Microb Ecol (1994) 28:19–37

MICROBIAL ECOLOGY

Heterotrophic Microbial Activity in Shallow Aquifer Sediments of Long Island, New York

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Received: 29 April 1993; Revised: 24 February 1994

Abstract. Bacterial numbers and activities (as estimated by glucose uptake and total thymidine incorporation) were investigated at two sites in Long Island, New York aquifer sediments. In general, bacterial activities were higher in shallow (1.5–4.5 m below the water table or BWT), oxic sediments than in deep (10–18 m BWT), anoxic sediments. The average total glucose uptake rates were 0.18 ± 0.10 ng gdw^{-1} h^{-1} in shallow sediments and 0.09 ± 0.11 ng gdw^{-1} h^{-1} in deep sediments; total thymidine incorporation rates were 0.10 ± 0.13 pmol gdw^{-1} h^{-1} and 0.03 ± 0.03 pmol gdw^{-1} h^{-1} in shallow and deep sediments, respectively. Incorporation of glucose was highly efficient, as only about 10% of added label was recovered as CO_2. Bacterial abundance (estimated from acridine orange direct counts) was $2.5 \pm 2.0 \times 10^7$ cells gdw^{-1} and $2.0 \pm 1.3 \times 10^7$ cells gdw^{-1} in shallow and deep sediments, respectively. These bacterial activity and abundance estimates are similar to values found in other aquifer environments, but are 10- to 1000-fold lower than values in soil or surface sediment of marine and estuarine systems. In general, cell specific microbial activities were lower in sites from Connetquot Park, a relatively pristine site, when compared to activities found in sites from Jamesport, which has had a history of aldicarb (a pesticide) contamination. To our knowledge, this is the first report of bacterial activity measurements in the shallow, sandy aquifers of Long Island, New York.

Introduction

Microbial assemblages in aquifers show remarkable physiological diversity and can exist in various subsurface environments ranging from shallow to deep (>200 m),

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pristine to contaminated, and from sand to clay [4, 7, 11, 14] systems. Aquifer microorganisms are primarily associated with biofilms around sediment particles and in general, metabolic activity and bacterial densities are higher in saturated, permeable sands than in dense, minimally permeable clays [35]. Given the limitations of selective growth media and plating techniques, the predominant aquifer microorganisms are thought to be aerobic or facultatively aerobic heterotrophs, mostly of *Pseudomonas* spp. [13, 14, 30]. Lithotrophic bacteria and microeukaryotes (e.g., protozoa, fungi, and algae) also occur in subsurface samples [13, 14, 37]. Manganese (Mn IV)- and iron (Fe III)-reducing aquifer bacteria [10, 28], as well as sulfate-reducing and methanogenic bacteria have been found in certain anoxic aquifer environments [29, 32].

Sediment bacteria play a major role in carbon cycling in various aquatic systems (e.g., lakes, salt marshes); however, relatively little is known about the contribution of heterotrophic bacterial processes to organic matter transformation in groundwater environments. Recent studies on subsurface sediments have either shown or suggested that bacterial populations are able to grow and survive in nutrient-poor conditions, can respond to a wide range of nutrient concentrations, and are able to use a variety of organic substrates such as glucose, glutamate, and amino acids [3, 14, 41].

One common feature of shallow aquifers is that they often exist in similar types of strata, i.e., sand and gravel layers of alluvial or unconsolidated sediments [15]. Measurements of bacterial metabolism and densities in one aquifer can thus contribute to a better understanding of bacterial processes in general and the role of subsurface bacteria in organic matter transformations. Within a specific aquifer, however, abundances and metabolic activities of microorganisms may be controlled by factors such as dissolved oxygen levels, and the amount and nature of organic input. Madsen and Bollag [29] found glucose and indole mineralization by aquifer bacteria to vary 100-fold in sediment cores taken from different depths within the same borehole; there was no correlation between the amount of substrate mineralized and the depth of the sample. The heterogeneity was attributed to variations in the distribution of aerobic bacteria, which had higher mineralization rates than anaerobic bacteria. This suggests that the distribution of aerobic vs. anaerobic bacteria and, subsequently, the amount of organic matter transformed, may be influenced by dissolved O_2 levels. Aquifer bacteria taken from the same geological layer but at different sites, showed higher glucose uptake and bacterial numbers in sediments with sewage input relative to pristine areas [18]. Bacterial abundances in sediments from several depths within the same borehole in a pristine aquifer were correlated to total organic carbon (TOC) [34]. These observations indicate that the amount of organic input can affect bacterial abundances and metabolic activities in the subsurface. In the Long Island aquifer system, dissolved O_2 levels and the amount of organic input can vary, but there have been no assessments of subsurface bacterial populations and their importance.

The present study sought to: (1) estimate bacterial numbers and heterotrophic activity in the Upper Glacial Aquifer (a shallow, sandy aquifer) of Long Island, New York in relation to other groundwater systems and, (2) examine site variability in bacterial numbers and metabolic activity at two locations with differing histories of organic (aldicarb, a pesticide) contamination. Sediments from each location were incubated under oxic and anoxic conditions to examine the potential for

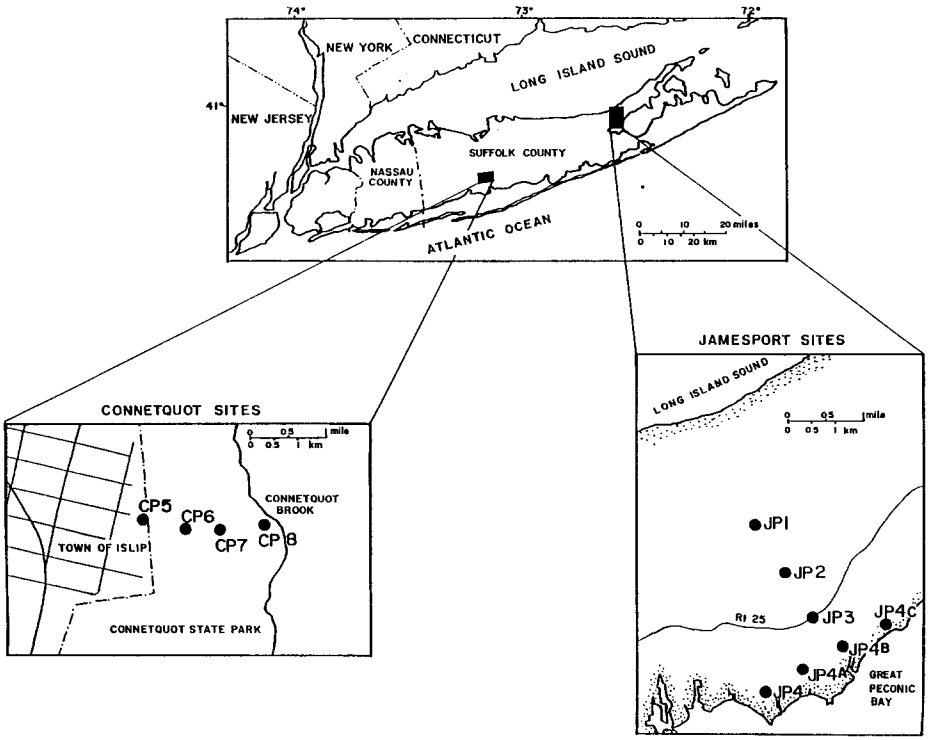

Fig. 1. Location of study area and core sites in Connetquot Park and Jamesport, Long Island, New York.

aerobic and anaerobic microbial activity. To our knowledge, this is the first such study in the Long Island aquifer system.

Materials and Methods

Description of Study Sites and Sampling Procedures

For this study, the location and sampling depths were based on preliminary profiles of pH, temperature, dissolved O_2 (DO), and aldicarb residues of pumped groundwater from monitoring wells maintained by the Suffolk County Department of Health Services. Sediment cores from the saturated portion of the Upper Glacial Aquifer of Long Island, New York were taken in two locations: Connetquot State Park, which had no previous aldicarb input, and Jamesport, which had high but variable aldicarb contamination. At each location, sampling sites were approximately 1 km apart (Fig. 1). Sediment cores were taken using a hollow stem auger drilling rig equipped with split barrel samplers (8.9 cm O.D.). Dissolved O_2 (DO), pH, and temperature of the cores (see Tables 1 and 2) were measured in the field using a DO meter (Orion model 800; Orion Research Inc., Cambridge, MA) and a pH meter (Corning model 105; Corning Inc., Corning, N.Y.) equipped with pH and temperature probes.

At each site, sediment cores were taken from two strata (1.5–4.5 m, designated "S" or shallow; and 10–18 m below the water table, designated "D" or deep) within the same borehole; cores from the shallow depth were always taken before cores from the deeper depth. The sediment samplers were then sealed and packed in ice for transport to the Marine Sciences Research Center, Stony Brook, New York.

The samplers were placed in a sterile hood and opened within 24 h after sampling. Both ends of the core were cut away and only the center portion of the core (parallel to the barrel) was carefully scooped and reserved for use in the experiments. Sediment cores from the upper stratum were incubated under oxic conditions while sediment cores taken from the lower stratum were incubated under anoxic conditions. One exception was site JP1 where sediment cores from 9.0 m below the water table (BWT) were incubated under oxic conditions, and sediment cores from 18 m and 60 m BWT were incubated under anoxic conditions. The "deep" sediments were not anoxic in situ (see Table 2); however, at each site, dissolved oxygen levels were lower in "deep" than in "shallow" sediments, and the "deep" sediments were incubated under anoxic conditions in order to determine the potential for anaerobic microbial activity. Sediments to be incubated under anoxic conditions were initially placed into N_2-gassed 500-ml glass Erlenmeyer flasks, the headspace flushed with N_2 and the flasks capped with butyl rubber stoppers. These sediments were then set up for the experiments and dispensed into incubation vials in an anaerobic glove bag under O_2-free conditions. All glass and plasticware were acid-washed and autoclaved prior to use in the experiments.

Bacterial Enumeration

To prepare samples for bacterial enumeration by epifluorescence microscopy, sediment samples (0.5 cc wet sediment diluted with 50 ml of autoclaved, 0.2 μm-filtered distilled water) were sonicated for 1 min to separate bacteria from sediment particles. After sonication, the supernatant was carefully pipetted off and buffered formalin added to a final concentration of 4%. The bacteria were enumerated using the acridine orange direct count (AODC) method as described by McDaniel and Capone [31]. Bacterial counts of the supernatant after further sonication of the same sediment sample were less than 2% of the counts taken from the initial supernatant.

Measurements of Bacterial Activity: [^{14}C]Glucose Uptake

The metabolic rate of natural microbial populations was assessed by a modification of the [^{14}C]glucose method as described in Bauer and Capone [5]. Sediment slurries (0.5 cc wet sediment/5 ml filtered [10.2 μm]) distilled water [FDW]) were dispensed into scintillation vials and spiked (final concentration, 14.4 ng g^{-1} dry sed wt) with D-[U-^{14}C]glucose (New England Nuclear, Boston, Mass., 1.1×10^{10} Bq $mmol^{-1}$ or 296 mCi $mmol^{-1}$) before capping the vials with butyl rubber stoppers. For experiments under anoxic conditions, sediment slurries were made with FDW that was bubbled with N_2 for 30 min; the headspace of the experimental vials was gassed with N_2 for 2 min at the start of incubation. The vials were incubated in a shaking water bath at 12°C. At each time point, three experimental vials were sacrificed and 0.4 ml of 2 N H_2SO_4 was injected into the sealed vials; the vial headspaces were flushed with air and the evacuated gas bubbled into 5.0 ml of Oxosol fluor (National Diagnostics, Somerville, N.J.) to trap any $^{14}CO_2$ evolved (defined as the respired fraction). The $^{14}CO_2$ trapping efficiency of this method was 91.8 ± 10.2% (n = 6). The slurry was filtered onto 0.2 μm pore-size filters (Millipore, Bedford, Mass.) and the residue washed several times with FDW before placing the filter into scintillation vials (defined as the incorporated fraction). Protosol (New England Nuclear) was added to the vials to digest organic matter. ScintiVerse II scintillation cocktail (Fisher Scientific, Fair Lawn, N.J.) and FDW were then added to make a homogeneous gel. Both kinds of samples were counted in a Liquid Scintillation Counter (Packard TriCarb 300C; Hewlett Packard Co., Rockville, MD). All experimental vials were run in triplicate.

To account for abiotic losses of the added radiolabeled glucose, formalin-killed controls (final concentration, 4%) were run in all experiments. At each time point, the controls were handled in the same manner as the experimental vials, with the headspace flushed into Oxosol and the sediment slurry filtered, washed, and made into a gel. During the course of the experiment, the controls had less than 0.1% and 0.2% of the total added label in the Oxosol and the ScintiVerse fractions, respectively. Of total radioactivity, approximately 30% (range, 0.5–50%) was associated with the cellular (solid-phase associated) and respired fractions by 24 h.

Measurement of Bacterial Activity: [^{3}H]Thymidine Incorporation

Bacterial activity was also estimated by total [methyl-^{3}H]thymidine incorporation (TTI). Slurries (prepared in a manner similar to that described above) were amended with [methyl-^{3}H]thymidine (final concentration, 12.1 ng g^{-1} dry sed wt, New England Nuclear, 3.1×10^{12} Bq $mmol^{-1}$ or 84.1 Ci $mmol^{-1}$) and incubated from 0 to 24–72 h at 12°C. Samples were extracted with ice-cold trichloroacetic acid (TCA) (final starting concentration, 5%) and the residues collected on 0.2 μm pore-size filters. The filters were dissolved in ethyl acetate and prepared for scintillation counting as described previously. All experimental vials were run in triplicate.

Formalin-killed controls (final concentration, 4%) were also run and treated in the same manner as the experimental vials. Radioactive counts in the controls were <0.2% of the total thymidine added at all time points. Of the total radioactive label added, approximately 2% (range, 0.3–3%) was associated with the cellular fraction after 24 h.

Data Analysis

Radioactive metabolism of glucose was estimated in the incorporated and respired (CO_2) fractions after subtracting radioactive counts in the abiotic controls. Total rates of glucose uptake were based on the sum of these fractions and were calculated by linear regression of glucose uptake uptake vs. time. Estimates of total thymidine incorporation were corrected for abiotic controls and TTI rates were calculated by linear regression of TTI vs. time. Because glucose uptake and TTI over time in Connetquot Park samples were biphasic (i.e., low activities followed by higher activities) over the length of the experiment (48–72 h), data from the first 24 h were used to determine initial rates of microbial activity. Standard errors of the rates were estimated from deviation around the regression lines. Total glucose uptake and thymidine incorporation rates per cell at each site were determined from the initial rates divided by bacterial densities. Location and site variability of total glucose uptake and TTI rates were examined using a blocked ANCOVA test; differences in microbial densities were tested using a two-way ANOVA. Pearson correlation coefficients for total glucose uptake rates, TTI rates, and bacterial numbers were also determined. All statistical analyses were conducted with SAS software (SAS Statistical Analysis Software, SAS Institute Inc., Cary, N.C.). Significance levels were set at $P < 0.05$ or less for all analyses.

Results

Field Conditions

At well sites in Connetquot Park and Jamesport, ambient temperature and pH were fairly uniform, but dissolved O_2 (DO) varied with depth (Table 1). Connetquot Park wells were sampled during the fall and were slightly colder (10.5–13.0°C) and more acidic (pH 4.9–5.7) than Jamesport wells, which were sampled during the spring and summer (11.0–17.3°C; pH 5.8–6.3). Dissolved O_2-levels varied from 1.3 to 8.6 ppm in Connetquot Park and from 0.5 to 9.8 ppm in Jamesport; at each site in both locations, DO levels were higher in samples from the shallow cores.

Microbial Activity and Counts: Connetquot Park Sites

Aquifer bacteria were metabolically active at all sites and depths in Connetquot and Jamesport locations. Uptake of glucose into cellular material and respiration to CO_2 were saturated at 10 ng gdw^{-1} (Fig. 2); a Wright-Hobbie transformation of initial

Table 1. Site characteristics for aquifer sediments[a]

Site	Depth BWT (m)	Depth from surface (m)	DO (ppm)	pH	Temp (°C)
Connetquot Park					
CP5S	1.5	12.0	8.0	4.9	13.0
CP6S	1.5	9.0	7.5	5.1	11.5
CP7S	1.5	6.0	8.6	5.2	10.5
CP8S	1.5	3.0	8.2	5.7	13.0
CP5D	18.0	28.5	4.2	5.3	13.0
CP6D	18.0	25.5	1.5	5.2	13.0
CP7D	18.0	24.0	3.4	5.7	12.0
CP8D	18.0	19.5	1.3	5.3	11.5
Jamesport					
JP1S	9.0	22.5	6.6	ND	17.3
JP2S	4.5	28.5	6.0	5.8	13.0
JP3S	4.5	14.5	6.6	5.3	14.5
JP4S	4.5	13.5	5.5	5.4	12.0
JP4AS	4.5	10.5	1.8	5.6	12.0
JP4BS	4.5	9.5	9.8	5.5	11.0
JP4CS	4.5	22.5	3.5	5.8	12.0
JP1D1	18.0	31.5	4.1	ND	13.3
JP1D2	60.0	73.5	3.5	ND	13.7
JP2D	10.0	34.0	5.1	6.6	12.2
JP3D	10.0	20.0	0.5	5.7	13.0
JP4D	10.0	19.0	0.7	5.5	12.0
JP4AD	10.0	16.0	0.5	5.3	11.5
JP4BD	10.0	15.0	1.2	5.7	12.0
JP4CD	10.0	28.0	3.2	6.3	12.0

[a]BWT, below water table; CP, Connetquot Park; JP, Jamesport; S, shallow; D, deep; ND, not determined

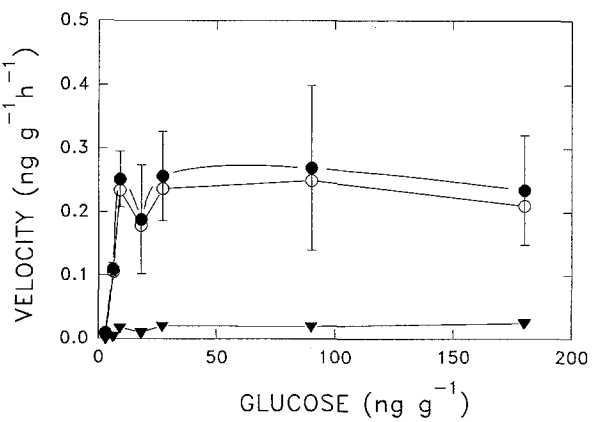

Fig. 2. Saturation of uptake and respiration with added glucose in oxic aquifer sediment from site CP6S. Samples incubated aerobically: respiration (▼); incorporation (○); total metabolism (incorporation plus respiration) (●). For clarity, *bars* for ±1 standard error (SE) are shown only for total metabolism.

Table 2. Bacterial activity measurements for Connetquot Park and Jamesport sites. Values are mean ± 1 SE

Site	Glucose[a] (pg $gdw^{-1}h^{-1}$)			TTI[a] fmol ($gdw^{-1}h^{-1}$)	Bacterial numbers (10^7 gdw^{-1})
	Respired	Incorporate	Total uptake		
CP5S	0	60 ± 11	60 ± 16	3.5 ± 0.6	1.8 ± 0.3
CP6S	0	87 ± 9	87 ± 17	14 ± 2	2.1 ± 0.8
CP7S	0.1 ± 0.1	73 ± 10	73 ± 18	26 ± 4	3.1 ± 0.2
CP8S	18 ± 4.3	300 ± 41	320 ± 44	74 ± 22	1.6 ± 0.2
CP5D	0	12 ± 3	12 ± 3	3.5 ± 0.2	1.4 ± 0.2
CP6D	0	10 ± 2	10 ± 4	2.5 ± 1.6	1.6 ± 0.1
CP7D	1.1 ± 0.8	42 ± 19	43 ± 20	20 ± 11	1.8 ± 0.2
CP8D	0	2.6 ± 0.1	2.6 ± 0.1	1.7 ± 0.2	0.9 ± 0.1
JP1S	6 ± 4	280 ± 20	280 ± 20	490 ± 34	3.0 ± 0.4
JP2S	21 ± 2	270 ± 23	290 ± 24	89 ± 11	2.8 ± 0.8
JP3S	25 ± 2	290 ± 24	320 ± 25	100 ± 11	7.9 ± 0.7
JP4S	18 ± 1	94 ± 10	110 ± 11	95 ± 7	2.5 ± 0.1
JP4AS	18 ± 1	110 ± 9	130 ± 11	73 ± 5	2.0 ± 0.2
JP4BS	20 ± 2	120 ± 8	140 ± 10	55 ± 3	0.7 ± 0.2
JP4CS	26 ± 2	150 ± 12	170 ± 14	60 ± 6	0.4 ± 0.1
JP1D1	7 ± 1	140 ± 9	140 ± 9	38 ± 4	0.6 ± 0.1
JP1D2	4 ± 1	44 ± 3	48 ± 4	10 ± 1	0.1 ± 0
JP2D	17 ± 1	240 ± 14	250 ± 16	25 ± 2	5.9 ± 0.4
JP3D	28 ± 2	320 ± 19	350 ± 20	40 ± 2	5.2 ± 0.9
JP4D	3 ± 1	23 ± 3	26 ± 3	45 ± 3	1.4 ± 0.4
JP4AD	5 ± 1	27 ± 2	32 ± 2	48 ± 5	3.9 ± 0.2
JP4BD	4 ± 1	49 ± 3	53 ± 4	61 ± 4	0.1 ± 0
JP4CD	7 ± 1	55 ± 4	62 ± 4	84 ± 4	1.5 ± 0.2

[a] Initial rates

glucose uptake rates vs. concentration yielded a turnover time of 25 h and a V_{max} of 250 pg $g^{-1}h^{-1}$.

Initial rates (0–24h) of total glucose uptake ranged from 2.6 to 320 pg $gdw^{-1}h^{-1}$; higher rates were found in shallow cores at all sites (Table 2). Except for CP8S and CP8D, there was little difference in glucose uptake among the sites along the 4-km transect in Connetquot Park. At CP8S there was a high initial rate over the first 24 h while at CP8D there was very low activity overall. TTI rates ranged from 1.7 to 74 pmol $gdw^{-1}h^{-1}$ and, except for site CP5 and CP7, higher rates were found in shallow cores. At CP5 and CP7, TTI rates were similar for shallow and deep cores. As with glucose uptake, there was a higher TTI rate at CP8S while the lowest activity was found at CP8D. Bacterial numbers were between 0.9 and 3.1 × 10^7 cells gdw^{-1}; more bacteria were found in shallow samples at all sites.

Microbial Activity and Counts: Jamesport Sites

Total glucose uptake over time varied by site in Jamesport. In shallow samples, there was a higher initial rate in glucose uptake over time in cores from the north-south transect (JP1, JP2, JP3) than in cores from the downgradient west-east

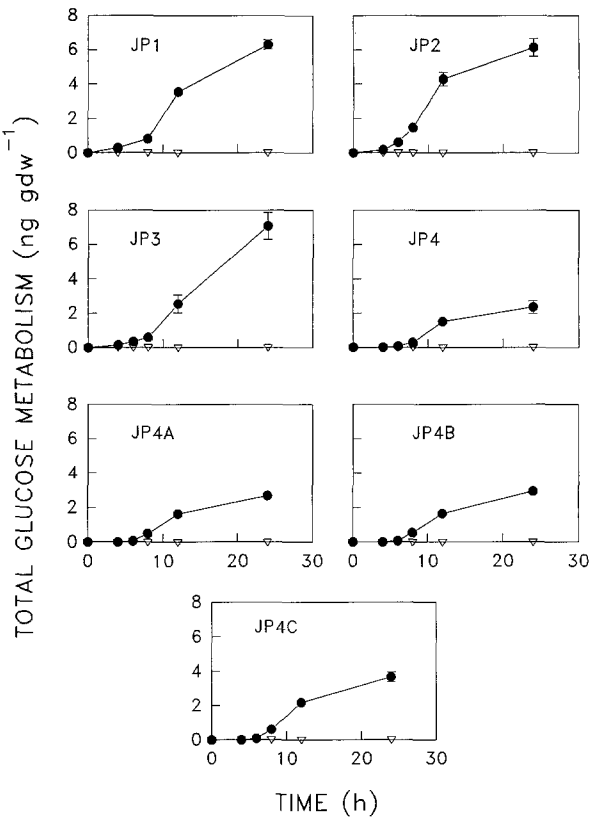

Fig. 3. Total glucose uptake over time for Jamesport sites, shallow only: total glucose metabolism (●); formalin-killed controls (▽). When present, *bars* are ±1SE.

transect (JP4, JP4A, JP4B, JP4C) (Fig. 3, Table 2). Activity increased after 8 h and was most pronounced in the north-south transect samples. In deep samples, except for JP1D2, the initial rate in total glucose uptake was higher in cors from the north-south transect than in cores from the west-east transect (Fig. 4). Glucose uptake activity was less at JP1D2 (60 m depth) than at JP1D1 (18 m depth) but was similar to activities found in the west-east transect. Increases in activity after the initial 8 h were also noted in the deep cores and were the most pronounced at JP1, JP2, and JP3.

Total thymidine incorporation in Jamesport was less variable than glucose uptake among sites. Except for JP1, there was little difference in TTI over time in the shallow cores within and between the two transects (Fig. 5). At JP1, there was a higher initial rate and highest overall activity. In the deep samples, except for JP4B and JP4C, there was little difference in TTI over time in cores within and between the two transects (Fig. 6). At JP4B and JP4C, there was a higher initial rate and overall higher TTI.

Initial rates of bacterial activity and biomass measurements at Jamesport are given in Table 2. Total glucose uptake rates ranged from 26 to 350 pg $gdw^{-1}h^{-1}$; except for sites JP2 and JP3, higher rates were found in shallow cores. At JP2 and JP3, glucose uptake rates were similar (within 1 SE) for shallow and deep samples.

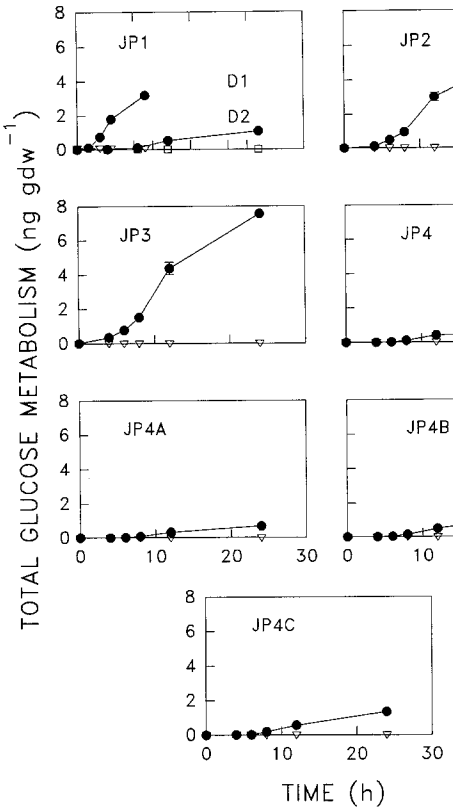

Fig. 4. Total glucose uptake over time for Jamesport sites, deep only. Total glucose metabolism (●); formalin-killed controls (▽). For JP1, (▽) and (□) are formalin-killed controls for D1 and D2, respectively. When present, *bars* are ±1 SE.

TTI rates were generally much higher than in Connetquot Park sites and ranged from 10 to 490 pmol gdw^{-1}h^{-1}. Except for site JP4B, higher rates were found in the shallow samples; at JP4B, TTI rates were similar for shallow and deep cores. Bacterial numbers were more variable than in Connetquot Park, ranging from 0.1 to 7.9 × 10^7 cells gdw^{-1}; except for JP2, JP4A, and JP4C, more bacteria were found in the shallow samples. At JP2, JP4A, and JP4C, bacterial abundance was higher in the deep samples.

When data are pooled from shallow and deep sediments, Connetquot Park and Jamesport locations, the average glucose uptake and TTI rates were 0.13 ± 0.09 ng gdw^{-1}h^{-1} (0.72 pmol g^{-1} h^{-1}) and 0.07 ± 0.10 pmol gdw^{-1}h^{-1}, respectively, while the average bacterial abundance was 2.4 ± 1.9 × 10^7 cells gdw^{-1}.

Cell Specific Activity: Connetquot Park vs. Jamesport Sites

In general, glucose uptake and thymidine incorporation rates per cell were higher in Jamesport than in Connetquot Park (Table 3), although not statistically significant at $P < 0.05$. Glucose uptake rate per cell ranged from 0.6 to 19 × 10^{-21} g cell^{-1}h^{-1} in Connetquot Park and 0.8 to 68 × 10^{-21} g cell^{-1}h^{-} in Jamesport; TTI rate per cell ranged from 0.2 to 4.7 × 10^{-21} mol cell^{-1}h^{-1} in Connetquot Park and

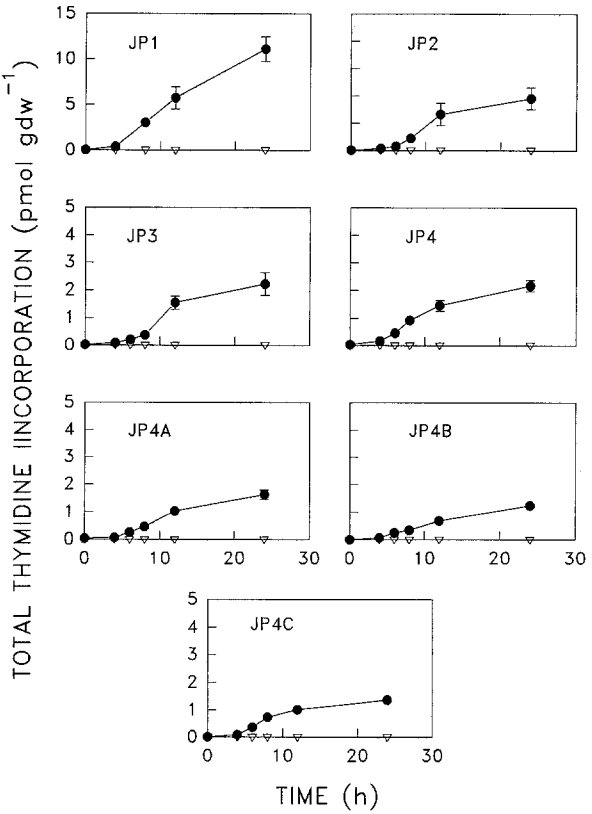

Fig. 5. Total thymidine incorporation (TTI) over time for Jamesport sites, shallow only: TTI (●); formalin-killed controls (▽). When present, *bars* are ±1 SE.

0.8 to 78 × 10^{-21} mol cell^{-1}h^{-1} in Jamesport. In Connetquot Park, except for CD7, glucose uptake rate per cell was higher in the shallow cores. At CP7, activity was similar for shallow and deep cores. Thymidine incorporation rate per cell was higher in the shallow cores at CP6 and CP8 and was similar for shallow and deep cores at CP5 and CP7. In Jamesport, glucose uptake rate per cell was more variable with higher activity occurring in shallow samples at sites JP2, JP4, JP4A, and JP4C. At the other Jamesport sites higher activity occurred in the deep cores. Except for JP4B, thymidine incorporation rate per cell was higher in shallow samples; at JP4B activity was higher in the deep sample.

Statistical Analyses

When initial glucose uptake and TTI rates were analyzed statistically for variability within the transect in Connetquot Park, bacterial activity rates were significantly different ($P < 0.05$) among the shallow cores. Similarly, rates of bacterial activity were significantly different among the deep cores within the transect. Glucose uptake and TTI rates in most cases were significantly higher ($P < 0.05$) in the shallow samples. Bacterial activity rates in Jamesport were significantly different ($P < 0.001$) among the shallow cores and among the deep cores within the two

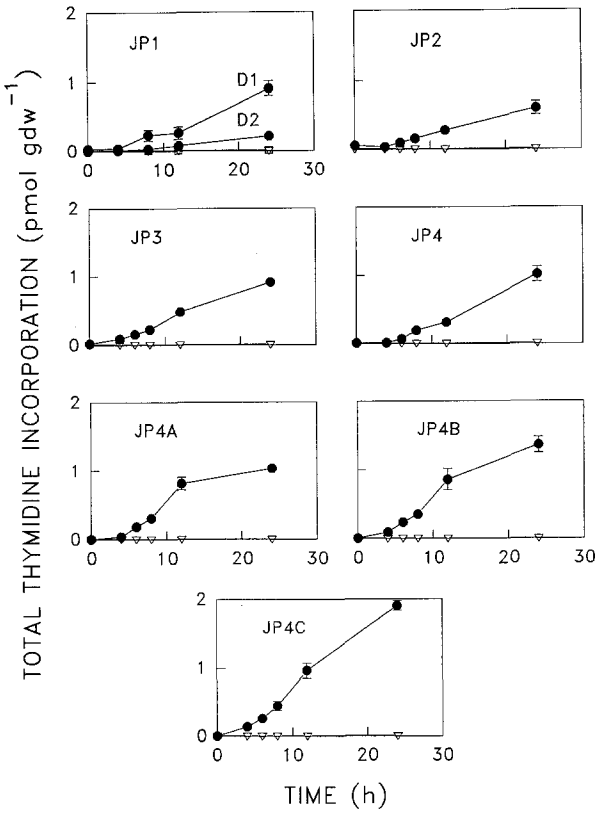

Fig. 6. Total thymidine incorporation (TTI) over time for Jamesport sites, deep only. TTI (●); formalin-killed controls (▽). For JP1, (▽) and (□) are formalin-killed controls for D1 and D2, respectively. When present, *bars* are ±1 SE.

transects. As with Connetquot Park, there was significantly higher ($P < 0.05$) bacterial activities in the shallow cores.

Variability between locations (Connetquot vs. Jamesport) in glucose uptake and TTI rates was also examined. Total glucose uptake rates were found to be statistically similar between the two locations, although TTI rates were found to be significantly higher ($P < 0.002$) in sediment samples from Jamesport. Two-way ANOVAs indicated no significant ($P < 0.05$) differences in bacterial numbers at all sites, depths, and locations tested.

Correlation coefficients were determined for combinations of total glucose uptake rates, TTI rates, and bacterial numbers (Table 4). Glucose uptake rates and bacterial numbers were significantly correlated ($P < 0.05$), while other combinations were not. Correlation coefficients between bacterial activity indices and densities and ambient environmental conditions were also determined (Table 4). Indices of bacterial activity (glucose uptake and TTI rates) and densities did not covary significantly with ambient DO and pH; bacterial numbers were also not significantly correlated to in situ temperature. However, both glucose uptake and TTI rates were significantly correlated ($P < 0.05$ and 0.001, respectively) to in situ temperature.

Table 3. Glucose metabolism and thymidine incorporation rates per cell at Connetquot Park and Jamesport sites

Site	Glucose (10^{-21}g cell^{-1}h^{-1})	Thymidine (10^{-21} mol cell^{-1}h^{-1})
CP5S	3.3	0.2
CP6S	4.1	0.7
CP7S	2.3	0.8
CP8S	19	4.7
CP5D	0.9	0.2
CP6D	0.6	0.2
CP7D	2.4	1.1
CP8D	0.3	0.2
JP1S	9.6	17
JP2S	10	3.2
JP3S	4.0	1.3
JP4S	4.5	3.9
JP4AS	6.4	3.7
JP4BS	19	7.7
JP4CS	47	17
JP1D1	23	6.1
JP1D2	48	10
JP2D	4.3	0.4
JP3D	6.6	0.8
JP4D	1.9	3.3
JP4AD	0.8	1.3
JP4BD	68	78
JP4CD	4.2	5.7

Table 4. Correlation coefficients for microbial activity and biomass estimates. Data from all sites (n = 22)

	Glucose[a] metabolism (r)	Total thymidine[a] incorporation (r)	Bacterial numbers (r)
Glucose[a] metabolism	1.00	0.34	0.62*
Total thymidine[a] incorporation		1.00	0.13
Bacterial numbers			1.00
Dissolved O_2	0.34	0.22	0.10
pH	0.21	0.12	0.14
Temperature	0.42*	0.75**	0.29

[a]Initial rates
*Significant at $P < 0.05$
**Significant at $P < 0.001$

Discussion

Bacterial metabolic activity and biomass were measurable in all samples from different depths, well sites, and locations in portions of the Long Island aquifer. Glucose uptake in oxic sediments from Jamesport became saturated at a glucose concentration of 10 ng g^{-1}; this result is similar to glucose uptake by bacteria in an

Table 5. Comparison of glucose metabolism rates, TTI rates, and bacterial numbers in selected aquifer environments

Site	Rate[a]		Bacterial numbers (10^6 cell g^{-1})	Source
	Glucose (pmol $g^{-1}h^{-1}$)	TTI		
Pristine aquifer sediment	1,300	–	–	[41]
Pristine aquifer sediment	–	–	10–100	[7]
Contaminated aquifer sediment	1.2	–	–	[1]
Fuel-oil contaminated groundwater	1.9	0.020	2.2	[22]
Uncontaminated groundwater	0.72	0.014	4.6	[22]
Aquifer sediment				
oxic	21–1000	–	–	[29]
anoxic	8.3–417	–	–	[29]
Granitic aquifer	–	–	0.03–0.66	[34]
Sandy aquifer	–	–	4.1–17.9	[42]
Sandy aquifer sediment			10–79	This study
oxic	0.33–1.8	3.5–110	–	This study
anoxic	0.06–1.9	1.7–49	–	This study

[a]Conversions from reported values, where needed, assume sediment particle mass density of 2.65 g ml^{-1} (from [15]). Glucose metabolism converted to moles

oil-contaminated aquifer (15 ng g^{-1}) [22]. Both the maximum glucose uptake rate (V_{max}) and turnover time (T_t) are 100–1000 times lower than V_{max} of 600–3400 ng g^{-1} h^{-1} and T_t of approximately 1 min reported for littoral sediments of an eutrophic lake [25]. Representative glucose turnover times in other aquatic systems include 10 min for polluted lake sediments [45] and 4.5 h for coastal marine waters [2]. Although a variety of sampling techniques and incubation conditions were used among the previous studies, it appears that in general, bacterial populations in aquifers have lower overall metabolic activities than populations in other aquatic environments.

The average glucose uptake and TTI rates (on a per gram dry sediment basis) and bacterial abundance in Long Island groundwater sediments are within the range found in other aquifer environments (Table 5). Estimates of glucose uptake rates determined in this study may be higher than that found in situ, as 10 n*M* glucose was added to the sediment slurries. To our knowledge, there have not yet been any measurements taken for in situ glucose in subsurface sediments, but due to the oligotrophic nature of most aquifers, 10 n*M* glucose may be greater than found in situ. In Long Island aquifer sediments, the average bacterial growth rate is 1.4×10^5 cells gdw^{-1} h^{-1}, assuming a conversion factor of 2×10^{18} cells mol^{-1} of thymidine incorporated [42]. If approximately 27% of the added thymidine is incorporated into DNA (as was found in Jamesport sediments and discussed later), then the bacterial growth rate is 3.7×10^4 cells gdw^{-1} h^{-1} in Long Island aquifer sediments. This bacterial growth rate is 10–30 times lower than that found in a pristine aquifer [42], and 40–16,000 times lower than in lake and seagrass sediments [39]. When bacterial activity was assessed on a per cell basis, glucose uptake and TTI rates are within the range found in a river [6] and in some marine sediments [5, 12] (Table 6). In general, however, glucose uptake and TTI rates per cell in aquifer sediments were lower than values found in other aquatic environments.

Table 6. Comparison of cell-specific glucose metabolism and TTI rates per cell in selected aquatic environments

Site	Glucose metabolism [mol(cell^{-1} h^{-1}) × 10^{-21}]	Thymidine uptake [mol(cell^{-1} h^{-1}) × 10^{-21}]	Source
River	0.34	—	[6]
River plume	2.2	—	[6]
Fuel-oil contaminated groundwater	72–2,056	54–210	[22]
Sewage contaminated aquifer sediments	1,056–1,389	—	[18]
Marine sediments	—	8–4	[12]
Marsh	—	4,000–11,000	[26]
Marsh			
oxic	2.5	102	[5]
anoxic	0.54	0.3	[5]
Aquifer sediments:			
oxic	0.013–0.26	0.2–17	This study
anoxic	0.002–0.38	0.2–78	This study
average	0.072	7.3	This study

The lower activities in aquifer sediments may be due to low dissolved organic carbon (DOC) concentrations (average, 0.1–1 mg liter^{-1}; [15]) in groundwater systems. Although DOC measurements do not distinguish between usable vs. refractory forms of carbon, in general, marine and estuarine sediments usually have a higher flux of biologically reactive organic matter than aquifer sediments, due, in part, to their proximity to primary production in overlying waters and at the sediment–water interface. In the subsurface environment, the more labile forms of organic carbon from terrestrial sources (e.g., leaf litter) may be intercepted by soil microorganisms, leaving less labile organic matter to percolate through to the underlying aquifer. The addition of labile organic carbon has been shown to increase the metabolic activity and the organic contaminant degradative capabilities of subsurface bacteria, suggesting that under in situ conditions, low metabolic activities and biomass are likely to be due to nutrient (in particular, carbon) limitation [8, 29, 40].

Bacterial activities, as estimated by initial rates of glucose uptake and TTI in Connetquot Park and Jamesport sites (Tables 2 and 3), were significantly higher ($P < 0.05$) in shallow, oxic sediments than in deep, anoxic sediments. The higher metabolic activity in shallow sediments is likely due to the greater growth yields of obligate and facultatively aerobic bacteria, which use a more energetically favorable electron acceptor than anaerobic bacteria. Higher rates of glucose and indole respiration were also noted in oxic vs. anoxic aquifer sediments by Madsen and Bollag [29]. An alternate explanation for the higher glucose uptake rates in oxic sediments is the more complete transformation of glucose in these sediments. In anoxic sediments, incomplete metabolism of glucose can lead to the formation of fermentation end products, which were not accounted for in the present study. King and Klug [25] found that ^{14}C-volatile fatty acids constituted the major fraction of [^{14}C]glucose uptake end products in eutrophic lake sediments. CO_2 as well as lactate, acetate, and propionate were formed as metabolic end products when glucose was added to methanogenic and Fe(III)-reducing river and groundwater

sediments [28]. However, in the oligotrophic milieu of most aquifer environments, including portions of the Upper Glacial Aquifer examined in this study, any fermentative end products should be rapidly utilized by various bacterial groups. Thus, it is likely that the significantly higher bacterial activities in shallow, oxic sediments is due to the more efficient growth yields of oxic bacteria.

Most of the radiolabel added as [^{14}C]glucose was incorporated into bacterial cellular biomass (ca. 90%) rather than respired over the period tested (Fig. 2, Tables 3 and 4). Of the two other studies that have measured bacterial [^{14}C]glucose uptake and respiration in aquifer sediments, both have also reported high (ca. 80%) incorporation into cellular material [1, 41], as has been reported for marine [33] and freshwater sediments [43, 45]. This is in contrast to observations in other aquatic systems where bacterial assimilation accounts for approximately 50–60% of the total glucose added [20, 24]. It is still not clear why aquifer bacteria incorporate high levels of glucose, but a possible explanation is the cellular storage of material in order to meet maintenance energy needs. Bacteria in olgiotrophic groundwater can store up to 10 times more poly-β-hydroxybutyric acid (PHB), an energy-rich polymer, than bacteria in enriched groundwater; when phosphate (a limiting nutrient in this case) was added to the cultures, the stored PHB was metabolized [8]. Further research is needed to determine the effects of nutritional status on uptake and assimilation of organic compounds by subsurface bacteria.

The incorporation of exogenous [^{3}H]thymidine into DNA by metabolically active bacteria can provide a sensitive measure of bacterial productivity [16]. However, not all of the radiolabel may be assimilated into DNA, as it can also be channeled to other macromolecules such as proteins and lipids [21, 27]. Nonspecific labeling due to bacterial catabolism of thymidine appears to be enhanced in oligotrophic environments [9, 17, 21]. In preliminary experiments with Long Island aquifer sediments, approximately 55 and 27% of the [^{3}H]thymidine added appeared in the protein and DNA fractions, respectively, with no apparent differences between oxic vs. anoxic incubations (J. Kazumi, unpublished data). Thus bacterial degradation of thymidine also appears to be occurring in these sediments. Nevertheless, TTI rates may still be useful as a relative index of metabolic activity of in situ microbial populations. Total thymidine incorporation rates were found to be significantly higher ($P < 0.002$) in Jamesport than in Connetquot Park sediments. The higher TTI rates in Jamesport sediments may be due to a number of factors, including the presence of nutrients that may otherwise be limiting, a metabolically more efficient bacterial population, and higher concentration of organic substrates. Of the limited field data collected for the current study, the major difference between the two locations is in the concentration of aldicarb, an organic pesticide. Previous studies in soils and salt marsh sediments indicate that aldicarb can be microbially metabolized and that portions of the molecule may serve as an energy source [23, 36, 38]. Jamesport sediments have a history of aldicarb input while Connetquot Park sediments do not. The higher TTI rates in Jamesport suggests that bacterial populations there are more metabolically active and, perhaps, even using aldicarb as an energy source.

Bacterial activity and abundances were found to be variable among sites within transects in Connetquot Park and Jamesport. Bacterial tracer studies in groundwater flow fields have shown higher densities of bacteria in sites closest to a sewage outfall vs. downgradient sites [17, 19]. Higher bacterial activities and contaminant

biodegradation potential have also been shown in contaminated sites upgradient of more pristine areas [17, 30]. In Connetquot Park and Jamesport transects, bacterial numbers did not decrease with distance from the site of organic input as would be expected. Madsen et al. [30] found small differences in total bacterial counts between pristine and contaminated sites, but higher protozoan counts in contaminated, saturated sediments. This suggests that protozoans are growing at the expense of bacteria in contaminated sediments, and that the lower than expected bacterial counts are due to loss via protozoan grazing. Although there was no apparent difference in bacterial numbers with distance from organic input, there was a relationship between bacterial activities and upgradient vs. downgradient sites. This was best shown with glucose uptake rates in the Jamesport transects. There were higher initial glucose uptake rates in the upgradient north-south transect closest to aldicarb input when compared to the downgradient east-west transect, which suggests that patterns of microbial activity may be related to hydraulic gradients and flows of organic contaminants. This relationship was not as apparent with TTI rates in the Jamesport transects nor with bacterial activity measurements in the Connetquot Park transect; more information on the nature and the biological reactivity of the organic compounds present in portions of the Long Island aquifer is required for this relationship to be confirmed.

Except for total glucose uptake rates and bacterial numbers, no other combinations of bacterial activity measurements and densities were significantly correlated ($P < 0.05$). Glucose uptake and thymidine incorporation rates were expected to covary as indicators of bacterial activity. However, glucose uptake and thymidine incorporation measure different aspects of bacterial activity; glucose uptake rates indicate the "heterotrophic potential" of a bacterial population while thymidine incorporation is a function of bacterial growth rates. Glucose uptake is one measure of heterotrophic activity, and high glucose uptake rates may not necessarily lead to the production of biomass and new cells. Microbial processes such as the hydrolysis of macromolecules, and uptake of other small dissolved molecules and inorganic nutrients are also required for growth to occur; limitations in any of these processes will limit bacterial growth rates [44]. Thus, heterotrophic activity (glucose uptake) and growth (thymidine incorporation) sometimes may be uncoupled, and in Long Island aquifer sediments, bacterial growth may be limited by factors (e.g., low levels of inorganic nutrients) other than labile carbon.

When glucose uptake and TTI rates and ambient environmental conditions (DO, pH, temperature) were analyzed, there were no significant relationships. Correlations between metabolic activity and ambient conditions may be difficult to interpret because the metabolic rates were estimated from sediment slurries maintained under laboratory conditions while the environmental data were collected in the field. Aquifer sediments taken from the deep depths were always incubated under anoxic conditions although the cores were not necessarily anoxic (see Table 4), and this may account for the poor relationship between bacterial activity rates and DO. However, there was a significant correlation ($P < 0.05$) between field or in situ temperature, and both glucose uptake and TTI rates; it is well established that bacterial activity rates are sensitive to changes in temperature. Thus, in some instances, estimates of bacterial activities may be correlated to field conditions. Other than with glucose uptake, bacterial numbers were not correlated with TTI rates nor ambient environmental measurements; in this study, densities were taken

as total AODC, which does not distinguish between metabolically active or inactive cells. Thus this result is not unexpected.

Subsurface sediment bacteria in the Upper Glacial Aquifer of Long Island had higher activities in shallow, oxic sediments than in deeper sediments that were anoxic. In general, bacterial activity and densities were low when compared to other aquatic environments (e.g., lakes, marshes), but were similar to other groundwater systems. Most of the metabolized glucose was associated with the cellular fraction rather than respired, suggesting that aquifer bacteria are carbon limited and may be storing metabolic products from glucose transformation as cellular biomass. Bacterial numbers and glucose uptake rates were not significantly different between Connetquot Park and Jamesport sites, although TTI rates were signficantly higher ($P < 0.002$) in the Jamesport sites.

Acknowledgments. Thanks to D. Kirchman and L. Young for comments and discussion on an earlier version of this manuscript. J. O'Neil and H. Wente provided sampling and technical assistance; S. Smith provided aid in the statistical analysis. Thanks also to S. Cary, R. Markel, and the drilling crew at the Suffolk County Department of Health Services. This work was supported by USGS Grant 14-88-0001-G1282 to D. Capone and by a Natural Sciences and Engineering Research Council (Canada) Fellowship to J. Kazumi.

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