplankton during treatment assays and those ingested by zooplankton and then released by sonication before chlorination treatment. Lack of colony growth on plate counts (TGE agar, 25°C) of these controls, compared to positive growth on plates from zooplankton treated with 10 mg liter\(^{-1}\) of free chlorine residual and then sonicated showed that bacteria associated with the zooplankton were protected from chlorination. These protected bacteria were presumed to be for the most part within the digestive system of the zooplankton. *Daphnia* continued to swim and move its filtering appendages for >2 min after immersion in chlorine, thus ensuring that even the interior of the carapace was exposed to the high free chlorine residuals. Because *Daphnia* is incapable of closing its carapace, all surfaces outside the

### Table 3. Bacterial abundances on 8 October 1987 estimated by epifluorescence direct counts (EDC) and standard plate counts of whole lake water vs. fractionated, chlorinated, and sonicated whole lake water containing released bacteria from within zooplankton.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>EDC (cells ml(^{-1}))</th>
<th>Whole water</th>
<th>Treated whole water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>4.1 (\times) 10(^6)</td>
<td>3.5 (\times) 10(^2)</td>
<td>1.1 (\times) 10(^4)</td>
</tr>
<tr>
<td></td>
<td>(1.3 (\times) 10(^6))(\dagger)</td>
<td>(1.9 (\times) 10(^3))</td>
<td>(6.4 (\times) 10(^2))</td>
</tr>
<tr>
<td>5.25</td>
<td>5.9 (\times) 10(^6)</td>
<td>1.1 (\times) 10(^3)</td>
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<td></td>
<td>(9.6 (\times) 10(^6))</td>
<td>(52.1)</td>
<td>(1.5 (\times) 10(^3))</td>
</tr>
<tr>
<td>5.75</td>
<td>5.9 (\times) 10(^6)</td>
<td>5.1 (\times) 10(^3)</td>
<td>1.0 (\times) 10(^4)</td>
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<td>(1.2 (\times) 10(^6))</td>
<td>(4.9 (\times) 10(^3))</td>
<td>(6.6 (\times) 10(^3))</td>
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</tbody>
</table>

* Spread plated on TGE agar and incubated at 20°C for 7 d. Abundances derived from mean number of all colony types visible after 7 d.

\(\dagger\) Numbers represent standard error of the means.
Table 4. Description and estimated density (No. ml-1) of macro- and microzooplankton found on 8 October 1987 in sequentially filtered fractions of whole lake water from 5.25 m before (B) and after (A) exposure to 10 mg liter-1 of NaOCl for 5 min. Numbers represent single counts of subsamples taken before and after chlorine treatment.

<table>
<thead>
<tr>
<th>Description</th>
<th>&gt;63 µm</th>
<th>63-20 µm</th>
<th>&lt;20 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrozooplankton</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daphnia</em></td>
<td>0.4</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>Copepods</td>
<td>0.2</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Nauplii</td>
<td>1.4</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td><em>Keratella</em></td>
<td>1.3</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td><em>Kellicottia</em></td>
<td>0.8</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td>Microzooplankton</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciliates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Halteria</em></td>
<td>-</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td><em>Uronema</em></td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>Cyclidium</em></td>
<td>-</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td><em>Oligotrich</em></td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Hymenostomes</td>
<td>-</td>
<td>-</td>
<td>31</td>
</tr>
<tr>
<td>Other</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Flagellates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Monas</em></td>
<td>-</td>
<td>-</td>
<td>121</td>
</tr>
<tr>
<td>Bodonid*</td>
<td>-</td>
<td>-</td>
<td>98</td>
</tr>
<tr>
<td><em>Rhodomonas</em></td>
<td>-</td>
<td>-</td>
<td>47</td>
</tr>
<tr>
<td>Dinoflagellate</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td><em>Cryptomonas</em></td>
<td>-</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>Cryptomonas†</td>
<td>-</td>
<td>-</td>
<td>69</td>
</tr>
<tr>
<td>Euglenoids</td>
<td>-</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td><em>Dinobryon</em>†</td>
<td>953</td>
<td>426</td>
<td>4</td>
</tr>
</tbody>
</table>

* Shown to be bacterivorous in Lake Oglethorpe (Sanders et al. 1989).
† Total colonial and single-celled forms.

animals were presumably exposed to the applied chlorine residuals. Epifluorescence microscopy of sonicated *Daphnia* showed that whole animals were disrupted, but filtering appendages and small pieces of carapace were present.

Microscopic examination confirmed detrital aggregates to be essentially absent from zooplankton fractions. Although the colonial alga *Dinobryon* was abundant (Table 4) in the 63-µm fraction, epifluorescence microscopy showed no evidence of bacterial attachment to the lorica. Furthermore, most loricas were empty or contained shrunken *Dinobryon* cells after chlorine treatment, indicating that even the insides of the loricas were exposed to chlorine.

Bacterial colony types cultured separately from untreated whole water and treated zooplankton fractions were enumerated for initial CFU ml-1, and streak plated onto separate, fresh TGE agar plates (25°C) for assessment of purity of morphological types. Many differing morphological types were observed on initial isolation of bacteria from the samples, but many of these colonies reverted to identical colony types upon subculture and were subsequently found to be of the same genus after biochemical identification. All colonies were grouped by their biochemical profiles and characterized at the generic level. Abundance data for colonies from initial isolation on TGE medium were grouped according to their identification profiles to achieve estimated CFU ml-1 of individual genera isolated at each depth. The percentage of total CFU ml-1 was calculated for each species isolated from untreated whole water or from treated zooplankton but not from both (Table 2), and from both whole water and treated zooplankton fractions (Table 1) at all depths.
Comparisons of bacterial isolates showed defined separation in the species composition of bacteria that could be cultured on TGE medium from untreated whole water and treated zooplankton at each depth and among depths (Table 2). Nine species (representing gram-positive and -negative organisms) were isolated exclusively from whole water samples at three depths and were not cultured from treated zooplankton fractions (Table 2). Greater than 80% of the bacteria isolated and enumerated (CFU ml⁻¹) from whole water were gram negative, with the flagellated bacteria *Alcaligenes* sp. and *Pseudomonas* sp. comprising >70% of these colonies (Tables 1 and 2). In comparison, 11 species were cultured from the treated zooplankton fractions (fractionated, chlorinated, neutralized, sonicated whole water) that were not cultured from whole water, and they included <30% of the total colonies isolated from these treatments (Table 2).

Six species were cultured from whole water and the treated zooplankton fractions (Table 1). All species except *Pseudomonas* sp. 8 had higher colony recovery upon initial isolation on TGE agar when cultured from the treated zooplankton fractions than from untreated whole water at the same depth (Table 1). The gram-negative bacterium *Acinetobacter* sp. was 1.37% CFU ml⁻¹ of the total bacteria cultured from whole water, but was 19.06% of the total colonies cultured from all treated zooplankton fractions. *Corynebacterium* sp. 3 showed the greatest differences in the proportion of total culturable colonies between whole water (2.75%) and the zooplankton treatment (25.40%). Corynebacteria also had the highest percentage of total CFU ml⁻¹ associated with the treated macrozooplankton fraction. Three species of *Corynebacterium* comprised ~45% of the total bacterioplankton cultured from all zooplankton fractions at all depths.

*Laboratory survival studies*—Laboratory survival experiments were designed with *D. ambigua*, bacterial species cultured from within treated macrozooplankton (*Corynebacterium* sp. 3 and 4, Table 1), and a bacterium cultured only from whole water (*Staphylococcus* sp. 2, Table 2). The *Corynebacterium* spp. were hypothesized to have survived ingestion and digestion, whereas *Staphylococcus* was presumed incapable of survival in *Daphnia*. Cultivation of bacteria from chlorine-treated and sonicated adult *D. ambigua* from the aquarium culture (starved in sterile, aged, filtered tapwater for 24 h) produced one colony (identified as a *Pseudomonas* sp.). This organism also was cultured from water sampled from the aquarium but was not isolated from the treated and sonicated *Daphnia* after the 2-h feeding period with experimental bacteria (*t = 0, Fig. 3*).

Although the three bacterial species were
in equal concentrations in the feeding suspension, abundances of CFU per Daphnia were different after the 2-h feeding period (t = 0, Fig. 3A). Corynebacterium sp. 4 and sp. 3, respectively, had 1 and 2 orders of magnitude higher CFU per D. ambigua than Staphylococcus sp. 2 at the end of the feeding period (t = 0, Fig. 3A; P < 0.0001).

The two corynebacterial species were cultured from inside D. ambigua at all periods from t = 30 min to 24 h (Fig. 3A). Although numbers of gut-retained bacteria cultured from within D. ambigua varied over the 24-h period, there was a significant increase (P < 0.01) in the corynebacterial CFU per D. ambigua between t = 0 and t = 24 h. In contrast, Staphylococcus sp. 2 had a significant reduction (P < 0.01) in CFU per D. ambigua from t = 0 to t = 1 h and could not be cultured from sonicated daphnids thereafter.

Staphylococcus sp. 2 survived longer in the gut of D. ambigua in the absence of the two corynebacterial strains (Fig. 3B). Although there was no significant difference in the CFU per D. ambigua of Staphylococcus cultured after the 2-h feeding period (t = 0) in the presence or absence of the corynebacterial species (Fig. 3A,B), Staphylococcus survived gut retention for 18 h longer in the absence of the two corynebacterial species but appeared to be eliminated after 19 h.

Selective feeding studies—The differences in CFU per D. ambigua of the two corynebacterial species and Staphylococcus sp. 2 after the 2-h feeding period could result from selective ingestion or mortality of the two morphologically different cell types (cultured rods and cocci). Selective ingestion was tested through feeding rate experiments with FLB of gram-positive rods (Corynebacterium sp. 3) and gram-positive cocci (Staphylococcus sp. 2).

Ingestion and clearance rates were calculated with Eq. 1 and 2, respectively, for each FLB type:

\[
\text{No. FLB ingested per Daphnia h}^{-1} = \text{No. FLB ml}^{-1} \times \text{ml sample/No. Daphnia sonicated (0.25 h)}^{-1}
\]

where 0.25 h was the duration of the FLB feeding experiment; No. ml h\(^{-1}\) animal\(^{-1}\)

No significant difference (P = 0.19) was found between ingestion rates of the FLB prepared from Corynebacterium sp. 3 and Staphylococcus sp. 2. Average ingestion rate of Corynebacterium sp. 3 was 2.4 \times 10^4 cells Daphnia\(^{-1}\) h\(^{-1}\) compared to 6.7 \times 10^4 for Staphylococcus sp. 2. Clearance rates for Corynebacterium sp. 3 and Staphylococcus sp. 2 FLB were 0.75 and 0.77 ml h\(^{-1}\) animal\(^{-1}\) and not considered significantly different (P = 0.29).

Bacterial inhibition experiments—Selective bactericidal mortality of each bacterial species in the presence of the other was determined by two methods. The stab-overlay method assayed the production of bacterial toxins by the test strain before inoculation of the indicator strain. The patch method assayed bacterial toxin production of the test strain in the presence of the indicator strain.

All patch and stab-overlay tests of the test strain Staphylococcus sp. 2 showed that there was no inhibition of either of the corynebacterial species by the Staphylococcus (data not shown). Conversely, both tests showed that the two corynebacteria inhibited Staphylococcus sp. 2. Plaque results from the tests showed that inhibition of Staphylococcus sp. 2 was greater when the bacteria were incubated in the presence of the corynebacteria (patch test).

Discussion

The number of bacteria capable of producing colonies on standard semisolid medium (agar plates) generally differs by several orders of magnitude from those enumerated by direct epifluorescence microscopy (see Roszak and Colwell 1987). The differences may be attributed to staining of dead or inactive cells, or the inability of certain cells to grow on the nutrients or form of the medium used to culture bacteria from the water. The latter “nonculturable” forms usually require highly enriched medium (e.g. amino acids and minerals) and special culture conditions.

Although planktonic bacteria are often
difficult to isolate on nutrient agar (Amy and Morita 1983; Pointdexter 1981), some planktonic bacteria may actively grow in conditions associated with surfaces and high nutrient concentrations on and within zooplankton. The association of bacteria with zooplankton may reduce some physical and chemical factors otherwise limiting bacterial growth and activity in the presence of substrate.

We found that for samples of Lake Oglethorpe water, heterotrophic plate counts on standard TGE medium were 3–4 orders of magnitude lower than total bacterial densities determined by the direct count method (Table 3). Conversely, we found an increase in cultured bacteria after fractionation of the water to concentrate zooplankton. The increase in diversity and abundance of culturable bacteria also occurred when free-living bacteria were eliminated by chlorination and neutralization and bacteria inside zooplankton were then released by sonication (Tables 1 and 2). No study to our knowledge has shown that attached or unattached nonspore-forming bacteria can survive 10 mg ml⁻¹ of free chlorine residual. Thus we concluded that bacterial survivors from chlorine treatment were protected within the zooplankton.

The increase in heterotrophic plate counts from fractionated zooplankton may have been due to a release of nutrients upon sonication which enhanced growth on the plates, high concentrations of culturable bacteria in the zooplankton guts, or a preadaptation to growth on surfaces and at high nutrient levels (i.e. within the gut of zooplankton). It could also be due to grazing concentrating species that were rare in the whole lake-water samples. Although these possibilities are not mutually exclusive, resistance to digestion is implied.

Specific differences in these cultured bacteria genera and abundance with depth from whole water and treated zooplankton were also observed (Tables 1 and 2). Bacterial species were clearly segregated by sampling depth. No species was found in common among depths from either those cultured inside or outside of zooplankton (Tables 1 and 2), and different species of the same genus were found at more than one depth. There is a high degree of species patchiness (microzonation) on a scale ≤0.5 m, despite the fact that at least some of the zooplankton in this lake have diel vertical migrations >0.5 m (Orcutt 1982).

Differences in bacterial densities with depth during thermal stratification have been reported in several lakes (McDonough et al. 1986; Simon 1987). Fliermans et al. (1975) concluded that differences in densities of individual bacterial species in the water column of lakes were due to the presence or absence of thermal stratification. We suggest that other factors may be involved that directly influence species composition and abundance. Spatial distribution and abundance of bacterial species observed in this study may be the result of differences in growth rates (shown in the susceptibility of cultivation) of different bacterial species or to selective factors caused by zooplankton grazing.

Colony morphology must be closely examined in the study of heterotrophic plate counts from aquatic environments because isolated single colonies (freshly isolated on semisolid agar) are often of mixed species that may not be differentiated. Also, a single species may produce several different colony types on the same plate when initially isolated from lake water, but then revert to a single morphological colony type upon subculture (Holder-Franklin 1986). Several isolates from Lake Oglethorpe exhibited this change in colony morphology after subculture and were subsequently identified to the same genus (Tables 1 and 2).

The fact that cultured whole water contained >80% gram-negative bacteria, whereas cultured sonicated zooplankton contained about equal concentrations of gram-positive and -negative bacteria (Tables 1 and 2) suggested that the zooplankton somehow selected gram-positive bacteria or that gram-positive bacteria became culturable when in association with zooplankton. Likewise, the flagellated, gram-negative bacteria *Alcaligenes* and *Pseudomonas* species composed >70% of the bacteria cultured from whole water, but *Alcaligenes* sp. could not be cultured from treated zooplankton, and the *Pseudomonas* species totaled <3% of cultured bacteria from treated zooplankton. These two flagellated species either were not ingested or were immedi-
ately rendered nonviable, presumably by rapid digestion.

It is unlikely that motility allowed the flagellated bacteria to avoid ingestion by zooplankton, since flagellated algae with greater swimming speeds are easily captured by *Daphnia*. Bacterial size was probably not an important factor in food capture. *Daphnia* ingested other bacteria of similar size (this study) as well as 0.57-μm microspheres (Sanders et al. 1989). Mortality upon ingestion is the more likely explanation of the inability to culture *Alcaligenes* and *Pseudomonas* species from sonicated zooplankton.

The differences in bacterial species composition observed from the field survival studies suggested that some species were capable of surviving in zooplankton (>63-μm size fraction) during the isolation procedures. *Daphnia ambigua* was used in laboratory studies because this species was a known bacterivore from Lake Oglethorpe (Pace et al. 1983; Sanders et al. 1989), was abundant during the sampling period, and was amenable to chlorine treatment. Also, other cladocerans have been shown to exert substantial grazing pressure on bacteria in some lakes (e.g. Kankaala 1988).

The bacteria found in both the lake water and the zooplankton size fraction were hypothesized to be resistant to digestion. The highest percentages of CFU ml^-1^ of bacteria cultured from the >63-μm fraction were gram-positive corynebacteria (Table 1). Bacterial species not cultured from treated zooplankton at the same depth were hypothesized to be selectively avoided, digested, or inhibited upon ingestion. A representative of this group was *Staphylococcus* sp. 2.

Our results from laboratory feeding studies indicate that the differential cultivation of bacteria from lake water and zooplankton was largely due to postigestion phenomena. We detected no selectivity between the cultured cocci and rods, although *D. ambigua* clearance rates on these cultured cells were about twice that determined for natural bacterioplankton with *Daphnia parvula* (Porter et al. 1983). The size of *Staphylococcus* and *Corynebacterium* in natural waters is not known, but it is likely that one or both are smaller than cultured forms. The insignificant difference (*P* > 0.1) between ingestion or clearance rates by *Daphnia* on these two cultured species may be explained by the observations that crustaceans are less efficient at filtering natural planktonic bacteria than algae (Porter et al. 1983; Pace et al. 1983; Güde 1988) and show no selectivity between small algae (*Chlamydomonas*) and large cultured bacteria (*Enterobacter*) (DeMott 1982).

Although laboratory experiments may eliminate selective ingestion as a major factor in the differential survival of bacteria, it is possible that *Daphnia* contains a greater proportion of natural rods than cocci in lake water. Peterson et al. (1978) observed similar differences between the proportion of large rods and small cocci in lake water and daphnid guts. A lower efficiency for cocci ingestion would not appear to be important because *Daphnia* can ingest microspheres smaller than the average size of natural bacterioplankton in Lake Oglethorpe (Sanders et al. 1989), and *D. ambigua* from culture tanks can ingest uncultured cocci as small as 0.31 μm (data not shown). Perhaps the differences in rods and cocci observed by Peterson et al. (1978) and us represent selective survival of the rods and not the cocci after ingestion.

We found that selective survival of bacterial rods and not cocci did occur when *D. ambigua* was fed a mixed suspension of cultured lake bacteria (Fig. 3A), suggesting that *Staphylococcus* was digested but corynebacteria were not. *Staphylococcus* sp. 2 was successfully cultured from within *D. ambigua* for several hours after ingestion in these experiments; however, when the crustaceans were initially fed this species alone, *Staphylococcus* survived gut retention in *D. ambigua* 18 h longer (Fig. 3B). Bacterial inhibition studies demonstrated allelopathy was a more likely contributor to mortality on *Staphylococcus* sp. 2 than digestion in the gut of *Daphnia*.

Microbial competition in the form of allelopathy has been shown to occur in phytoplankton and zooplankton (see Pace 1988). Bacterial inhibitors have been shown to oc-
cur in marine waters (Massey 1970), and seasonal variations of bacterial inhibitors in lake water have been reported to influence bacterial survival (Klein and Alexander 1986). Coliforms are also affected by bacteriocinlike substances that may hinder these populations in drinking water distribution systems (Means and Olson 1981). Although dilution in the ambient environment may render secreted toxins by other organisms ineffective against bacteria (Klein and Alexander 1986), high concentrations of bacterial toxins produced by ingested bacteria could exist in zooplankton guts, thereby increasing the likelihood of pronounced allelopathic effects during periods of intense bacterivory. Bacterial allelopathy inside zooplankton could have a greater effect on bacterial species composition than that of the continued exposure of bacterial species to dilute toxins in the water column.

It is possible that the corynebacteria may have also enhanced digestion of the Staphylococcus. Hadas et al. (1983) found that bacterial microflora associated with Daphnia magna seemed to be partially responsible for digestion of Escherichia coli. They showed that there was enhanced alkaline phosphatase and β-galactosidase activity in cocultures of E. coli and Aeromonas hydrophila isolated from D. magna guts compared to monocultures of the same bacteria, suggesting enzyme activity for the digestion of E. coli. We did not determine whether the corynebacteria enhanced digestion of Staphylococcus, but it was evident from the disappearance of Staphylococcus from Daphnia guts after 18 h that digestion was possible in the absence of the corynebacteria (Fig. 3B).

Microhabitats such as the guts of zooplankton are likely to be important sites of bacterial interactions and may contribute to the control of bacterioplankton community structure. Survival and enrichment of bacteria after ingestion may be another survival strategy (Roszak and Colwell 1987) by which bacteria utilize nutrient-rich microzones within and around zooplankton in an otherwise nutrient-poor environment in the pelagic zones of freshwater ecosystems. Dependence on zooplankton for nutrient enrichment may also be a direct mechanism of growth regulation that induces seasonal changes in bacterial populations due to zooplankton composition and abundance.

To date, bacterioplankton are considered to be a "black box" in most food-web studies. Abundance and productivity are determined for the bacterial community as a whole, through total direct cell counts and incorporation rates of radiotracers, respectively. Grazing rates determined by uptake of prelabeled tracer cells, microspheres, or bacterioplankton assume that tracers are ingested nonselectively with the bacterial assemblage. Additionally, it is assumed that all ingested cells are digested and assimilated with equivalent efficiency. If some bacteria survive ingestion, then grazing rates determined by tracer methods may overestimate grazing mortality on bacterial populations.

Our experiments were limited by current methods to differentiate between culturable and viable but nonculturable forms within the total bacterioplankton population. Although these studies do not address the quantitative importance of resistance to digestion in the total bacterioplankton community, it is likely that resistance to digestion is an important survival mechanism of some species of bacteria in freshwater ecosystems. Further study will be required to determine the impact that this form of survival has on total mortality of bacteria from grazing and the effects on the total bacterioplankton community.

References


Sieburth, J. M., and P. G. Davis. 1982. The role of heterotrophic nanoplanckton in the grazing and
Announcement

Nominations for the 1992 Lindeman Award

Nominations from ASLO members are invited for the 1992 Lindeman Award, to be presented at the Aquatic Sciences Meeting in Santa Fe, New Mexico, 9–14 February 1992. This award is presented annually in honor of Raymond L. Lindeman (1915–1942) and is intended to recognize an outstanding paper written by a young scientist.

The initial gift to create a fund for this award was made in 1986 by Lindeman's colleague in graduate school, Charles B. Reif, of Wilkes College, Pennsylvania. Lindeman received his Ph.D. in March 1941 from the University of Minnesota, and in September 1941 he began postdoctoral work with G. Evelyn Hutchinson at Yale. His career was cut short by death in April 1942. The paper for which he is most remembered was published posthumously (1942. The trophic-dynamic aspect of ecology. Ecology 23: 399–418). This paper has since become the foundation for research on the flow of energy in plant and animal communities. (For more information about Lindeman, see Cook, R. E. 1977. Science 198: 22–26; Reif, C. B. 1986. Bull. Ecol. Soc. Am. 67: 20–25.)

Eligible papers must deal with the aquatic sciences, be written in English by an author no older than 35 in 1990, and must have been published in a 1990 volume of a peer-reviewed journal. Nominations, which should consist of a copy of the paper nominated and a brief letter describing its impact on the field, should be sent to Sallie W. Chisholm, Chair, R. M. Parsons Lab., Bldg. 48, Massachusetts Institute of Technology, Cambridge 02139. To ensure full consideration, nominations should be received by 15 November 1992.

Previous Lindeman Award Recipients


