

Report to the
MASSACHUSETTS BAYS PROGRAM

**SURVIVAL AND DEPOSITION OF FECAL INDICATOR
BACTERIA IN SEDIMENTS OF BOSTON HARBOR**

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MASSACHUSETTS BAYS PROGRAM

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FOREWORD

The roots of the Massachusetts Bays Program extend back to 1982, when the City of Quincy filed suit against the Metropolitan District Commission and the Boston Water and Sewer Commission over the chronic pollution of Boston Harbor, Quincy Bay, and adjacent waters. Outdated and poorly maintained sewage treatment plants on Deer Island and Nut Island were being overwhelmed daily by sewage from the forty-three communities in the Metropolitan Boston area. Untreated and partially treated sewage were spilling into Boston Harbor.

Litigation over the pollution of Boston Harbor culminated in 1985 when the United States Attorney filed suit on behalf of the Environmental Protection Agency against the Commonwealth of Massachusetts for violations of the Federal Clean Water Act. The settlement of this suit resulted, in 1988, in the creation of the Massachusetts Water Resources Authority, the agency currently overseeing a multi-billion dollar project to repair and upgrade Metropolitan Boston's sewage treatment system. In addition, the settlement resulted in the establishment of the Massachusetts Environmental Trust - an environmental philanthropy dedicated to improving the Commonwealth's coastal and marine resources. \$2 million in settlement proceeds are administered by the Trust to support projects dedicated to the restoration and protection of Boston Harbor and Massachusetts Bay.

The Trust provided \$1.6 million to establish the Massachusetts Bays Program, a collaborative effort of public officials, civic organizations, business leaders, and environmental groups to work towards improved coastal water quality. The funding was used to support both a program of public education and a scientific research program focussing on the sources, fate, transport and effects of contaminants in the Massachusetts and Cape Cod Bays ecosystem. To maximize the efficiency of limited research funding, the sponsored research program was developed in coordination with research funded by the MWRA, the United States Geological Survey, and the Massachusetts Institute of Technology Sea Grant Program. The study described in this report characterizes the survival of bacterial indicators in sediments and provides recommendations on the use of counts in sediments as a measure of public health risk.

In April, 1990, following a formal process of nomination, the Massachusetts Bays Program became part of the National Estuary Program. The additional funding provided as part of this joint program of the Environmental Protection Agency and the Commonwealth of Massachusetts is being used to continue a coordinated program of research in the Massachusetts Bays ecosystem, as well as supporting the development of a comprehensive conservation and management plan for the coastal and marine resources of Massachusetts and Cape Cod Bays.

The information in this document has been subject to Massachusetts Bays Program peer and administrative review and has been accepted for publication as a Massachusetts Bays Program document. The contents of this document do not necessarily reflect the views and policies of the Management Conference.

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EXECUTIVE SUMMARY

This report summarizes the survival and the effect of environmental factors that affect the survival of the two standard bacterial counts, fecal coliforms and enterococci, in Boston Harbor sediment. The work was conducted under the mission of Projects 3 (transport and retention of contaminants) and Project 4 (bioaccumulation and biotransformation of contaminants) of the Massachusetts Bays Program (MBP). The impetus for the work stems from acknowledged shortcomings of the fecal coliform and enterococcus counts as they are used for monitoring the public health risks in recreational waters and shellfish in marine environments. Regulatory agencies still rely on the fecal coliform and/or enterococcus count as a measure of water quality. Previous work in this laboratory and other laboratories has shown that these indicators of fecal contamination probably survive longer in sediments as compared to their shorter survival in the overlying water. This work was undertaken to better characterize the survival of bacterial indicators in sediments and to provide recommendations on the use of counts in sediments as a measure of public health risk.

Project Summary

The major findings of the report are that both fecal coliforms and enterococci survived significantly longer in sediments than the values reported by others in seawater and that fecal coliform

counts decreased more rapidly than enterococci in sediments. Laboratory experiments demonstrated that grazing is an important factor in the decline of enterococcus counts in sediments. With less impact than grazing, decreases in fecal coliform and enterococcus counts were accelerated by increasing temperature and increasing salinity. In field experiments, counts of bacterial-indicators seeded in surface intertidal sediments declined faster than in corollary laboratory experiments, which indicated that washout and/or sunlight may also be important factors in the decline of counts. Preliminary experiments demonstrated that 10 to 20% of the fecal indicators present in raw sewage could settle within 2 hours. The settling was not affected by salinity nor the concentration of sewage. These settling characteristics also appeared to occur in field experiments.

Recommendations

These results indicate that counts of fecal coliforms and enterococci in sediments have promise as an improved measure of water quality in Boston Harbor and similar ecosystems. It is plausible to assume that if fecal coliforms and enterococci settle onto sediments and survive for prolonged periods, then potential disease-causing agents associated with fecal waste can also settle and survive. The complex and rapid water circulation patterns in Boston Harbor when coupled with the relatively low frequency of sampling feasible for assessing the bacteriological quality of the Harbor, point to the potential use of employing

sediment counts as a more consistent measure of chronic fecal contamination. It is recommended that this alternative measure be pursued. While newer approaches based on emerging biotechnology may be the methods of choice in the future, there are still many obstacles to overcome in the next few years before these methods will be standardized, widely available, and cost-effective. *Clostridium perfringens* spore counts are too conservative to use in assessing risk to public health. F-specific RNA bacteriophage of *E. coli* and other viruses are good markers for sewage but their methods are involved and the counts are still of dubious value. The use of sediment fecal coliform or enterococcus counts would be a relatively simple alternative to current practices.

INTRODUCTION

Historical Data/Information

Both scientists and environmental managers have questioned the use of coliform counts as indicators of fecal pollution and the presence of disease-causing organisms in marine environments. Fecal coliforms die off rapidly in seawater (Carlucci and Pramer 1959; Gameson and Saxon 1967); often more readily than the pathogens themselves (Lessard and Sieburth 1983). Thus the absence of fecal coliforms does not ensure public safety from disease-causing bacteria and enteric viruses derived from fecal contamination (Goyal 1983). In addition, fecal coliforms are not useful as indicators of potential disease-causing bacteria such as pathogenic vibrios, which are indigenous to coastal ecosystems (Joseph *et al.* 1982). Enterococci have been suggested as alternative indicators for fecal coliforms because they survive longer than coliforms in seawater (Borrego *et al.* 1983; Evison and Tosti 1980; O'Malley *et al.* 1982; Pettibone *et al.* 1987), but they too do not assure the absence of viruses or vibrios.

Despite its shortcomings as an indicator of fecal contamination and thus as an indicator of public health risk in coastal ecosystems, the coliform count, and more recently enterococci counts, are still among the sole measures of water quality used to manage recreational and commercial waters. In large part, their utility has been validated through a long history of widespread use for water and wastewater. Among the advantages of

using fecal bacterial counts is low cost, standardization, and ease of use. In addition, the methods do not require highly-trained personnel. However, they are not commonly used for aquatic sediments.

The use of sediment fecal indicator bacteria counts instead of, or in addition to, water column counts could provide a significant improvement over current methodology for monitoring the impact of fecal contamination on coastal ecosystems. Previous work from Boston Harbor conducted in my laboratory (Shiaris *et al.* 1987) and in freshwater and marine ecosystems from other laboratories (LaLiberte and Grimes 1982; Matson *et al.* 1978; Rittenberg *et al.* 1958; Van Donsel and Geldreich 1971) has demonstrated that sediments contain fecal indicator bacteria at much higher densities than overlying waters. There is evidence that fecal coliforms and fecal enterococci survive longer in sediments than overlying water (Marino and Gannon 1991; Roper and Marshall 1979; Shiaris *et al.* 1987). Sediment particles may protect them from predation (Burton *et al.* 1987; Grimes 1980) and high organic matter in sediments compared to the water column may promote their longevity (Gerba and McLeod 1976) and supply them with protective osmoregulatory compounds (Gauthier *et al.* 1991).

Thus, employing sediment indicator counts instead of, or in addition to, water column counts may provide additional advantages for assessing the public health status of coastal ecosystems. One of the major deficiencies of using fecal bacteria as indicators in the water column is their transitory nature and rapid die-off. The term "die-off" here refers to loss of culturability on standard coliform enumeration culture media,

either by loss of the ability to divide and form colonies or by the death of the cell. Their absence in the water column at a given sampling time may belie sporadic episodes of fecal contamination. Estuaries, like rivers, are subject to constant water movement and intermittent sewage inputs, which may lead to deceptively low counts of indicator bacteria in the water column. As in rivers, sediments are more likely to accurately reflect the recent history of fecal pollution in estuarine environments. Since human pathogens could be introduced into the water column by resuspension (Grimes 1975, 1980), sediments near sewage outfalls must be considered a potentially hazardous reservoir of sewage-borne diseases.

Matson *et al.* (1978) suggested monitoring fecal coliform counts in riverine sediments. This approach may be even more useful in coastal sediments (Shiaris *et al.* 1987) because of the increased die-off of fecal bacteria in seawater. Valiela *et al.* (1991) suggested that sediment fecal coliform counts were better indicators of shellfish contamination than counts from overlying waters, which did not correlate well with counts from shellfish. Thus, the use of sediment fecal bacterial counts may provide significant improvement over water column counts in coastal ecosystems. Several laboratories (Gerba and McLeod 1976; Matson *et al.* 1978; Sayler *et al.* 1975; Shiaris *et al.* 1987; Valiela *et al.* 1991) have reported higher densities of fecal indicator bacteria in marine sediments than in overlying waters. In at least two reports the densities of fecal coliforms and enterococci in sediments appeared to be a function of the prevailing current and wind directions from known sources (Loutit and Lewis

1985; Rittenberg et al. 1958). In Savin Hill Cove, an intertidal mud flat in the Dorchester Bay section of Boston Harbor, my coworkers and I (Shiaris et al. 1987) reported a significant correlation of fecal coliforms and enterococci counts in sediments with proximity to a combined sewage outfall (CSO). The sediment counts were reproducible over a two-day period, while counts in the overlying water column were several orders of magnitude lower or below the limits of detection. Thus, it appears that routine monitoring of sediments could provide a more accurate measure of chronic fecal contamination. They may be more reliable indicators of the importance of a CSO or storm drain as a source of fecal contamination. Sediment counts are less dependent on short-term fluctuations in wind direction, wind speed, tidal direction, and tidal velocity. In some instances, for example dredging activities, violent storms, or human activity around bathing beaches, it is likely that sediments themselves are a major source of fecal bacteria to the overlying waters (Grimes 1975, 1980).

A clear distinction should be made between the possible use of sediment fecal coliform counts and sediment *Clostridium perfringens* spore counts. The *C. perfringens* spore count is a useful indicator of human fecal waste (Akama and Otani 1970) when spores are present at high densities in the environment (Bisson and Cabelli 1979). *C. perfringens* spore counts are particularly useful as tracers of sewage movement and deposition (Emerson and Cabelli 1982), but since they are very long-lived bacterial endospores, they have only limited use as an indicator of public health. A good indicator of the risk of communicable disease

should "behave" like the pathogens themselves. *Clostridium perfringens* is too long-lived for that purpose. However, in conjunction with fecal coliform/enterococcus counts, they could provide valuable information on the nature and source of fecal waste inputs into the environment (Emerson and Cabelli 1982).

The relatively high fecal indicator counts in sediments could be due to two factors: (1) high rate of deposition from the overlying water column, and (2) increased survival of fecal bacteria in marine sediments as compared to the overlying seawater. The latter hypothesis is more likely to be important since the sediment counts remain stable for days (Shiaris *et al.* 1987), even when counts in the overlying water are low.

The rapid decrease of fecal bacteria, in particular, fecal coliforms, in seawater with time has been attributed to the effects of salinity (Goyal *et al.* 1977), predation (Enzinger and Cooper 1976), toxic metals (Jones 1964), temperature (Gameson 1984; Larsen and Willeberg 1984; Rhoades and Kator 1988), starvation, solar irradiation (Barcina *et al.* 1990; Fujioka *et al.* 1981; Kapuscinski and Mitchell 1982) and competition by indigenous bacteria (Carlucci and Pramer 1959; Mitchell 1968). The nature of the decrease in numbers is dependent on the methods of enumeration. Traditional fecal coliform and enterococcus counts are based on the ability of the bacterium to be cultured (*i.e.*, grow and divide) in a bacteriological medium. Resulting colonies on filters and agar surfaces are called colony-forming units (CFU). Fecal indicators and potential pathogens, which may not be indigenous to temperate aquatic environments, may go into a nonculturable state (Grimes *et al.* 1986; Roszack and Colwell

1987; Xu *et al.* 1982). In some cases, nonculturable pathogens can still retain their metabolic activity and even infectivity (McFeters and Stuart 1972; Roszack and Colwell 1987). Thus, CFUs could underestimate the threat to human public health.

A recent study on *Escherichia coli* and *Enterococcus faecalis* by González *et al.* (1992) sheds light on the relationship between decrease in fecal indicator counts and culturability in natural seawater. They reported that two factors caused a decrease in the CFUs of these two indicators: grazing by protozoa and the transformation of the cells to a nonculturable state. The decrease in total numbers (both culturable and nonculturable) was attributable to predation by protozoa alone. Their work confirmed the importance of protozoan grazing on enteric bacteria in marine waters as established previously (Enzinger and Cooper 1976; McCambridge and McMeekin 1979, 1980). However, in sediments, predacious bacteria and bacteriophage (Garcia-Lara *et al.* 1991) do not seem to play a significant role.

The effect of environmental parameters on fecal bacterial survival in sediments is less well documented. The increased levels of organic matter in sediments may play a major role (Gerba and McLeod 1976). High organic matter was associated with the increased uptake of glycine betaine by *E. coli* in marine sediments (Gauthier *et al.* 1991). Since glycine betaine is an osmoregulator, it may play a role in the increased survival of *E. coli* in marine sediments. The interaction of fecal bacteria with clay particles may also increase their survival (Burton *et al.* 1987; Grimes 1980). However, in one attempt to correlate 12

environmental variables with coliform densities in estuarine sediments, no correlation was found (LaBelle *et al.* 1980).

Objectives of this investigation

The major objective of the work reported here was to examine the survival of fecal coliforms and enterococci in coastal sediments. The work was undertaken as a first step to develop an improved indicator of public health risk from fecal contamination in coastal ecosystems.

The rationale for assessing enteric bacterial counts in sediments was two-fold. First, if fecal indicator bacterial survival is longer in the sediments than the water column, then sediment counts should provide a more sensitive and reliable measure of chronic fecal contamination than counts based on the vagaries of water column movement and the short-lived nature of indicators. Second, the proposed approach is based on well-established protocols of culturing bacterial indicators. Newer approaches such as DNA probes (Green *et al.* 1991) or sensitive polymerase chain reaction methods (Bej *et al.* 1990, 1991) should be the methods of choice in the future; however, there are many obstacles to overcome in the next few years before these methods could become standardized, widely available, and cost-effective. *C. perfringens* spore counts are too conservative to use in assessing risk to public health (Bisson and Cabelli 1980). F-specific RNA bacteriophage of *E. coli* are good markers for sewage but their methods are involved and the numbers are still of dubious value (Havelaar 1991). The use of sediment fecal coliform or enterococcus counts would be a relatively simple alternative to current practices.

The specific tasks which are designed to meet the major objective were to:

1. determine the survival rate of fecal coliforms and fecal enterococci in sediments of Boston Harbor.
2. determine how these bacterial indicators are deposited in sediments.
3. determine the environmental parameters which affect the survival of bacterial indicators of fecal pollution.

MATERIALS AND METHODS

Sample sites. Three areas in Boston Harbor were used as sample or experimental sites for fecal indicator bacteria studies (Fig. 1). Four sites in Savin Hill Cove (SHC) were located near the Fox Point combined sewer outlet (CSO). SHC1 was west of the CSO, SHC2 within 10 meters of the CSO, and sites SHC3 and SHC4 were south and southwest of the CSO. Initial field work and most laboratory experiments were done with muddy sediments taken from SHC2 for several reasons: fecal coliform and enterococci counts were high at this site; the site is located conveniently near the laboratory; and, other investigators have conducted studies in and around the Fox Point CSO.

Two sites, NR1 and NR2, were sampled for presence of fecal indicator bacteria. They were initially included as potential experimental sites because there are several storm drains along the Neponset River. These two sites were not used for any further experiments.

The muddy sediment site, WR2, in the Weir River near World's End, Hingham, was chosen for field survival experiments because background fecal coliform and enterococci counts were relatively low compared to other potential sites in the Boston Harbor. These sediments were seeded with raw sewage and decline in counts was observed over a period of several weeks. Cores were incubated at ambient temperature in the dark. Therefore, it was crucial that the initial loads and inputs of indicator bacteria were negligible.

Sediment cores. Sediment corers were constructed from 2-inch diameter polycarbonate tubing cut to 35-cm lengths. They were used to sample sediments and for laboratory and field survival experiments. For laboratory experiments, corers were gently pushed into sediments within 3 to 5 cm from the top. They were removed from sediments at low tide and both ends were capped. Cored sediments were placed into chest coolers and kept at ambient temperature (with water from the site) for immediate transport to the laboratory. Prior to removal, sediment temperature and overlying water salinity (Beckman RS-5 Salinometer) were measured. pH was determined in the laboratory from core subsampled porewater.

For field experiments, corers were pushed by hand approximately 20 cm into the sediment, allowing 15 cm to protrude. An 82-mm diameter Nylon membrane (0.45- μ m pore-size) was secured to the top of the core with an O-ring or rubber band to prevent fresh particle settling onto the core surface.

Similarly, autoclaved (sterile) sediments were placed in cores of varying diameters (from 6 to 65 mm diameter) to determine the appropriate core diameter for survival and deposition rate experiments. Cores were placed into Fox Point (site SHC2) sediments, removed 12 days later, and enumerated for enterococci, but not fecal coliforms.

Sample processing. Sediment was extruded through the top of the core by pushing a tight-fitting plunger from the bottom of the corer. The top 1 cm was aseptically removed and placed in a preweighed mini-blender to obtain the wet weight. The sediment was diluted (1:10 wt/vol) with autoclaved estuarine

water and homogenized in a Waring blender for 20 s to free bacteria from sediment particles (see Results section). Homogenized samples were serially diluted in peptone water, and appropriate dilutions were filtered through a 0.45- μ m pore-size membrane (Gelman Sciences, Inc.). Filters were prepared in triplicate.

Fecal coliform counts. Fecal coliforms were enumerated by standard membrane filtration methods (Clesceri et al. 1989) as amended for sediments (Shiaris et al. 1987). To enumerate fecal coliforms, filters were placed in 50-mm culture dishes on MF support pads (Millipore Corp.) saturated with mFC broth supplemented with 1% (wt/vol) rosolic acid. Dishes were incubated for 24 h while submerged in a circulatory water bath at $44.5 \pm 0.2^\circ\text{C}$. Only typical blue colonies were picked for isolation and further tested on commercial API® identification kits (Analytab Products, Plainview, NY) to verify that typical blue colonies were fecal coliforms.

Fecal coliform enumerations were conducted with newer membrane filter media formulation to evaluate their efficiency for counting sediment fecal coliforms. Medium m-7FC with 4-methylumbelliferyl- β -D-glucuronide (MUG; Reasoner et al. 1979) and m-T7 agar (LeChevallier et al. 1983) were examined. The m-7FC/MUG medium contained (g/L): proteose peptone #3 (Difco), 4.0; yeast extract (Difco), 3.0; lactose, 10; mannitol, 5; sodium chloride, 7.5; sodium dodecyl sulphate, 0.2; sodium desoxycholate, 0.1, bromocresol purple, 0.35; phenol red, 0.3; agar, 15.0. The medium was autoclaved, cooled, and amended with filter-sterilized MUG (Sigma) to a final concentration of 50 μ g/ml. The

m-T7 agar contained (g/L): proteose peptone #3, 5.0; lactose, 20.0; yeast extract, 3.0; Tergitol 7 (25% solution; Sigma), 0.4 ml; polyoxyethylene ether W-1 (Sigma), 5.0; bromthymol blue, 0.1; bromcresol purple, 0.1; agar, 15.0. The medium was autoclaved, cooled, and amended with 0.1 g/L penicillin G.

Enterococci counts. Enterococci were enumerated by standard methods (Clesceri et al. 1989) as amended for sediments (Shiaris et al. 1987). To enumerate enterococci, filters were placed on KF streptococcal agar supplemented with 0.1% (wt/vol) triphenyl-tetrazolium chloride or on m-Enterococcus agar and incubated for 45 to 51 h at $35 \pm 0.5^\circ\text{C}$. Typical red colonies were counted. All counts were done with the aid of a dissecting microscope on filters containing between 30 and 300 colony-forming units (CFU). Random colonies were picked for isolation and further testing by Gram staining and by inoculating commercial API® CH kits designed for the identification of streptococci.

Effective particle size fraction of fecal bacteria. An attempt to determine the effective particle size of fecal bacteria was done by gently filtering water samples through a series of filters with varying pore size. Counts were done on the filtrate and filter-bound fecal indicators. Samples were taken from overlying water, from Nut Island sewage treatment plant effluents, and from a CSO to determine if the particle association of fecal bacteria changes during residence time in the estuary. Estuarine water samples and samples of sewage treatment plant effluent (prechlorinated) and combined sewage were separated into a settleable solid and water phase (Clesceri et al. 1989). One liter of sample was placed into an Imhoff cone and allowed to

settle for several hours. The sides were gently stirred with a sterile glass rod. A subsample of the settled solids was drawn off by pipette. Particles in the water phase were further differentiated by filtering through a series of polycarbonate membrane filters under very gentle conditions (vacuum pressures under 5 inches of mercury). Fractions were $\geq 20 \mu\text{m}$, $\geq 10 \mu\text{m}$, $\geq 1 \mu\text{m}$, and $\leq 1 \mu\text{m}$. The samples were resuspended in a peptone buffer and counts of fecal bacteria were performed as described above.

Survival of indicator bacteria in sediments: field experiments. The experimental design originally proposed for determining die-off in the field was to place cores into sediments and fit them with filters to prevent further sedimentation of coliforms and enterococci. Preliminary experiments in the field indicated that the small pore-size membranes ($0.45 \mu\text{m}$) necessary to exclude the fecal indicator bacteria also restricted advective flow within the core, which resulted in chemical changes. This was obvious in the decreased depth of O_2 penetration into the sediments as estimated from sediment color (light grey when oxidized and black when reduced).

A different approach was attempted here: to remove an intact section of contaminated sediment from site SHC2 and place it in a comparable muddy location, WR2, except that WR2 did not receive as much coliform- and enterococcus-laden sedimentation. This approach did not work because it was logistically difficult to move enough intact surface sediment to another site without considerable perturbation.

Thus an alternative field approach was employed. A $0.75 \times 0.75 \text{ m}^2$ plexiglass box (open top and open bottom) was constructed

and placed in relatively clean intertidal sediments at WR2. The box was 50 cm tall. On the flood tide as water began to fill the box, raw sewage (40 liters) from the Nut Island Sewage Treatment Plant was gently added to provide an inoculum of fecal indicator bacteria. The box was left in place for 2 tidal cycles and removed. Although the sewage did not percolate into the fine-grained sediments, a significant portion of the fecal indicators present in the sewage were deposited onto the surface of the sediments in the course of two tidal cycles. The corners of the box were marked with plastic stakes so replicate cores could be taken at subsequent low tides within the original boundaries of the box. Control cores were taken outside the box boundaries to account for extant densities of fecal indicator bacteria at the WR2 site. Enterococci and fecal coliforms were enumerated every several days and in control (unseeded) plots.

Effect of temperature and salinity on the survival of fecal bacteria in sediments. The focus was on three major parameters which seem to affect the rate of indicator bacteria survival in sediments: temperature, salinity, and grazing by protozoans.

A 3 x 3 matrix was used to examine the effect of temperature and salinity on survival. These experiments were conducted in sterilized sediments seeded with raw sewage and incubated in the laboratory to isolate the temperature and salinity effects from other environmental parameters. Sediments from site WR2 were homogenized, diluted 1:10 with distilled water or saline water to provide three salinities: 0 (effectively less than 1), 15, and 30 ‰. Sediment slurries (100 g) were placed in 1 liter beak-

ers fitted with parafilm covers and autoclaved. Triplicate beakers of each salinity were incubated at 4, 12, and 25°C. All sediments were inoculated with 10 ml raw sewage from Nut Island STP. The non-sterile controls, which were incubated at 25°C and 30‰ salinity, displayed survival curves typical of previous experiments.

Grazing of fecal indicator bacteria by benthic protozoans.

The study was conducted on a benthic community at the low-tide level of a well-protected tidal flat in Savin Hill Cove (site SHC2). The sediment was composed of a fine mud; the salinity of overlying water was about 30‰; and the day-time temperature at the mud surface was 20°C. The experiment was conducted in May, 1991.

Four strains of enteric bacteria were used as model prey. *Enterococcus faecalis* ATCC 27274, *Enterococcus faecium* ATCC 19434, and two sewage treatment plant isolates of *Escherichia coli* were grown separately in tryptic soy broth (Difco, MI) at 35°C. After a 24-h incubation, bacteria were centrifuged (10,000 x g for 10 min) and washed 3 times in 0.05 M Na₂HPO₄ in 0.85% NaCl solution. The suspension of *E. faecalis* was mixed with the suspension of *E. faecium*. Similarly, the two *E. coli* strains were combined. Coliforms were stained with DTAF, 5-([4,6-dichlorotriazin-2-yl]amino)fluorescein (Sigma Chemical Co., St. Louis, MO), after Sherr et al. (1987), for 2-h at 60°C with 2 mg of DTAF. The enterococcus mixture was stained with acridine orange (0.01% final concentration) for 2 h at 60°C. After staining, bacteria were washed 3 times with the Na₂HPO₄-NaCl solution and resuspended in filter-sterilized seawater from the sample

site. The seawater was filter-sterilized by passing it through a 0.2- μm pore-sized Millipore (Bedford, MA) or Nuclepore (Pleasanton, CA) filter. In order to avoid clumps and obtain homogeneously dispersed cells, mixtures were filtered through 1.2- μm pore-size Nuclepore filters and sonicated for 1 to 2 sec. The density of fluorescently-labelled coliforms was adjusted to 4×10^8 cells/ml, and the density of fluorescently-labelled enterococci was adjusted to 3×10^8 cells/ml.

Intact sediments were obtained by hand-coring with a 47-mm i.d. polycarbonate core (four replicates) and the cores were transported to the laboratory within one hour of collection. The sediment was carefully extruded through the top of the core and the top 2-cm horizon was sliced off. The core slice (4 replicates) was placed onto a scintered-glass Millipore filter-unit with a 42-mm filtration surface diameter. The coliform mixture (6 ml) and enterococcus mixture (5 ml) were carefully layered on top of the sediments. Negative pressure was applied from beneath to replace the pore-water in the sediment sections with the fluorescently-labeled bacteria (FLB) mixtures. The fluorescently-labeled coliform and fluorescently-labeled enterococcus densities were 23% and 13% of the total native bacteria density, respectively.

The FLB-inoculated sediments were incubated at 22°C for 1 hour. During this incubation, subsamples were taken from each filter unit by coring the sediments with 8-mm i.d. cores to a 1-cm depth. Thus, the present work was restricted to the community inhabiting the upper one centimeter of the sediment. Subsamples were taken at 15-, 30-, 45-, and 60-min of incubation from each

of the 4 replicates. Subsamples consisted of 2 cores per time point for counting ciliates and the meiobenthos, and a single core per time point for flagellates. Subsamples were immediately preserved with cold glutaraldehyde (2% vol/vol, final concentration), and stored at 4°C in the dark prior to microscopic observations.

Microbenthic and meiobenthic organisms were extracted from preserved samples with Percoll (Sigma Chem. Co.), a silica gel, according to the methods of Alongi (1986) and Schwinghamer (1981). Percoll (5 ml) was mixed with each sediment subsample (approximately 6 cm³ final volume), and centrifuged at 500 x *g* for 10 min. The top layer, a mixture of Percoll and pore-water containing benthic organisms, was carefully pipetted off the sediment and fresh Percoll was added. The procedure was repeated 3 times with each sample, and the 3 fractions were combined. These mixtures were filtered through either 0.45- μ m or 3- μ m pore-size cellulose nitrate Millipore filters, so that all organisms of interest extracted from the sample were collected onto a single filter.

Filters were covered with prewarmed (35°C) coverslips containing a drop of melted agar (2% vol/wt solution). After a 5- to 15-sec incubation in a freezer (-20°C) the agar hardened, and coverslips could be removed. Thus, organisms collected on filters were protected by a thin layer of agar from being washed out during following treatments. The entire procedure is described in more detail in the protocol of Montagnes and Lynn (1987). Filters were further dehydrated in a standard series of progressive ethanol solutions: 30, 50, 70, 96, 96, 100, 100% (5

min in each), followed by immersing filters twice in xylene (5 min each). This process results in almost completely transparent filters (Montagnes and Lynn 1987). Treated filters were either embedded in Canadian balsam (permanent slides), or placed on a microscopic slide between two drops of low-fluorescence immersion oil and covered with coverslips (temporary slides).

In addition, several filters representing one subsample from each replicate were stained with 4',6-diamidino-2-phenylindole (DAPI; Sigma Chem. Co.), at a final concentration of 5 $\mu\text{g/ml}$ before immersion in agar. These filters were used to examine and count microflagellates by epifluorescence microscopy on a Zeiss Standard microscope equipped with a Zeiss filter set (BP 365/10 exciter filter, FT 390 beam splitter, LP 395 barrier filter; 1250x magnification). By scanning 100 to 150 microscopic fields per slide, 20 to 35 total microflagellates were examined per slide. Additional filters were stained with nigrosin (Borror 1968) for ciliate identification according to Small and Lynn (1985).

On all other slides, micro- and meiobenthic organisms were detected, identified, counted, and measured under brightfield phase-contrast microscopy on a Zeiss Standard microscope (150x to 1250x magnification). For nematode identification, the guide by Platt and Warwick was used (1983). Ingested FLB were counted in each benthic animal by epifluorescence microscopy (Zeiss filter set: BG 12 exciter filter, FI 450 beam splitter, and barrier filter 47; x1250 magnification) without disturbing the position of the slide. A total of 31 to 74 ciliates, 25 to 88 nematodes, 15 to 25 copepods, 2 to 15 other metazoans was examined on each

filter. The 4 replicates employed in the grazing experiments were sampled at 4 time points; thus 16 filters were examined for flagellates and 32 filters (2 subsamples at each time point for each replicate) were examined for ciliates and meiobenthos.

The ingestion rates of native bacteria were then calculated assuming that native bacteria were consumed in proportion to consumed FLB:

$$I = I_{\text{FLB}} \frac{N_{\text{N.B.}} + N_{\text{FLB}}}{N_{\text{N.B.}}} ;$$

where, I is the grazing rate on native bacteria (bacteria/individual grazer/h); I_{FLB} is the grazing rate on FLB determined in the experiment (FLB consumed/individual grazer/h); $N_{\text{N.B.}}$ is the field density of native bacteria (bacteria/cm³); and N_{FLB} is the concentration of added FLB (FLB/cm³).

For enumeration of native bacteria, four subsamples were taken at the beginning of incubation with 8-mm i.d. cores. Samples were fixed with 3 ml formaldehyde (2% final concentration), incubated with tetrasodium pyrophosphate (10 mM final concentration), and sonicated 3 times for 10 s each (Velji and Albright 1985). After proper dilution, samples were stained with DAPI at a final concentration of 5 µg/ml (Porter and Feig 1980). Each sample was filtered through a 0.2-µm pore-size black Nuclepore filter and washed several times with filter-sterilized (0.2-µm pore-size Nuclepore filters) seawater. Bacteria were detected by epifluorescence microscopy on a Zeiss Standard microscope equipped with a Zeiss DAPI filter set (see above). At

least 200 cells per slide in at least 10 microscopic fields were counted.

Statistical analysis. Data were routinely transcribed from data notebooks to Lotus 1-2-3 files for recordkeeping and transformation into computer data files for use with statistical packages. Instat, a PC-based statistical software package was used for preliminary analysis (Student's t-test and Wilcoxon's two-sample test and binomial test) and MINITAB, a package on UMass/Boston Vax-11 mainframe computer was used for analysis of variance and regression analyses.

RESULTS

Comparison of media and optimization of methods for enumerating fecal coliforms and enterococci in sediments

Comparison of microbiological media. Of the various media examined for optimal differential recovery of indicator bacteria, mFC medium (Difco) was found superior for fecal coliforms in sediments, and m-Enterococcus medium (Difco) was found superior for enterococci in sediments (Table 1). All media were tested as

TABLE 1. Comparison of microbiological media for enumerating fecal coliforms and enterococci in muddy sediments from Fox Point, Savin Hill Cove, Dorchester Bay, MA.

Medium	Fecal Indicator Counts ^a (cfu ^b /ml)	
	Fecal Coliforms	Enterococci
m-FC	18.0 x 10 ³ (5.4 x 10 ³) ^c	-
m-7FC/MUG	8.5 x 10 ³ (3.3 x 10 ³)	-
m-T7	9.1 x 10 ³ (4.8 x 10 ³)	-
m-Enterococcus	-	9.6 x 10 ³ (2.0 x 10 ³)
KF-Streptococcus	-	5.5 x 10 ³ (1.2 x 10 ³)

^a Average of 3 sediment samples taken in January 1990

^b cfu, colony-forming units

^c standard error of the mean, S.E.

agar media in conjunction with membrane filtration methods. All subsequent fecal indicator numbers reported here were derived

from enumeration on these two media. Both media are recommended by the American Public Health Association's *Standard Methods for the Examination of Water and Wastewater* (Clesceri et al. 1989) for enumeration of indicator bacteria in water samples.

Blending and sonication of sediments prior to membrane filtration. Because sediment bacteria are associated with particles, their densities are typically underestimated by viable counting techniques such as membrane filtration. Therefore, time-series of Waring blending and sonication of sediment dilutions was performed prior to spread plating to separate indicator bacteria from particles. Sediments were diluted 1:10 in sterile peptone water and Waring blended or sonicated for 5, 10, 15, 20, 30, 45, 60, 90, and 120 seconds. The optimal blending time, the time that gave the highest fecal coliform counts in the sediments was 20 seconds. Sonication was not as effective as disruption by a Waring blender. Therefore, sediments were routinely blended for 20 sec prior to further serial dilutions and membrane filtration.

Identification of presumed fecal coliforms and enterococci from membrane filters. Random colonies of presumptive fecal coliforms were picked from m-FC plates and identified with routine morphological, physiological, and biochemical tests by the API 20E strip for the identification of Gram negative fermentative rod-shaped bacteria. Samples were obtained from Fox Points cores used to determine survival of indicator bacteria (see below). Of 45 typical blue colonies examined (Table 2), 98% were fecal coliforms: 58% were *Escherichia coli*, 36% were *Klebsiella pneumoniae*, and only one isolate was *Enterobacter aerogenes*.

Only one isolate was not identified. There was no obvious change in the ratio of the 3 fecal coliform species with survival time in the core; however, the samples were too small for statistical confidence testing.

TABLE 2. Identification of presumptive fecal coliforms (typical blue colonies) on m-FC medium. Colonies were picked, streaked for isolation, and identified with API-20E strips.

Day	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>E. aerogenes</i>	NI ^a	Other
0	3	2	1	0	0
7	4	4	0	1	0
14	6	3	0	0	1 ^b
21	6	4	0	0	0
35	7	3	0	0	0

^a NI, Not identified by API 20E system

^b *Enterobacter amnigenus*

Random colonies of presumptive enterococci were also picked from m-Enterococcus enumeration plates for Fox Point core samples. The enterococci were streaked for isolation and identified with routine morphological, physiological and biochemical tests by the API CH strip for the identification of streptococci (Table 3). The most common isolate was *Enterococcus faecium* (46%), followed by *Enterococcus faecalis* (28%). However, other gram positive cocci were identified: *Streptococcus avium*, *Streptococcus mutans*, and *Streptococcus bovis*. Of the 54 isolates, 9 (17%) could not be identified with the routine analyses, although they were Gram-positive cocci. One of the recognized problems of the enterococcus count, is that many bacterial species (at least

2 genera) could be represented, but the sources of enterococci are not well known. The types and frequencies of the isolates did not appear to change with time in laboratory die-off experiments (see below) with the possible exception of *S. bovis*, which was detected only after 35 days.

TABLE 3. Identification of presumptive enterococci (typical red or pink colonies) on m-Enterococcus medium. Colonies were picked, streaked for isolation, and identified with API-CH strips.

Day	<i>E. faecium</i>	<i>E. faecalis</i>	<i>S. avium</i>	<i>S. bovis</i>	<i>S. mutans</i>	NI ^a
0	5	3	0	0	0	1
7	4	4	1	1	0	2
14	5	3	0	0	0	3
21	6	2	1	0	0	2
35	5	3	0	1	1	1

^a NI, Not identified by API CH system

Spatial distribution and variation of indicator bacteria in surface sediments

Densities of bacterial indicators in Boston Harbor sites. Several sites in Savin Hill Cove, the Neponset River, and the Weir River were sampled in December 1989 - January 1990 (Fig. 1). All sites sampled had muddy intertidal sediments. The densities of fecal indicator bacteria in the surficial sediments were determined (Table 4).

TABLE 4. Fecal coliform densities in the surface sediments of intertidal sites in Boston Harbor.

Location	Site No.	Bacterial Counts (cfu/g wet wt sediment)	
		Fecal Coliforms	Enterococci
Savin Hill Cove	SHC1	4.2×10^3 (1.9) ^a	2.8×10^3 (0.9)
	SHC2	7.1×10^3 (3.3)	1.4×10^3 (0.5)
	SHC3	2.0×10^3 (1.3)	1.2×10^3 (0.9)
	SHC4	3.9×10^3 (2.2)	2.3×10^3 (1.2)
Neponset River	NR1	2.5×10^4 (8.7)	9.4×10^3 (7.2)
	NR2	1.9×10^4 (9.5)	5.7×10^3 (4.8)
Weir River	WR1	< 60	< 60
	WR2	< 60	< 60

^a (standard deviation, $\times 10^3$)

Site SHC2, the Savin Hill Cove site closest to the Fox Point CSO was chosen for further laboratory experiments and initial field experiments because it is close and convenient to the laboratory and the fecal indicator counts were high.

Ultimately site WR2 in the Weir River was used for field experiments to determine fecal indicator survival because the field densities were relatively low (below detection). In this approach sediments were seeded with fecal bacteria and monitored numbers under conditions in which natural fecal bacterial deposition was negligible. The site was within 1 hour of the laboratory by automobile or boat, so samples were processed within 2 hours of sampling.

Spatial distribution and variation of bacterial indicators in surface sediments. Nine random samples were taken from a 1.0 m² grid (10 x 10 cells) that had been placed on the sediment surface at site SHC2 (Fig. 1). The area of each sample, of 100 possible samples, was approximately 10 cm². Enumeration of fecal indicator bacteria indicated considerable spatial variation for both fecal coliforms and enterococci on this scale in the surface sediments (Table 5). The mean density of fecal coliforms was 12.4×10^3 CFU/g; the standard deviation was 9.5×10^3 ; and the standard error of the mean was 2.2×10^3 . The mean density of enterococci was 8.8×10^3 CFU/g; the standard deviation was 6.3×10^3 ; and the standard error of the mean was 1.5×10^3 . Therefore, the difference between mean fecal coliforms and mean enterococci was not significant at the 95% level of confidence.

Using the Student's statistic (t) distribution:

$$x \pm t_{1 - (\alpha/2)} s / \sqrt{n}, \text{ where } x = \text{mean}$$

s = standard deviation

$\alpha = 0.95$ (95% confidence)

then,

$$Q(x) = t_{0.975} s / \sqrt{n}, \text{ where } Q = \text{percent precision desired}$$

n = number of replicates

TABLE 5. Spatial distribution of fecal indicator bacteria in Fox Point surface sediments.

Grid No.	Replicate	Bacterial Counts (cfu/g wet wt sediment)	
		Fecal Coliforms	Enterococci
6	1	4.9×10^3	22.0×10^3
	2	4.3×10^3	18.4×10^3
12	1	8.6×10^3	5.5×10^3
	2	9.1×10^3	6.3×10^3
18	1	2.7×10^3	1.4×10^3
	2	3.1×10^3	1.7×10^3
35	1	2.5×10^3	1.4×10^3
	2	1.9×10^3	1.7×10^3
36	1	11.4×10^3	2.0×10^3
	2	13.0×10^3	2.7×10^3
51	1	26.5×10^3	12.2×10^3
	2	28.6×10^3	12.0×10^3
57	1	8.0×10^3	10.8×10^3
	2	11.7×10^3	9.3×10^3
60	1	21.1×10^3	13.7×10^3
	2	29.1×10^3	14.4×10^3
99	1	18.3×10^3	10.6×10^3
	2	21.7×10^3	11.9×10^3

It was determined that 3 replicate cores are required to achieve less than an order of magnitude precision (significance testing between means). This means that triplicate cores are necessary to detect a significant difference ($p < 0.05$) between two means at a precision of $\pm 100\%$ of the mean. Increasing the replication would not be feasible and the returns in precision diminish. To achieve 75% precision, 8 replicates are needed, and 17 replicates are needed for 50% precision. Therefore triplicate samples were used for field sampling.

Effect of temperature and salinity on survival of indicator bacteria in sediments: laboratory experiments

Cores were taken from sediments adjacent to the Fox Point CSO, site SHC2 (Fig. 1) in February 1990 (Fig. 2) and in July 1990 (Fig. 3). The ambient surface sediment temperatures were 5°C in February and 28°C in July. The time elapsed for the fecal indicator bacteria counts to decrease by 90% , or T_{90} , is given in Table 6. Enterococci survived longer than fecal coliforms at

TABLE 6. T_{90} values for fecal coliforms and enterococci in cores taken from Savin Hill Cove site SHC2.

Date	Temperature (°C)		T_{90} (days)	
	Site	Incub.	Fecal Coliforms	Enterococci
February	5	4	11	75
	5	23	11	35
July	28	4	20	65
	28	23	6	70

either incubation temperature. Also, the decrease in fecal coliform counts in cores was accelerated by higher temperatures (Figs. 2 and 3). A similar trend was not obvious for enterococci counts. The conclusion that was drawn from these data is that enterococci survived longer in intertidal sediments than fecal coliforms.

Distribution of indicators with depth in Savin Hill Cove sediments

Replicate cores were taken in Fox Point sediments (site SHC2) along a transect from the CSO: at 5, 10, and 20 m. Cores were subsampled for bacterial indicator counts at 2-cm depth intervals (Fig. 4). Fecal coliform densities decreased rapidly below the top 2 cm. Fecal coliforms were not detected at 4 cm depth at the 2 sites closest to the CSO. In contrast, enterococcus densities decreased less rapidly than fecal coliforms and were detected as deep as 10 cm. Assuming that mixing is inconsequential at 4 cm in these sediments, this is further evidence that enterococci survive longer in marine environments, perhaps with a T_{90} on the order of months in sediments.

Survival of indicator bacteria in sediments: field experiments

Surface sediments at site WR2 (Weir River) were seeded with raw sewage and the decline of bacterial indicators was observed during July, September, and December 1990, and April 1991 as shown in Figs. 5, 6, 7, and 8, respectively. Background counts for both fecal coliforms and enterococci (surrounding sediments) were <100 CFU/ g wet wt at all times during the sampling year. The surface sediment temperatures and estimated T_{90} values are given in Table 7.

TABLE 7. Surface sediment temperatures and T_{90} values for fecal coliform and enterococci survival in seeded Weir River surface sediments.

Month	Surface Sediment Temp (°C)	Bacteria in Raw Sewage (CFU/ml)		T_{90} (days)	
		FC ^a	EC ^b	FC	EC
July	23 - 29	5.9×10^8	2.4×10^6	1.8	4.7
September	21 - 26	3.1×10^8	5.7×10^6	1.8	2.0
December	8 - 12	9.0×10^7	2.6×10^6	1.3	5.8
April	7 - 10	1.9×10^8	4.3×10^6	10	>20

^a FC, fecal coliforms

^b EC, enterococci

Approximately, 10% of the indicator bacteria that were added to the sediment as raw sewage were deposited and still present one day later (the first day of sampling). Initial fecal coliform counts were higher than enterococci, which reflected the higher ratio of fecal coliforms to enterococci in the raw sewage of Nut Island Sewage Treatment Plant. However, the T_{90} values for fecal coliforms were consistently shorter than the values for enterococci. Surface sediment temperature (Table 7) was not significantly correlated to T_{90} values ($p > 0.05$). Except for April, the T_{90} values for both fecal coliforms and enterococci were lower than values determined in cored sediments laden with fecal indicator bacteria that were incubated in the laboratory (see Table 6).

Deposition of indicator bacteria

Effect of core size. No differences in deposition rate of enterococci were found based on core diameter. The deposition rate (uncorrected for loss of CFU during the incubation time) was 23 enterococcus CFU cm⁻² day⁻¹. No statistically significant differences in deposition rates were found among similar size cores placed a meter apart at site SHC2, nor among variances of these rates. Thus it appears that enterococcus deposition was uniform on a scale of meters at this site.

The results of the field work (Table 7) indicated that a significant portion of the fecal indicator bacteria settled out of raw sewage in a relatively short period of time, thus settling experiments were done employing Imhoff cones. Approximately 22% of fecal coliforms (Fig. 9) but only 9% of enterococci (Fig. 10) settled out of the water column within 2 hours. Varying ratios of sewage to seawater (Fig. 11) did not affect the proportion of fecal indicators that settled in 2 hours. The ratio of fecal coliforms to enterococci that settled was also unaffected by seawater (Fig. 11). In other words, varying salinity and bacterial densities did not significantly affect settling characteristics.

Effect of environmental parameters on fecal indicator survival in sediments: laboratory experiments

Effect of temperature and salinity on survival of indicator bacteria in sediments. A 3 x 3 matrix was used to examine the interactive effect of temperature and salinity on survival. These experiments were conducted in seeded sterile sediments in the laboratory to isolate the temperature and salinity effects from other environmental parameters. Results are shown in Figs. 12 and 13; the key to symbols is given in Fig. 13. The non-sterile controls, which were incubated at 25°C and 30‰ salinity, displayed survival curves typical of previous experiments. As before (Figs. 2 and 3), the enterococci survive significantly longer than fecal coliforms in these sediments. What is striking in these experiments compared to previous experiments, is the extended survival of both fecal coliforms and enterococci that were incubated in sterile sediments. The fecal coliform counts decreased about two orders-of-magnitude in the non-sterile controls (Fig. 12), while during the same time period, counts in all the experimental autoclaved sediments did not change. A similar trend can be seen for enterococci (Fig. 13), except that enterococci counts decreased only by a 1/2 order-of-magnitude in five days.

Temperature, to a greater degree than salinity, intensified the decline of both fecal coliforms and enterococci counts. Neither temperature nor salinity significantly affected indicator counts until 20 days for fecal coliforms (Fig. 12) and 49 days for enterococci (Fig. 13). Results of 2-way analysis of variance for day 49 counts are shown for fecal coliforms in Table 8 and for enterococci in Table 9. Temperature accounted for 60% of the variation in fecal coliform counts and salinity for 19%

(Table 8). Similarly, temperature accounted for 68% of the variation in enterococci counts and salinity for 8%, but there were significant non-additive effects between temperature and salinity for the enterococcus counts (Table 9).

TABLE 8. Results of analysis of variance of fecal coliform survival on day 49 in sediment slurries.

Source	DF ^a	SS ^b	MS ^c	F-value	p ^d
Temperature	2	963	481	28.26	0.0001
Salinity	2	315	158	9.25	0.002
Interaction	4	37	9	0.54	0.71
Error	18	306	17		
Total	26	1622			

^a DF, degrees of freedom

^b SS, sum of squares

^c MS, mean squares

^d p, probability

TABLE 9. Results of analysis of variance of enterococci survival on day 49 in sediment slurries.

Source	DF ^a	SS ^b	MS ^c	F-value	p ^d
Temperature	2	774	387	44.23	<0.0001
Salinity	2	92	46	5.27	0.016
Interaction	4	117	29	3.35	0.032
Error	18	157	9		
Total	26	1140			

^a DF, degrees of freedom

^b SS, sum of squares

^c MS, mean squares

^d p, probability

Effect of grazing on indicator bacteria in sediments. Savin Hill Cove site SHC2 surface sediment samples contained bacteria, microphytobenthic species (not studied), protozoans, nematodes, harpacticoid copepods and their nauplii, and gastrotrichs. The extant bacteria were mostly rod-shaped cells at a density of 7.5×10^8 cells/cm³ (s.e. $\pm 2.2 \times 10^8$). Protozoans were represented by microflagellates, ciliates, and foraminiferans. Microflagellates, mostly small round cells 2 to 6 μ m in diameter, outnumbered all other protozoans; their mean density was 1.1×10^4 cells/cm³. The ciliated protozoan assemblage was relatively poor in both abundance and species diversity. The two species *Prorodon* sp. and *Chlamidodon* sp. represented 40% of the total ciliate density of only 84 individuals/cm³. Foraminiferans were rare; their density was less than 10 individuals/cm³.

Meiobenthic species composition was typical for intertidal communities. Nematodes were the predominant group with a total density of 118 individuals/cm³. Three species numerically dominated the nematodes: *Metoncholaimus* sp., *Comesoma* sp., and *Monhystera* sp. Harpacticoid copepods represented the second most abundant metazoan group with a total density of 42 individuals/cm³. Other meiobenthic animals were rare relative to nematodes and harpacticoids (total density < 20 individuals/cm³, Table 10).

TABLE 10. Benthic animal field densities, their grazing rates on FLB and calculated grazing rates on field bacteria.

Organism	Field Density	Ingestion rates	Ingestion
rates	(ind ^a /cm ³)	(FLCol ^b /ind/h)	
(bac ^c /ind/h)			
Microflagellates	1.1x10 ⁴ (0.8x10 ⁴) ^d	1.2 (4.0)	5
Ciliates			
<i>Prorodon</i> sp.	18 (10)	169 (147)	704
<i>Chlamidodon</i> sp.	16 (22)	162 (128)	675
Other ciliates	50 (40)	26 (59)	108
Foraminiferans	< 10	0	0
Nematodes			
<i>Metoncholaimus</i> sp.	14 (14)	126 (128)	525
Other nematodes	104 (58)	5 (31)	21
Harpacticoid copepods	42 (48)	10 (32)	42
Nauplii	18 (20)	8 (12)	33
Gastrotriches	< 10	0	0

^a ind, individuals

^b FLCol, fluorescently-labelled coliforms

^c bac, bacteria

^d ± standard error of the mean in parenthesis

Bacterivory by the micro- and meiobenthos. Almost all animals examined ingested fluorescently-labelled bacteria (FLB), but the rates of FLB ingestion varied by over 2 orders of magni-

tude. The number of ingested fluorescently-labeled coliforms by *Prorodon* sp., *Metoncholaimus* sp., and other nematodes increased steadily with time (Fig. 14). In contrast, the number of fluorescently-labeled coliforms encountered in microflagellates, *Chlamidodon* sp., unidentified ciliates, and nauplii did not maintain a steady increase beyond the 15-min incubation period. Therefore, ingestion rates of all animals were calculated from the number of fluorescently-labeled coliforms consumed in 15 min (Table 10).

Two ciliates, *Prorodon* sp. and *Chlamidodon* sp., and a nematode, *Metoncholaimus* sp., had the highest coliform ingestion rates, 126 to 169 FLB/h. Other ciliates, as well as other nematodes, consumed either few fluorescently-labeled coliforms or none, with a resulting grazing rate of <5 to 26 FLB/h. Microflagellates, harpacticoid copepods and their nauplii also had low rates of FLB consumption, 1.2 to 10 fluorescently-labeled coliforms/h. On one occasion only, however, a harpacticoid copepod specimen with the gut filled with thousands of fluorescently-labeled enterococci was observed. Since it was the only specimen out of hundreds of harpacticoid copepods examined that contained FLB, that datum was omitted from further calculations. Other groups of benthic animals, foraminiferans and gastrotriches, did not contain FLB.

Differences in ingestion rates were also distinguishable at the genus level (Table 10). Thus, the grazing rates on FLB were not only group-specific, but, also, at least genus-specific: the two most abundant ciliate genera (represented probably by a single species each) consumed fluorescently-labeled coliforms at

rates statistically higher ($p < 0.01$, Wilcoxon two-sample test) than the ingestion rates of other ciliates (Table 10). Similarly, *Metoncholaimus*, a nematode, consumed fluorescently-labeled coliforms at a significantly higher rate ($p < 0.01$) than other nematodes.

Benthic animals appeared to select between fluorescently-labeled enterococci and fluorescently-labeled coliforms. Unfortunately, acridine orange-labeled enterococci lost their fluorescence within 1 to 3 days following the completion of the experiment, so that the data comparing coliform consumption with enterococcus consumption were available only for ciliates, because they were processed and enumerated first. The binomial test indicates that the ratio of fluorescently-labeled coliforms/fluorescently-labeled enterococci in digestive vacuoles of *Prorodon* sp. and *Chlamidodon* sp. (155:1 and 74:1, respectively) was significantly different ($p < 0.01$) from the expected ratio (5:3). Some unidentified ciliates contained a 5:4 ratio of fluorescently-labeled coliforms/fluorescently-labeled enterococci, which was not significantly different from expected. Some ciliates, therefore, discriminated between enterococci and coliforms with preference for rod-shaped coliforms while others consumed different FLB without selection.

DISCUSSION

Fecal coliforms and enterococci in Boston Harbor sediments

Several lines of evidence demonstrated that enterococci survive considerably longer in coastal sediments as compared to fecal coliforms. In laboratory core survival experiments, which used naturally contaminated sediments next to Fox Point CSO, the T_{90} values for enterococci were 3- to 12-fold longer than the T_{90} values for fecal coliforms (Table 6). Similarly, T_{90} values for enterococci were 1.1- to 4.5-fold longer than the T_{90} values for fecal coliforms in field experiments in Weir River sediments seeded with raw sewage (Table 7). Further evidence was provided by depth profiles of sediments near Fox Point CSO (Fig. 4). Fecal coliforms were confined to the top 2 to 4 cm, while enterococci were detected down to 9 cm. The prolonged survival of enterococci compared to fecal coliforms mirrors the trend reported for seawater (Borrego *et al.* 1983; Evison and Tosti 1980; O'Malley *et al.* 1982; Pettibone *et al.* 1987), which suggests that similar factors contribute to their relative decline in both habitats.

Enterococci have been suggested as alternative indicators for fecal coliforms in marine environments because they survive longer than fecal coliforms in brackish water. This work extends this survival relationship to Boston Harbor sediments. Cabelli *et al.* (1982) found a significant correlation between enterococcus counts, perhaps due to their prolonged survival in seawater,

and swimming-associated gastroenteritis. This has prompted some coastal regulatory agencies to adopt an enterococcus standard for coastal water quality. However, caution and perhaps further investigation is warranted. In a recent publication, Fleisher (1991) reanalyzed Cabelli and workers' epidemiological data that supports United States federal bacteriological water quality criteria governing marine recreational waters and found that the original statistical analysis was flawed. He concluded that there is no scientific basis for current standards based on enterococcus counts. I suggest, from arguments developed in this section, that an examination of the relationship of enterococcus counts to swimming-related illness might provide a better standard.

In addition to investigating the relationship of sediment enterococci to water quality, the nature and distribution of enterococci in coastal waters and sediments should be examined in greater detail. In these experiments, only 76% of the counts reported as "enterococci" were from bacterial species with a human fecal origin (Table 3). The counts were performed on m-Enterococcus medium, which is superior to KF-Streptococcus medium (A.P. Dufour, 1980, Abstr. Ann. Meet. Am. Soc. Microbiol., p. 205). Similar results between the two media were obtained in Boston Harbor sediments (Table 1). This suggests that there may be sources of enterococci other than sewage to Boston Harbor sediments. Their significance and contribution to sediment (and water) enterococci densities should be understood if the enterococcus count is employed.

The survival time of culturable fecal coliforms and enterococci in Boston Harbor sediments was long when compared to reported survival times in seawater (Gerba and McLeod 1976; Matson *et al.* 1978; Roper and Marshall 1979; Sayler *et al.* 1975; Shiaris *et al.* 1987). Survival for fecal coliforms, as measured by T_{90} values, ranged from 6 to 20 days in cores (Table 6) and 1.3 to 10 days in seeded sediments in the field (Table 7). For enterococci, values ranged from 35 to 75 days in cores, and 2 to >20 days in seeded sediments in the field. In general, estimates of T_{90} from data in the literature are in the hour to day range for seawater and in the days to weeks range for sediments. T_{90} is a crude estimate which does not take into account any environmental conditions, lag times, or the nature of the kinetics of the decline of indicator counts; however, it is still useful as a general measure.

The prolonged survival of both indicators in sediments as compared to overlying seawater indicates that surface sediment indicator counts may be a more useful measure of water quality than counts in the overlying water for management of coastal waters. Sediments from sites known to be near fecal contamination sources displayed consistently elevated densities of fecal coliforms and enterococci. Sediments from Savin Hill Cove, which is contaminated by the Fox Point CSO, and sediments from the Neponset River, which has several major storm drains in the area, had $>10^3$ fecal indicator bacteria per g sediment at all times sampled over a 2-year period. In contrast, sediments in the Weir River near World's End, a site distant from direct inputs of fecal contamination other than from boating, always had

less than 100 fecal indicator bacteria per gram of sediment. This supports previous work that sediment counts may be more reliable than water column counts in Boston Harbor (Shiaris *et al.* 1987) and in other freshwater and marine ecosystems (LaLiberte and Grimes 1982; Matson *et al.* 1978; Rittenberg *et al.* 1958; Van Donsel and Geldreich 1971).

Boston Harbor and other coastal ecosystems, like rivers, are subject to constant water movement and intermittent sewage inputs. If monitoring is not done at a frequency equal to or shorter than the variability of inputs and water movement, the mean indicator bacteria counts in the water column will be severely underestimated. As in rivers, sediments are more likely to accurately reflect the recent history of fecal pollution in estuarine environments. The potential usefulness of sediment counts was recently demonstrated in a river system with known point sources of sewage input. The sediment fecal coliform counts were more consistent with time and thus more reflective of chronic sewage contamination as compared to the high variable counts in the overlying water (G.W. Pettibone and K.N. Irvine, 1992, Population dynamics of indicator bacteria associated with combined sewer overflows and bed sediment in the Buffalo River, New York, Abstracts of the Ann. Meet. of the Am. Soc. Microbiol., p. 298). One of the major deficiencies of using fecal bacteria as indicators in the water column is their transitory residence time and rapid decline. Their absence in the water column at a given sampling time does not necessarily provide high confidence that they will be absent in the water a short time later.

The work reported here supports the suggestions of Matson et al. (1978), Shiaris et al. (1987), and most recently, Valiela et al. (1991) that monitoring fecal coliform counts, and perhaps enterococci, in sediments could provide significant improvement over water column counts in coastal ecosystems. Several laboratories (Gerba and McLeod 1976; Matson et al. 1978; Sayler et al. 1975; Shiaris et al. 1987) have reported higher densities of fecal indicator bacteria in marine sediments than in overlying waters.

Settlement of fecal indicator bacteria

The presence of high sediment densities of fecal coliforms and enterococci adjacent to sources of contamination was borne out with experiments to determine settling characteristics (Figs. 9 and 10). A significant proportion of fecal coliforms (>20%) and enterococci (almost 10%) could settle out of sewage and onto the bottom in areas nearby sewage inputs. The settling was not affected by salinity nor by fecal indicator bacteria densities. In spite of the water movement from tidal flow over the intertidal zone, significant (ca. 10%) settling of fecal coliforms and enterococci was consistently observed in Weir River sediments that were seeded with sewage as compared with control areas.

To my knowledge, only one study has been reported in the scientific literature on the deposition of fecal bacteria in sediments (Milne et al. 1986). This work by Milne and coworkers was conducted in bottles and suggested that fecal coliforms

rapidly adsorbed to added suspended particles. If fecal bacteria remain free as unattached particles during their residence in the water column, depositional rates should be very low and they could be transported to great distances in coastal systems. A typical *E. coli* cell is rod-shaped with the approximate dimensions of 1.1-1.5 x 2.0-6.0 μm (Brenner 1984) and weighs about 9.5×10^{-13} g (Ingraham et al. 1983). However, if all or some of the fecal bacteria in the water column are attached to larger particles, their rate of deposition would be much greater and related to the local source(s) of input. The proportion of bacteria which are attached to particles in marine environments can vary greatly, from less than 1% in the pelagic (Hobbie et al. 1972) to 98% in estuaries (Goulder 1977). Nearshore waters tend to have an increased percentage of attached bacteria (Bell and Albright 1982), and it is likely that at least some percentage of fecal indicator bacteria are also attached (Milne et al. 1986). Several unsuccessful attempts to determine the effective particle size of fecal coliforms and enterococci in sewage were made in this study. But indirect evidence, the distribution of fecal coliforms and enterococci in the intertidal sediments of Savin Hill Cove with respect to a combined sewage outfall, suggests a rapid deposition rate in these waters (Rittenberg et al. 1958; Shiaris et al. 1987).

It is likely that sediments themselves are a major source of fecal bacteria to the overlying waters (Grimes 1975, 1980). The T_{90} values of field experiments (Table 7) were much shorter than laboratory experiments (Table 6). A plausible explanation is that indicator bacteria were physically removed from surface

sediments by resuspension from tidal action, storm activity, and agitation by rainfall. Since human pathogens could be introduced into the water column by resuspension (Grimes 1975, 1980), sediments near sewage outfalls must be considered a potentially hazardous reservoir of sewage-borne diseases. The field approach used here represents a significant improvement over the few previous attempts that used laboratory strain inocula and autoclaved sediments in chambers to measure survival of fecal bacteria in sediments. Here, the intact sediment fecal bacteria in a system that achieves minimal disruption to the sediment integrity under ambient environmental conditions indicated that washout and/or inactivation by sunlight (Barcina *et al.* 1990; Fujioka *et al.* 1981; Kapuscinski and Mitchell 1982) may be important factors in the densities of indicator bacteria in the field.

Effect of environmental factors on fecal indicator survival

Increasing temperature accelerated the decline of fecal coliforms and enterococci counts in surface sediments. This relationship has been demonstrated by others in aqueous systems (Larsen and Willeberg 1984; Rhoades and Kator 1988). The detrimental effect was more pronounced for fecal coliforms than for enterococci. In naturally contaminated cores incubated in the laboratory (Figs. 2 and 3), decline of fecal coliform counts was faster at 23° than at 4°C. Temperature was also a factor in sterile sediments seeded with raw sewage (Figs. 12 and 13); however, survival was greatly extended when sediments were first

sterilized. It appears that a major factor contributing to the reduction of indicator counts in sediments is the living components, *i.e.*, microfauna, meiofauna, or macrofauna. Removal of grazing pressure, and to a lesser extent competition with autochthonous flora (Carlucci and Pramer 1959; Mitchell 1968), are the most likely explanations for the enhanced survival. Therefore the temperature effect in natural sediments is most likely an indirect effect. In other words, grazing and competition are stimulated in the field with increasing temperature. The temperature effect was not distinct in the field studies, most likely because other factors had a larger effect.

Increasing salinity also decreased survival of culturable fecal coliforms and enterococci. As before, the effect was much more pronounced for fecal coliforms than for enterococci (Tables 8 and 9). The rapid decline of fecal bacterial counts, in particular fecal coliforms, in seawater has been attributed to the toxic effects of salinity (Goyal *et al.* 1977).

Enterococci were preferentially ingested by ciliates, heterotrophic flagellates, and photosynthetic flagellates as compared to fecal coliforms. In the seawater systems that were used, the fecal coliforms appeared to clump to each other and to organic particles in the water. This may account for their preferential escape from protozoan predators.

Only a few studies have been conducted on benthic grazing because, in part, the methodology for studying benthic bacterivory is fraught with difficulties (Carman 1990; Carman, K.R. *et al.* 1989; Dobbs *et al.* 1989; Fallon *et al.* 1983; Montagna 1983; Montagna and Bauer 1988; Tremaine and Mills 1987). Approaches

that have been used to quantify micro- and meiobenthic grazing rates on benthic bacteria can be described as either indirect or direct. The indirect approach is based on inhibitors that selectively block bacterial growth and/or animal consumption of bacteria (Montagna and Bauer 1988; Sanders and Porter 1986; Sherr *et al.* 1986). The grazing rates on bacteria are calculated from the decrease in bacterial densities. The rates are dependent on the specificity of the inhibitors, but to a varying degree, some of the non-target organisms are sensitive and some of the target organisms are resistant to the inhibitors. Thus, this approach can cause considerable uncertainty.

Bacterivory can be measured directly by amending sediments with bacteria labelled with either radioactive elements (Hargrave 1970; Penon *et al.* 1991) or fluorochromes (Kemp 1988; Sherr *et al.* 1987). Alternatively, bacteria can be selectively labelled *in situ* with [³H]thymidine on the assumption that bacteria but not eukaryotic organisms become labeled with appropriate concentrations of [³H]thymidine (Findlay *et al.* 1984; Hollibaugh *et al.* 1980; Montagna and Bauer 1988). This approach is attractive because it perturbs the community less than the other methods and it allows simultaneous measurement of bacterial production, but it generates other problems. The shortcomings include high rates of non-specific binding of [³H]thymidine, radiolabel accumulation by grazers bodies via non-feeding processes, and intensive uptake of the label by enteric and epicuticular bacteria (or nongrazed bacteria) living inside and on the surface of potential grazers (Montagna and Bauer 1988). Similar problems were encountered

when ^{14}C -labelled tracers were used for measuring herbivory (Blanchard 1991; Carman 1990; Montagna 1983).

In Boston Harbor sediments, bacterivory was measured in micro- and meiobenthos using fluorescently-labeled bacteria, fecal coliforms and enterococci, as model food items. This technique is direct since only FLB within the digestive vacuoles/guts of predators were counted. Much of the uncertainty inherent in grazing rates calculated by indirect approaches (Fallon *et al.* 1986; Tremaine and Mills 1987) is averted. A major advantage of this approach is the ability to collect all potential grazers on one filter per sample for individual examination, eliminating the need for time-consuming hand-sorting.

As it is commonly employed, it is not possible to identify all the grazers with certainty on the same filter used to determine FLB consumption. Black polycarbonate filters are used to minimize background fluorescence, but they are not optically transparent. Thus, reliable identification is hampered and protozoans are typically lumped into a single group (Novitsky 1990) or, at best, into two groups, microflagellates and ciliates (Kemp 1988). The use of cellulose membrane filters cleared in xylene allows, in a single microscopic field, simultaneous enumeration of consumed FLB by epifluorescent microscopy and identification of the grazer. An additional advantage of cellulose membranes is the ability to distinguish between internal FLB and FLB absorbed on the outer surface of the grazer's body.

The modified technique yielded considerable information about benthic grazing in the muddy tidal flat but none of the techniques available are without flaws. Two aspects of the FLB

technique could lead to bias in the determination of grazing rates. First, the addition of FLB to the sediment can disrupt the community and influence animal feeding rates and behavior in an unpredictable manner (Carman *et al.* 1989; Dobbs *et al.* 1989). Of the various approaches to amending the sediment, the pore-water replacement technique is one of the least disruptive (Carman *et al.* 1989; Dobbs *et al.* 1989). Second, discrimination of prey based on size or type by grazers can influence the estimate of total bacterivory. Influence by prey size and shape in food selection has been well documented at least in planktonic protozoans (Andersson *et al.* 1986; Chrzanowski and Simek 1990; Gonzalez *et al.* 1990; Sherr *et al.* 1990; Turley *et al.* 1986). Therefore, the choice of bacteria for FLB preparation may be crucial. Allochthonous cocci- and rod-shaped FLB were used to account for size- and shape variations in prey, although most of the data obtained is based on consumption of coliforms only. However, rod-shaped bacteria were predominant in the benthos.

High background fluorescence of cellulose filters makes visualization of the smallest protozoans, the microflagellates, more difficult since the filters must be stained with a fluorescent stain to adequately visualize them. Therefore, the original technique of Sherr *et al.* (1987) is more appropriate for studying flagellate bacterivory.

While technically difficult, FLB could still be counted in small protozoans to provide an estimate of microflagellate grazing rates on FLB (Table 10). The estimate is on the low end of the range of rates reported in the literature for planktonic species (2 to 363 bacteria flagellate⁻¹ h⁻¹, (Andersen and

Sorensen 1986; Bloem *et al.* 1989; Daggett and Nerad 1982; Davis and Sieburth 1984; Fenchel 1982; Kopylov *et al.* 1980; Landry *et al.* 1984; McManus and Fuhrman 1988; Sherr *et al.* 1983; Weisse 1981), but comparable to the rate of benthic bacterivory (2 bacteria/flagellate/h) estimated by Kemp (1990). Unless the flagellate feeding rate was severely underestimated, the density of microflagellates in the present community was probably too low to affect bacterial abundances. The flagellate to bacteria ratio in the muddy tidal-flat was 1:70,000. Kemp (1990) suggested that a ratio greater than 1:1,000 is necessary to affect bacterial abundance. It is estimated that the entire microflagellate assemblage could potentially consume only 0.2% of the bacterial standing stock per day.

In contrast to the microflagellates, the ciliates were voracious grazers of bacteria (Table 10). At least some ciliate species, especially in planktonic communities, are intensive bacterivores (Bernard and Rassoulzadegan 1990; Gonzalez *et al.* 1990; Rivier *et al.* 1985; Sherr *et al.* 1987, 1989; Sherr and Sherr 1987). High ciliate grazing rates on sediment bacteria (37 to 600 bacteria/ciliate/h) were reported in a few benthic studies that focussed on ciliate grazing (Fenchel 1975, Kemp 1988). Despite high grazing rates by individual ciliates, their role in balancing bacterial production in the muddy tidal-flat was probably negligible. The total ciliate abundance at the time of the experiment was low (Table 10), and the entire ciliate assemblage could potentially consume only 0.1% of the bacterial standing stock per day.

Similarly, grazing by the meiobenthos did not appear to have an effect on bacterial dynamics. Most of the meiobenthic species contained FLB in their guts, but only one nematode species, *Metoncholaimus* sp., consumed FLB at high rates (Table 10). The ability of both nematodes and harpacticoid copepods to consume sediment bacteria is well documented in a number of habitats (Carman et al. 1985; Carman and Thistle 1989; Coull and Palmer 1984; Decho and Castenholz 1986; Montagna 1984; Montagna and Bauer 1988; Rieper 1978, 1982; Schiemer 1982; Tietjen 1980; Tietjen and Lee 1973). At extant densities in the tidal-flat sediments, they could potentially account for the consumption of only 0.03% per day of the bacterial standing stock. Other nematode species and the harpacticoid copepods essentially disregarded bacterial prey. Taking a bacterial turnover value of 10 d from previous studies (Kemp 1987, 1990), bacterivorous ciliates and meiobenthic species must be 10^4 individuals/cm³ to balance the bacterial production by estimated grazing rates. This is an unrealistically high density. Rather, these results support the findings of Montagna et al. (1983), who reported an absence of spatial correlations between bacterial and meiofauna abundances. The ability of the meiobenthos to balance bacterial production has been shown (Montagna 1984), but in that case the predominant grazing pressure was attributed to polychaetes. The data provide additional evidence, that on muddy tidal-flats, micro- and meiobenthos may not influence bacterial numbers by grazing. If macrofaunal feeding has also only a marginal effect on bacterial abundances (Bianchi and Levinton 1984; Kemp 1987; Levinton and Bianchi 1981; Moriarty and Pollard 1982), the major

part of the bacterial production in the muddy tidal flat sediments might not be directly influenced by grazing at all.

The relatively low grazing rates that were found in sediments is in contrast to grazing impacts in the overlying water column in marine waters. Protozoan grazing is an important source of bacterial mortality in the sea (Azam *et al.* 1983; Fenchel 1982). Among protozoans, heterotrophic flagellates are believed to be the most important grazers of bacteria (Anderson and Fenchel 1982; McManus and Fuhrman 1988), while ciliates are considered to graze mostly on nano-sized eukaryotic organisms (Bernard and Rassoulzadegan 1990; Sherr *et al.* 1990). The importance of protozoan grazing and the transformation to a non-culturable state in the decline of fecal coliforms and enterococci counts in marine waters was recently demonstrated by González *et al.* (1992). The decrease in total numbers (both culturable and nonculturable) was attributable to predation by protozoa alone. Their work confirmed the importance of protozoan grazing on enteric bacteria in marine waters as established previously (Enzinger and Cooper 1976; McCambridge and McMeekin 1979, 1980). Predacious bacteria and bacteriophage (Garcia-Lara *et al.* 1991) do not seem to play a significant role. It appears that the decreased predation pressure on fecal indicator bacteria in sediments as compared to the water column, may explain in large part the prolonged survival of culturable fecal indicator bacteria in sediments.

While predation pressure is lower in sediments than in overlying water, the grazing experiments show that protozoans do graze the indicator bacteria and that grazing is still an impor-

tant factor in the slow decline of indicator counts in the sediments. The increased decline of both indicator counts in non-sterile sediment slurries (Figs. 12 and 13), support this conclusion. However, other factors may play a role. Sediments may prevent or retard the transition of culturable indicator bacteria into nonculturable forms (Grimes *et al.* 1986; Roszack and Colwell 1987; Xu *et al.* 1982). The interaction of fecal bacteria with clay particles may also, by similar mechanisms, increase their survival (Burton *et al.* 1987; Grimes 1980). Recently, Gauthier *et al.* (1991) demonstrated that glycine betaine, an osmoregulator present in marine sediments, could play a role in the increased survival of *E. coli*. Finally, increased levels of organic matter in sediments may play a major role (Gerba and McLeod 1976), perhaps even allowing growth and division (Marino and Gannon 1991). High organic matter was associated with the increased uptake of glycine betaine by *E. coli* in marine sediments (Gauthier *et al.* 1991).

CONCLUSIONS

The major conclusions of this study were:

1. Enterococci survived significantly longer than fecal coliforms in Boston Harbor surface sediments. This was demonstrated consistently in laboratory and field experiments, as well as from depth profiles.
2. Boston Harbor sediments near known sources of fecal contamination had consistently high densities of fecal coliforms and enterococci ($> 10^3$ CFU/g), while densities in sediments not near contamination sources were low ($< 10^2$ CFU/g).
3. A significant proportion of fecal coliforms ($>20\%$) and enterococci ($>10\%$) sediment from sewage within 2 hours. The sedimentation is not affected by salinity nor fecal indicator bacteria densities.
4. Reduced grazing by protozoans, probably through association of fecal indicator bacteria with particles, is a major reason that fecal indicator bacteria survive longer in sediments as compared to the water column, where grazing pressure can be intense. However, grazing in sediments still appears to be a factor in the observed decline of fecal indicator bacteria counts in sediments.
5. Survival of fecal coliforms and enterococci in sediments is significantly affected by temperature, and to a lesser extent, salinity.
6. Counts of fecal coliforms and enterococci in sediments represent a potentially superior method for assessing chronic

contamination of coastal environments by fecal-derived
contamination than water column counts.

LITERATURE CITED

- Alongi, D.M.** 1986. Quantitative estimates of benthic protozoa in tropical marine systems using silica gel: a comparison of methods. *Est. Coast. Mar. Sci.* **23**:443-450.
- Akama, L., and S. Otani.** 1970. *Clostridium perfringens* as the flora intestine of health persons. *Jpn. J. Med. Sci. Biol.* **23**:161-175.
- Andersen, P., and T. Fenchel.** 1985. Bacterivory by microheterotrophic flagellates in seawater samples. *Limnol. Oceanogr.* **30**:198-202.
- Andersen, P., and H.M. Sorensen.** 1986. Population dynamics and trophic coupling in pelagic microorganisms in eutrophic coastal waters. *Mar. Ecol. Prog. Ser.* **33**:99-109.
- Andersson, A., U. Larsson, and H. Hagstrom.** 1986. Size-selective grazing by a microflagellate on pelagic bacteria. *Mar. Ecol. Prog. Ser.* **33**:51-57.
- Azam F, T. Fenchel, J.G. Field, J.S. Gray, L.A. Meyer-Reil, and F. Thingstad.** 1983. The ecological role of water column microbes in the sea. *Mar. Ecol. Prog. Ser.* **10**:257-263
- Barcina, I., J.M. González, J. Iriberry, and L. Egea.** 1990. Survival strategy of *Escherichia coli* and *Enterococcus faecalis* in illuminated fresh and marine systems. *J. Appl. Bacteriol.* **68**:189-198.
- Bej, A.K., M.H. Mahbubani, R. Miller, J.L. DiCesare, L. Haff, and R.M. Atlas.** 1990. Multiplex PCR amplification and immobilized capture probes for detection of bacterial pathogens and indicators in water. *Mol. Cell. Probes* **4**:353-365.

- Bej, A.K., S.C. McCarty, and R.M. Atlas.** 1991. Detection of coliform bacteria and *Escherichia coli* by multiplex polymerase chain reaction: comparison with defined substrate and plating methods for water quality monitoring. *Appl. Environ. Microbiol.* **57**:2429-2432.
- Bej, A.K., R.J. Steffan, J. DiCesare, L. Haff, and R.M. Atlas.** 1990. Detection of coliform bacteria in water by polymerase chain reaction and gene probes. *Appl. Environ. Microbiol.* **56**:307-317.
- Bell, C.R., and L.J. Albright.** 1982. Attached and free-floating bacteria in a diverse selection of water bodies. *Appl. Environ. Microbiol.* **43**:1227-1237.
- Bernard, C., and F. Rassoulzadegan.** 1990. Bacteria or microflagellates as a major food source for marine ciliates: possible implications for the microzooplankton. *Mar. Ecol. Prog. Ser.* **64**:147-155.
- Bianchi, T.S., and J.S. Levinton.** 1984. The importance of microalgae, bacteria, and particulate organic matter in the somatic growth of *Hydrobia totteni*. *J. Mar. Res.* **42**:31-442.
- Bisson, J.W., and V.J. Cabelli.** 1980. *Clostridium perfringens* as an indicator of water pollution. *J. Water Pout. Control Fed.* **52**:241-248.
- Blanchard, G.F.** 1991. Measurement of meiofauna grazing rates on microphytobenthos: is primary production a limiting factor? *J. Exp. Mar. Biol. Ecol.* **147**:37-46.
- Bloem, J., C. Albert, M.-B.J. Bar-Gilissen, T. Berman, and T.I. Cappenberg.** 1989. Nutrient cycling through phytoplankton,

- bacteria and protozoa, in selectively filtered Lake Vechten water. *J. Plankton Res.* **11**:119-132.
- Borrego, J.J., F. Arrabal, A. deVincente, L.F. Gomez, and P. Romero.** 1983. Study of microbial inactivation in the marine environment. *J. Water Pollut. Control Fed.* **55**:297-302.
- Borror, A.C.** 1968. Nigrosin-HgCl-Formalin: a stain-fixative for ciliates (Protozoa, Ciliophora). *Stain. Tech.* **43**:293-294.
- Brenner, D.J.** 1984. Enterobacteriaceae. pp. 408-516. In, N.R. Krieg and J.G. Holt (eds), *Bergey's Manual of Systematic Bacteriology, Volume 1*. Williams and Wilkens, Baltimore.
- Burton, G.A. Jr, D. Gunnison, and G.R. Lanza.** 1987. Survival of pathogenic bacteria in various freshwater sediments. *Appl. Environ. Microbiol.* **53**:633-638.
- Cabelli, V.J., A.P. DuFour, L.J. McCabe, and M.A. Levin.** 1982. Swimming-associated gastroenteritis and water quality. *Am. J. Epidemiol.* **115**:606-616.
- Carlucci, A.F., and D. Pramer.** 1959. Factors affecting survival of bacteria in seawater. *Appl. Microbiol.* **7**:388-392.
- Carman, K.R.** 1990. Radioactive labeling of a natural assemblage of marine sedimentary bacteria and microalgae for trophic studies: an autoradiographic study. *Microb. Ecol.* **19**:279-290.
- Carman, K.R., F.C. Dobbs, and J.B. Guckert.** 1989. Comparison of three techniques for administering radiolabeled substrates to sediments for trophic studies: uptake of label by harpacticoid copepods. *Mar. Biol.* **102**:119-125.

- Carman, K.R., and D. Thistle.** 1985. Microbial food partitioning by three species of benthic copepods. *Mar. Biol.* **88**:143-148.
- Chrzanowski, T.H., and K. Šimek.** 1990. Prey-size selection by freshwater flagellated protozoa. *Limnol. Oceanogr.* **35**:1429-1436.
- Clesceri, L.S., A.E. Greenberg, and R.R. Trussell (Eds).** 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition. American Public Health Association, Washington, D.C.
- Coull, B.C., and M.A. Palmer.** 1984. Field experimentation in meiofaunal ecology. *Hydrobiologia* **118**:1-19.
- Decho, A.D., and R. Castenholz.** 1986. Spatial patterns and feeding of meiobenthic harpacticoid copepods in relation to resident microbial flora. *Hydrobiologia* **131**:87-96.
- Daggett, P., and T.A. Nerad.** 1982. Axenic cultivation of Bodo edax and Bodo ancinatus and some observations on feeding rates in monoxenic culture. Abstr. 30, *J. Protozool.* **29**:290-291.
- Davis, P.G., and J.M. Sieburth.** 1984. Estuarine and oceanic microflagellate predation of actively growing bacteria: estimation by frequency of dividing-divided cells. *Mar. Biol. Prog. Ser.* **19**:237-246.
- Dobbs, F.C., J.B. Guckert, and K.R. Carman.** 1989. Comparison of three techniques for administering radiolabeled substrates to sediments for trophic studies: incorporation by microbes. *Microb. Ecol.* **17**:237-250.

- Dufour, A.P., E.R. Strickland, and V.J. Cabelli.** 1981. Membrane filter method for enumerating *Escherichia coli*. Appl. Environ. Microbiol. **41**:1152-1158.
- Emerson, D.J., and V.J. Cabelli.** 1982. Extraction of *Clostridium perfringens* spores from bottom sediment samples. Appl. Environ. Microbiol. **44**:1144-1149.
- Enzinger, R.M., and R.C. Cooper.** 1976. Role of bacteria and protozoa in the removal of *Escherichia coli* from estuarine waters. Appl. Environ. Microbiol. **31**:758-763.
- Evison, L.M., and E. Tosti.** 1980. An appraisal of bacterial indicators of pollution in seawater. Prog. Water Technol. **12**:591-599.
- Fallon, R.D., S.Y. Newell, and C.S. Hopkinson.** 1983. Bacterial production in marine sediments: will cell-specific measures agree with whole-system metabolism? Mar. Ecol. Prog. Ser. **11**:119-127.
- Fenchel, T.** 1975. The quantitative importance of the benthic microfauna of an arctic tundra pond. Hydrobiologia **46**:445-464.
- Fenchel, T.** 1982. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. Mar. Biol. Prog. Ser. **8**:225-231.
- Fenchel, T.** 1982. Ecology of heterotrophic microflagellates IV Quantitative occurrence and importance as bacterial consumers. Mar. Ecol. Prog. Ser. **9**:35-42.
- Fleisher, J.M.** 1991. A reanalysis of data supporting U.S. federal bacteriological water quality criteria governing marine recreational waters. Res. J. Wat. Pollut. Control Fed. **63**:259-265.

Fujioka, R.S., H.H. Hashimoto, E.B. Siwak, and R.H.T. Young.

1981. Effect of sunlight on survival of indicator bacteria in seawater. *Appl. Environ. Microbiol.* **41**:690-696.

Gameson, A.L.H., and J.R. Saxon. 1967. Field studies on effect of daylight on mortality of coliform bacteria. *Water Res.* **1**:279-295.

Garcia-Lara, J., P. Menon, P. Servais, and G. Billen. 1991.

Mortality of fecal bacteria in seawater. *Appl. Environ. Microbiol.* **57**:885-888.

Gauthier, M.J., G.N. Flatau, and V.A. Breittmayer. 1991. Protective effect of glycine betaine on survival of *Escherichia coli* cells in marine environments. *Water Sci. Technol.* **24**:129-132.

Gerba, C.P. and J.S. McLeod. 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. *Appl. Environ. Microbiol.* **32**:114-120.

González, J.M., J. Iriberry, L. Egea, and I. Barcina. 1992.

Characterization of culturability, protistan grazing, and death of enteric bacteria in aquatic ecosystems. *Appl. Environ. Microbiol.* **58**:998-1004.

González, J.M., E.B. Sherr, and B.F. Sherr. 1990. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *Appl. Environ. Microbiol.* **56**:583-589.

Goulder, R. 1977. Attached and free-living bacteria in an estuary with abundant suspended solids. *J. Appl. Bacteriol.* **43**:399-405.

- Goyal, S.M.** 1983. Indicators of viruses, p. 211-230. In, G. Berg (ed.), Viral pollution of the environment. CRC Press, Inc., Boca Raton, Fla.
- Goyal, S.M., C.P. Gerba, and J.L. Melnick.** 1977. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas coast. Appl. Environ. Microbiol. 34:139-149.
- Green, D.H., G.D. Lewis, S. Rodtong, and M.W. Loutit.** 1991. Detection of fecal pollution in water by an *Escherichia coli* uidA gene probe. J. Microbiol. Meth. 13:207-214.
- Grigsby, P. and J. Calkins.** 1979. The inactivation of a natural population of coliform bacteria by sunlight. Photobiology 31:291-294.
- Grimes, D.J.** 1975. Release of sediment-bound fecal coliforms by dredging. Appl. Microbiol. 29:109-111.
- Grimes, D.J.** 1980. Bacteriological water quality effects of hydraulically dredging contaminated upper Mississippi River bottom sediment. Appl. Environ. Microbiol. 39:782-789.
- Grimes, D.J., R.W. Atwell, P.R. Brayton, L.M. Palmer, D.M. Rollins, D.B. Roszack, F.L. Singleton, M.L. Tamplin, and R.R. Colwell.** 1986. The fate of enteric pathogenic bacteria in estuarine and marine environments. Microbiol. Sci. 3:324-329.
- Hargrave, B.T.** 1970. The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). J. Anim. Ecol. 39:427-437.
- Havelaar, A.H.** (editor). 1991. Bacteriophages as model viruses in water quality control. Wat. Res. 25:529-545.

- Hobbie, J.E., O. Holm-Hansen, T.T. Packard, L.R. Pomeroy, R.W. Sheldon, J.P. Thomas, and W.J. Wiebe. 1972. A study of the distribution and activity of microorganisms in ocean water. *Limnol. Oceanogr.* 17:544-555.
- Hollibaugh, J.T., J.A. Fuhrman, and F. Azam. 1980. Radioactive labeling of natural assemblages of bacterioplankton for use in trophic studies. *Limnol. Oceanogr.* 25:172-181.
- Ingraham, J.L., O. Maaløe, and F.C. Neidhardt. 1983. Growth of the bacterial cell. Sinauer Associates, Sunderland, MA.
- Jones, G.E. 1964. Effect of chelating agents on the growth of *Escherichia coli* in seawater. *J. Bacteriol.* 87:483-499.
- Joseph, S.W., R.R. Colwell, and J.B. Kaper. 1982. *Vibrio parahaemolyticus* and related halophilic vibrios. *Crit. Rev. Microbiol.* 10:77-124.
- Kapuscinski, R.B., and R. Mitchell. 1982. Sunlight-induced mortality of viruses and *Escherichia coli* in coastal seawater. *Environ. Sci. Technol.* 17:1-6.
- Kemp, P.F. 1987. Potential impact on bacteria of grazing by a macrofaunal deposit-feeder, and the fate of bacterial production. *Mar. Ecol. Prog. Ser.* 36:151-161.
- Kemp, P.F. 1988. Bacterivory by benthic ciliates: significance as a carbon source and impact on sediment bacteria. *Mar. Ecol. Prog. Ser.* 49:163-169.
- Kemp, P.F. 1990. The fate of bacterial production. *Rev. Aquat. Sci.* 2:109-124.
- Kopylov, A.I., T.I. Mamayeva, and S.F. Batsanin. 1980. Energy balance of the colorless flagellate *Parabodo attenuatus* (Zoomastigophora, Protozoa). *Oceanology* 20:705-708.

- LaBelle, R.L., C.P. Gerba, S.M. Goyal, J.L. Melnick, I. Cech, and G.F. Bogdan.** 1980. Relationships between environmental factors, bacterial indicators, and the occurrence of enteric viruses in estuarine sediments. *Appl. Environ. Microbiol.* **39**:588-596.
- LaLiberte, P., and D.J. Grimes.** 1982. Survival of *Escherichia coli* in lake bottom sediment. *Appl. Environ. Microbiol.* **43**:623-628.
- Landry, M.R., L.W. Haas, and V.L. Fagerness.** 1984. Dynamics of microbial plankton communities: experiments in Kaneohe Bay, Hawaii. *Mar. Ecol. Prog. Ser.* **16**:127-133.
- Larsen, J.L., and P. Willeberg.** 1984. The impact of terrestrial and estuarial factors on the density of environmental bacteria (Vibrionaceae) and faecal coliforms in coastal water. *Zbl. Bakt. Hyg., I. Abt. Orig. B* **179**:308-323.
- LeChevallier, M.W., S.C. Cameron, and G.A. McFeters.** 1983. New medium for improved recovery of coliform bacteria from drinking water. *Appl. Environ. Microbiol.* **45**:484-492.
- Lessard, E.J., and J.M. Sieburth.** 1983. Survival of natural sewage populations of enteric bacteria in diffusion and batch chambers in the marine environment. *Appl. Environ. Microbiol.* **45**:950-959.
- Levinton, J.S., and T.S. Bianchi.** 1981. Nutrition and food limitation of deposit feeders. I. The role of microbes in the growth of mud snails (Hydrobiidae). *J. Mar. Res.* **39**:531-545.
- Loutit, M.W., and G. Lewis.** Faecal bacteria from sewage effluent in sediments around an ocean outfall. *New Zealand J. Mar. Freshwater Res.* **19**:179-185.

- Marino, R.P., and J.J. Gannon.** 1991. Survival of fecal coliforms and fecal streptococci in storm drain sediment. *Water Res.* **25**:1089-1098.
- Matson, E.A., S.G. Horner, and J.D. Buck.** 1978. Pollution indicators and other microorganisms in river sediment. *J. Water Pollut. Control Fed.* **50**:13-19.
- McCambridge, J. and T.A. McMeekin.** 1979. Protozoa predation of *Escherichia coli* in estuarine waters. *Water Res.* **13**:659-663.
- McCambridge, J. and T.A. McMeekin.** 1980. Effect of temperature on activity of predators of *Salmonella typhimurium* and *Escherichia coli* in estuarine waters. *Aust. J. Mar. Freshwater Res.* **31**:851-855.
- McFeters, G.A., and D.G. Stuart.** 1972. Survival of coliform bacteria in natural waters: field and laboratory studies with membrane-filter chambers. *Appl. Microbiol.* **24**:805-811.
- McManus, G.B., and J.A. Fuhrman.** 1988. Clearance of bacteria-sized particles by natural populations of nanoplankton in the Chesapeake Bay outflow plume. *Mar. Ecol. Prog. Ser.* **42**:199-206.
- Milne, D.P., J.C. Curran, and L. Wilson.** 1986. Effects of sedimentation on removal of faecal coliform bacteria from effluents in estuarine water. *Water Res.* **20**:1493-1496.
- Mitchell, R.** 1968. Factors affecting the decline of nonmarine microorganisms in seawater. *Water Res.* **2**:535-543.
- Montagna, P.A.** 1983. Live controls for radioisotope food chain experiments using meiofauna. *Mar. Ecol. Prog. Ser.* **12**:43-46.

- Montagna, P.A.** 1984. In situ measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. *Mar. Ecol. Prog. Ser.* **18**:119-130.
- Montagna, P.A., and J.E. Bauer.** 1988. Partitioning radiolabeled thymidine uptake by bacteria and meiofauna using metabolic blocks and poisons in benthic feeding studies. *Mar. Biol.* **98**:101-110.
- Montagna, P.A., B.C. Coull, T.L. Herring, and B.W. Dubley.** 1983. The relationship between abundances of meiofauna and their suspected microbial food. *Est. Coast. Shelf Sci.* **17**:381-394.
- Montagnes, D.J.S., and D.H. Lynn.** 1987. A quantitative protargol stain (QPS) for ciliates: method description and test of its quantitative nature. *Mar. Microb. Food Webs* **2**:83-93.
- Moriarty, D.J.W., and P.C. Pollard.** 1982. Diel variation of bacterial productivity in seagrass (*Zostera capricorni*) beds measured by the rate of thymidine incorporation into DNA. *Mar. Biol.* **72**:165-173.
- Novitsky, J.A.** 1990. Protozoa abundance, growth, and bacterivory in the water column, on sedimenting particles, and in the sediment of Halifax Harbor. *Can J. Microbiol.* **36**:859-863.
- O'Malley, M.L., D.W. Lear, W.N. Adams, J. Gaines, T.K. Sawyer, and E.J. Lewis.** 1982. Microbial contamination of continental shelf sediments by wastewater sludge. *J. Water. Pollut. Control Fed.* **54**:1311-1317.
- Penon, F.J., J. Martínez, J. Vives-Rego, and J. García-Lara.** 1991. Mortality of marine bacterial strains in seawater. *Antone van Leeuwenhoek* **59**:207-213.

- Pettibone, G.W., S.A. Sullivan, and M.P. Shiaris.** 1987. Comparative survival of antibiotic-resistant and -sensitive fecal indicator bacteria in estuarine water. *Appl. Environ. Microbiol.* **53**:1241-1245.
- Platt, H.M., and R.M. Warwick.** 1983. Freelifving marine nematodes. Cambridge University Press, Cambridge, UK.
- Porter, K.G., and Y.S. Feig.** 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25**:943-948.
- Reasoner, D.J., J.C. Blannon, and E.E. Geldreich.** 1979. Rapid seven hour fecal coliform test. *Appl. Environ. Microbiol.* **38**:229-336.
- Rhoades, M.W., and J. Kator.** 1988. Survival of *Escherichia coli* and *Salmonella* spp. in estuarine environments. *Appl. Environ. Microbiol.* **54**:2902-2907.
- Rieper, M.** 1978. Bacteria as food for marine harpacticoid copepods. *Mar. Biol.* **45**:337-346.
- Rieper, M.** 1982. Feeding preferences of marine harpacticoid copepods for various species of bacteria. *Mar. Ecol. Prog. Ser.* **7**:303-307.
- Rittenberg, S.C., T. Mittwer, and D. Ivler.** 1958. Coliform bacteria in sediments around three marine sewage outfalls. *Limnol. Oceanogr.* **3**:101-108.
- Rivier, A., D.C. Brownlee, R.W. Sheldon, and F. Rassoulzadegan.** 1985. Growth of microzooplankton: a comparative study of bacterivorous zooflagellates and ciliates. *Mar. Microb. Food Webs* **1**:51-60.

- Roper, M.M., and K.C. Marshall.** 1979. Effects of salinity on sedimentation and of particulates on survival of bacteria in estuarine habitats. *Geomicrobiol. J.* **1**:103-116.
- Roszack, D.B., and R.R. Colwell.** 1987. Survival strategies of bacteria in the natural environment. *Microbiol. Rev.* **51**:365-379.
- Sanders, R.W., and K.G. Porter.** 1986. Use of metabolic inhibitors to estimate protozooplankton grazing and bacterial production in a monomictic eutrophic lake with an anaerobic hypolimnion. *Appl. Environ. Microbiol.* **52**:101-107.
- Sayler, G.S., J.D. Nelson Jr., A. Justice, and R.R. Colwell.** 1975. Distribution and significance of fecal indicator organisms in the upper Chesapeake Bay. *Appl. Microbiol.* **30**:625-638.
- Schiemer, F.** 1982. Food dependence and energetics of free-living nematodes. II. Life history parameters of *Caenorhabditis briggsae* (Nematoda) at different levels of food supply. *Oecologia (Berl.)* **54**:122-128.
- Schwinghamer, P.** 1981. Extraction of living meiofauna from marine sediments by centrifugation in a silica sol-sorbitol mixture. *Can. J. Fish. Aquat. Sci.* **38**:476-478.
- Sherr, B.F., E.B. Sherr, T.L. Andrew, R.D. Fallon, and S.Y. Newell.** 1986. Trophic interactions between heterotrophic protozoa and bacterioplankton in estuarine water analyzed with selective metabolic inhibitors. *Mar. Ecol. Prog. Ser.* **32**:169-179.
- Sherr, B.F., E.B. Sherr, and T. Berman.** 1983. Grazing, growth, and ammonium excretion rates of a heterotrophic microflagel-

- late fed with four species of bacteria. Appl. Environ. Microbiol. **45**:1196-1201.
- Sherr, B.F., E.B. Sherr, and R.D. Fallon.** 1987. Use of monodispersed, fluorescently labeled bacteria to estimate *in situ* protozoan bacterivory. Appl. Environ. Microbiol. **53**:958-965.
- Sherr, B.F., E.B. Sherr, J. McDaniel, J. Gonzalez, and R. Hanson.** 1990. Size selective grazing by bacterivorous protozoa: implication for bacterial production. EOS **71**:162.
- Sherr, E.B., F. Rassoulzadegan, and B.F. Sherr.** 1989. Bacterivory by pelagic choreotrichous ciliates in coastal waters of the NW Mediterranean Sea. Mar. Ecol. Prog. Ser. **55**:235-240.
- Sherr, E.B., and B.F. Sherr.** 1987. High rates of consumption of bacteria by pelagic ciliates. Nature (Lond.) **325**:710-711.
- Shiaris, M.P., A.C. Rex, G.W. Pettibone, K. Keay, P. McManus, M.A. Rex, J. Ebersole, and E. Gallagher.** 1987. Distribution of indicator bacteria and *Vibrio parahaemolyticus* in sewage-polluted intertidal sediments. Appl. Environ. Microbiol. **53**:1756-1761.
- Small, E.B., and D.H. Lynn.** 1985. Phylum Ciliophora Doflein, 1901, p. In J.J. Lee, S.H. Hutner, and E.C. Bovee (ed.) An illustrated guide to the Protozoa. Society of the protozoologists, Lawrence, KS.
- Tietjen, J.H.** 1980. Microbial-meiofaunal interrelationships: a review. Microbiology 1980:335-338.
- Tietjen, J.H., and J.J. Lee.** 1973. Life history and feeding habits of the marine nematode, *Chromadora macrolaimoides* Steiner. Oecologia **12**:303-314.

- Tremaine, S.C., and A.L. Mills.** 1987. Inadequacy of the inhibitor cycloheximide in studies of protozoan grazing rates on bacteria at the freshwater-sediment interface. *Appl. Environ. Microbiol.* **53**:1969-1972.
- Turley, C.M., R.C. Newell, and D.B. Robins.** 1986. Survival strategies of two small marine ciliates and their role in regulating bacterial community under experimental conditions. *Mar. Ecol. Prog. Ser.* **33**:59-70.
- Valiela, I., M. Alber, and M. LaMontagne.** 1991. Fecal coliform loadings and stocks in Buttermilk Bay, Massachusetts, USA, and management implications. *Environ. Manag.* **15**:659-674.
- Van Donsel, D.J., and E.E. Geldreich.** 1971. Relationships of salmonellae to fecal coliforms in bottom sediments. *Water Res.* **5**:1079-1087.
- Velji, M.I., and L.J. Albright.** 1985. Microscopic enumeration of attached marine bacteria of seawater, marine sediment, fecal matter, and kelp blade samples following pyrophosphate and ultrasound treatments. *Can J. Microbiol.* **32**:121-126.
- Weisse, T.** 1989. The microbial loop in the Red Sea: dynamics of pelagic bacteria and heterotrophic nanoflagellates. *Mar. Ecol. Prog. Ser.* **55**:241-250.
- Xu, H.S., N. Roberts, F.L. Singleton, R.W. Attwell, D.J. Grimes, and R.R. Colwell.** 1982. Survival and viability of non-culturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. *Microb. Ecol.* **8**:313-323.

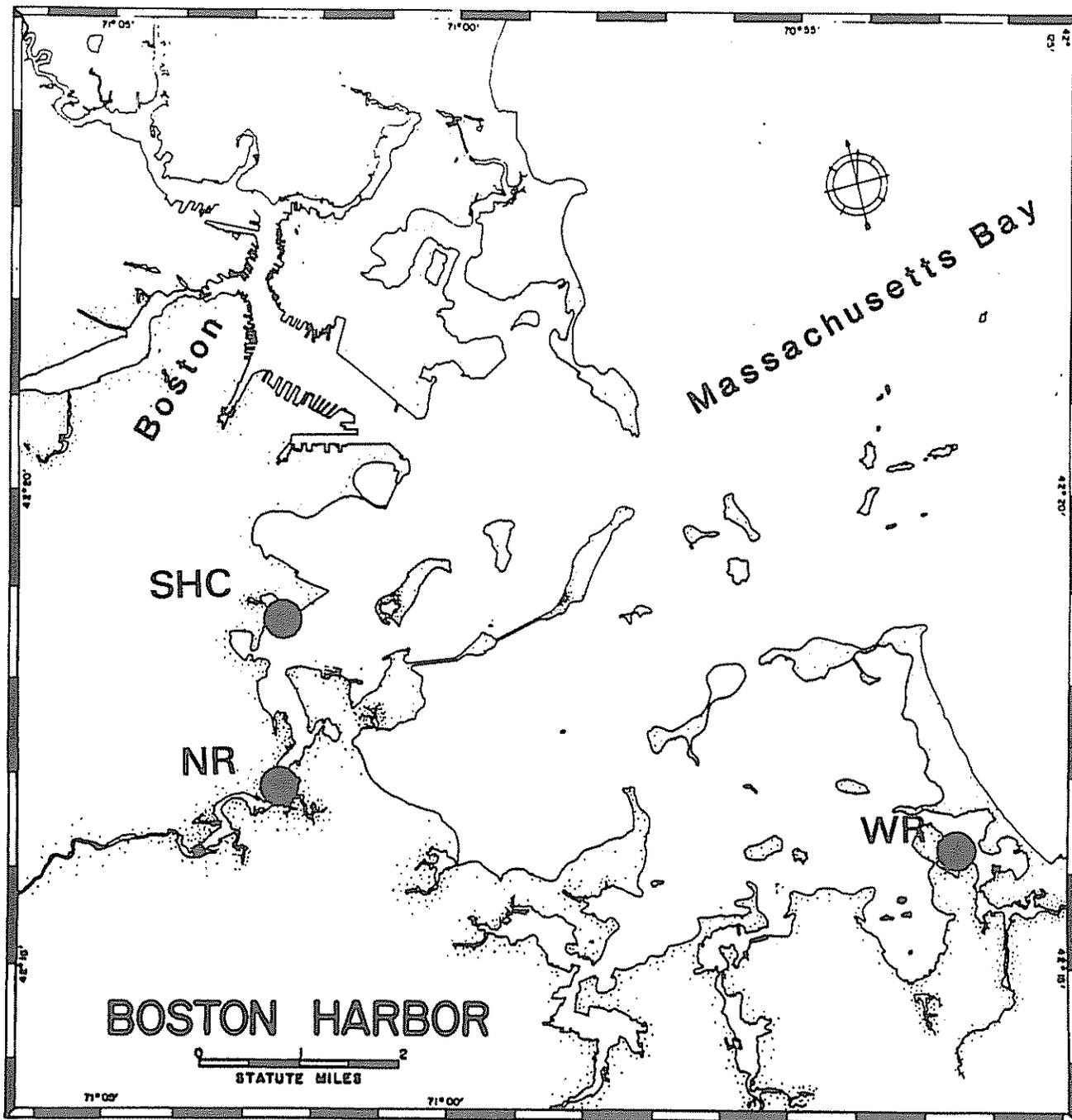


FIG. 1. Location of sample site in Boston Harbor, Massachusetts.

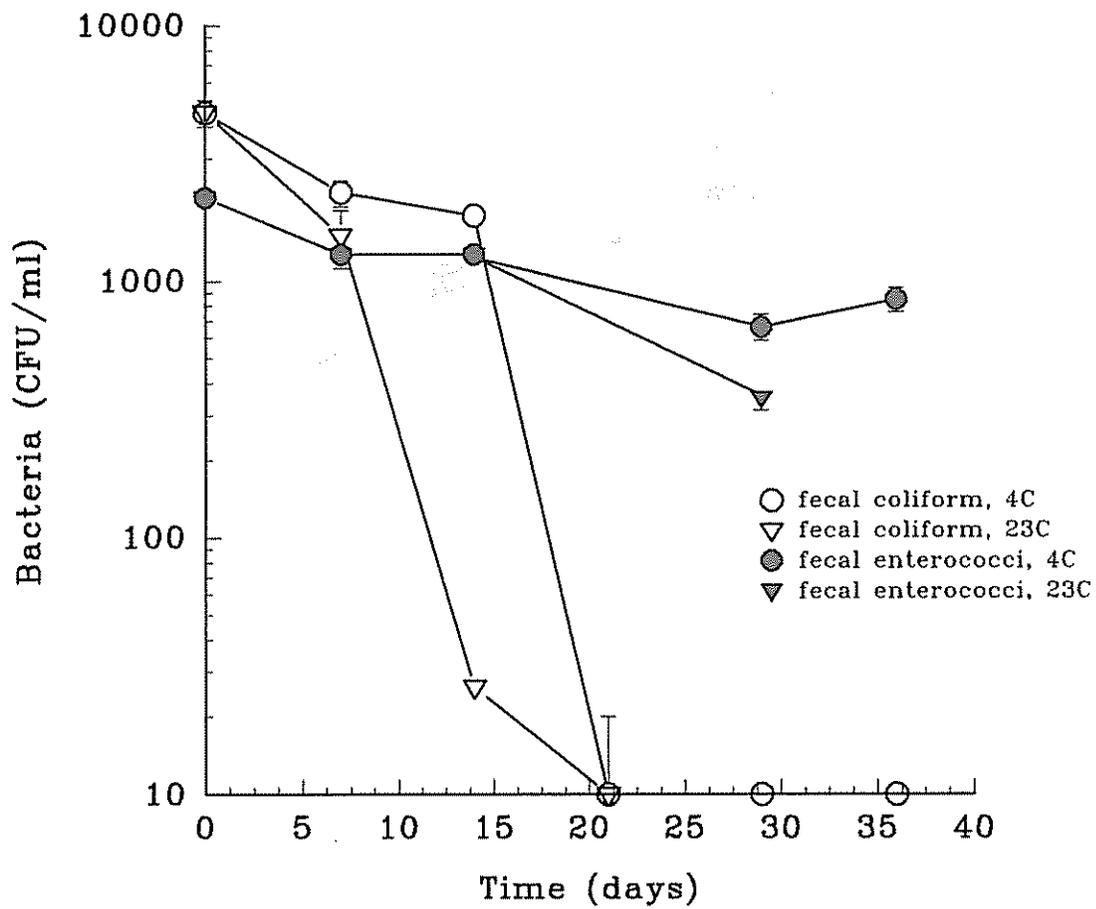


FIG. 2. Survival of fecal coliforms and enterococci in cores from Savin Hill Cove, SHC2, surface sediments in February, 1990.

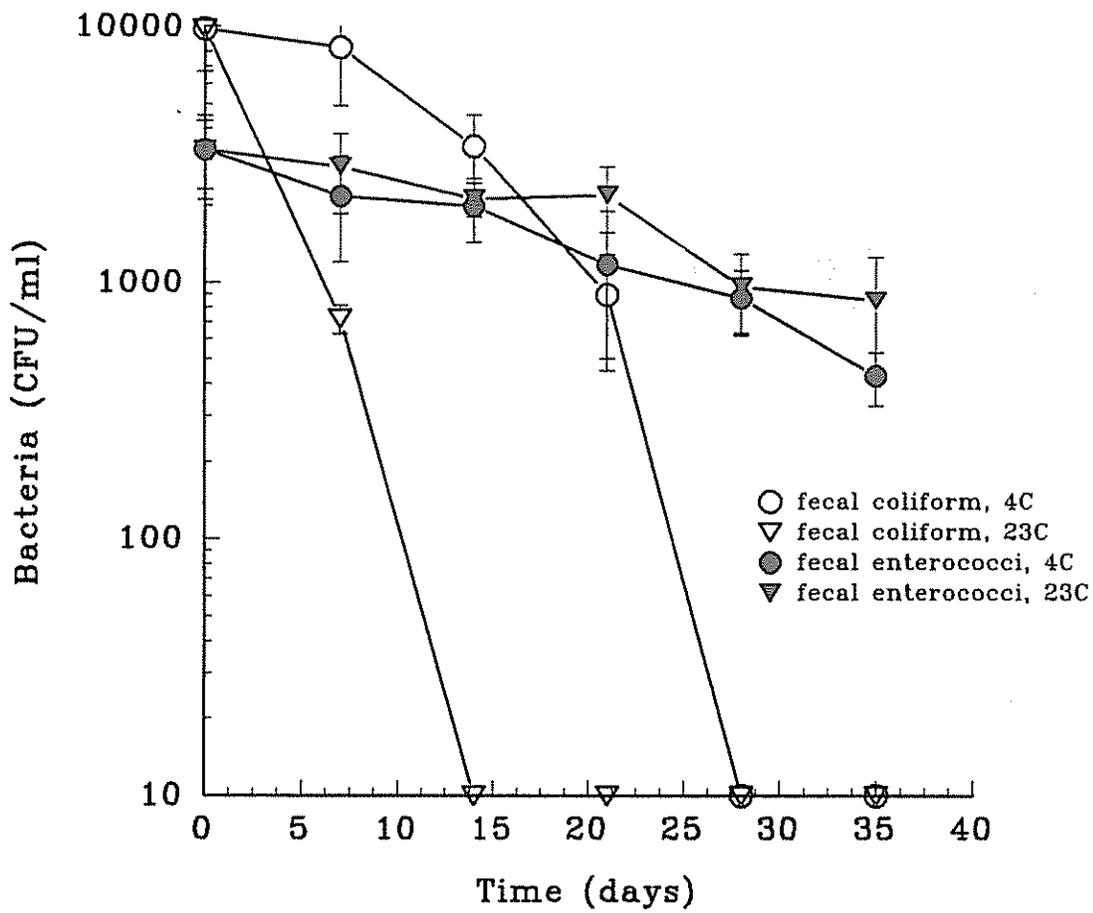


FIG. 3. Survival of fecal coliforms and enterococci in cores from Savin Hill Cove, SHC2, surface sediments in July, 1990.

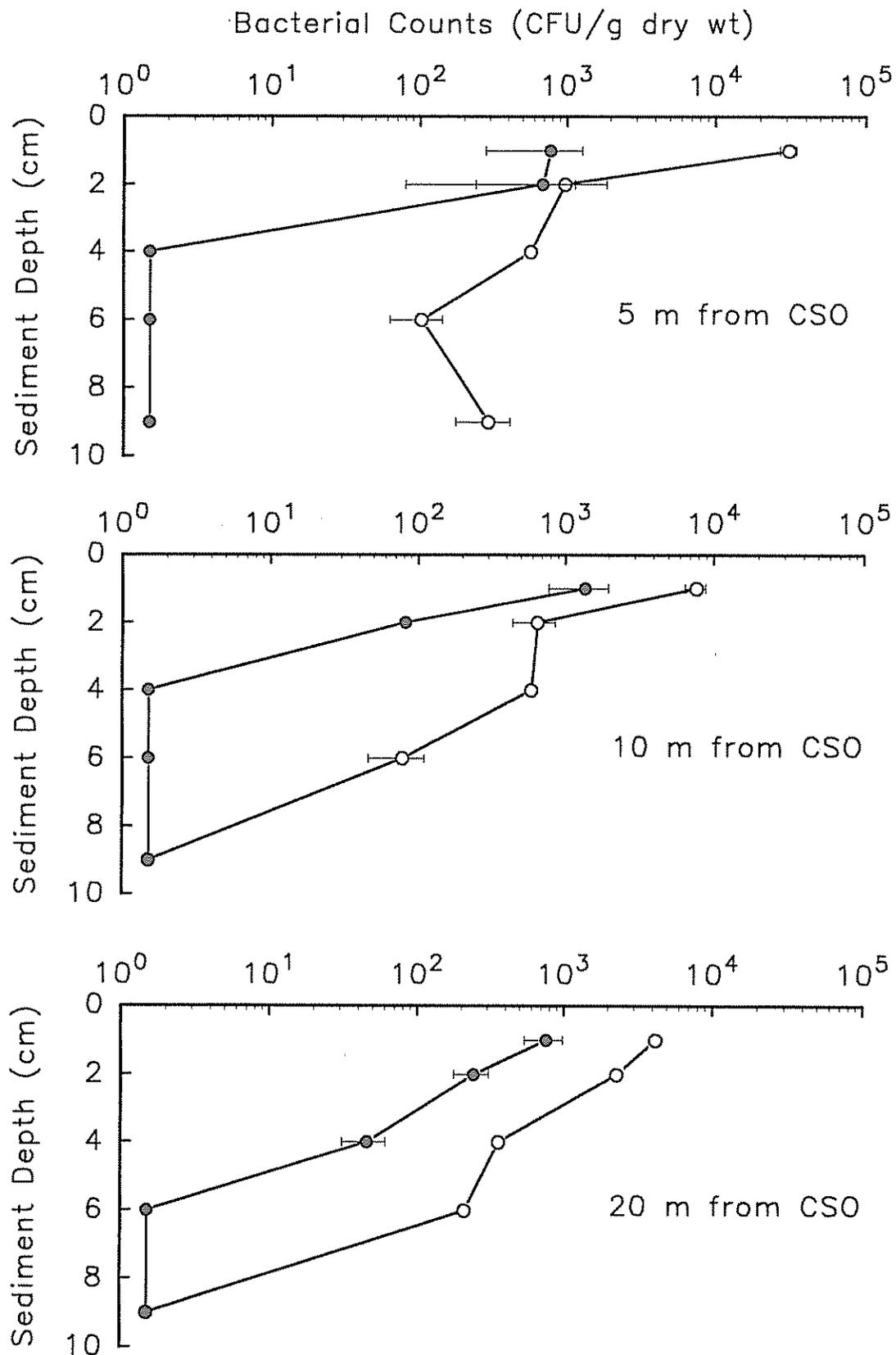


FIG. 4. Distribution of fecal coliforms (●) and enterococci (○) with depth in Savin Hill Cove, SHC2, sediments.

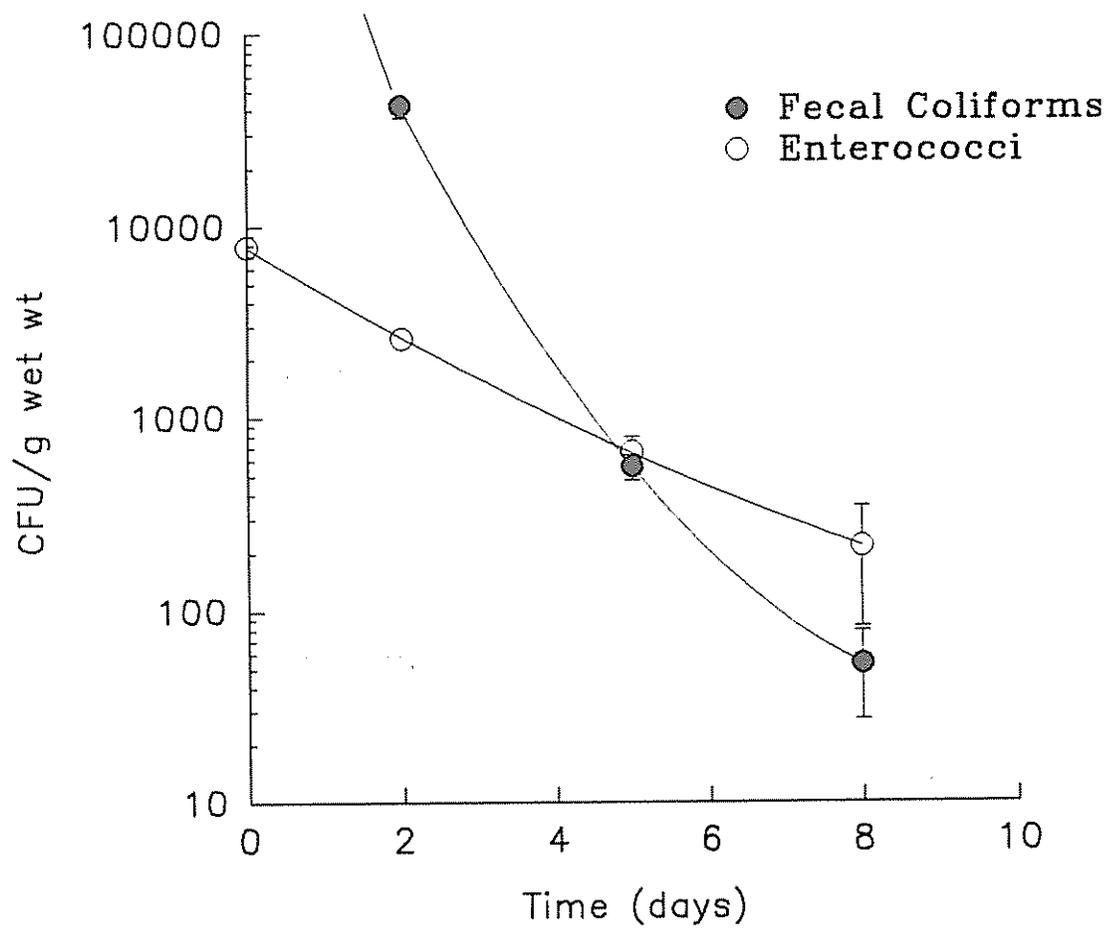


FIG. 5. Survival of seeded fecal coliforms and enterococci in Weir River, WR2, surface sediments in the field - July 1990.

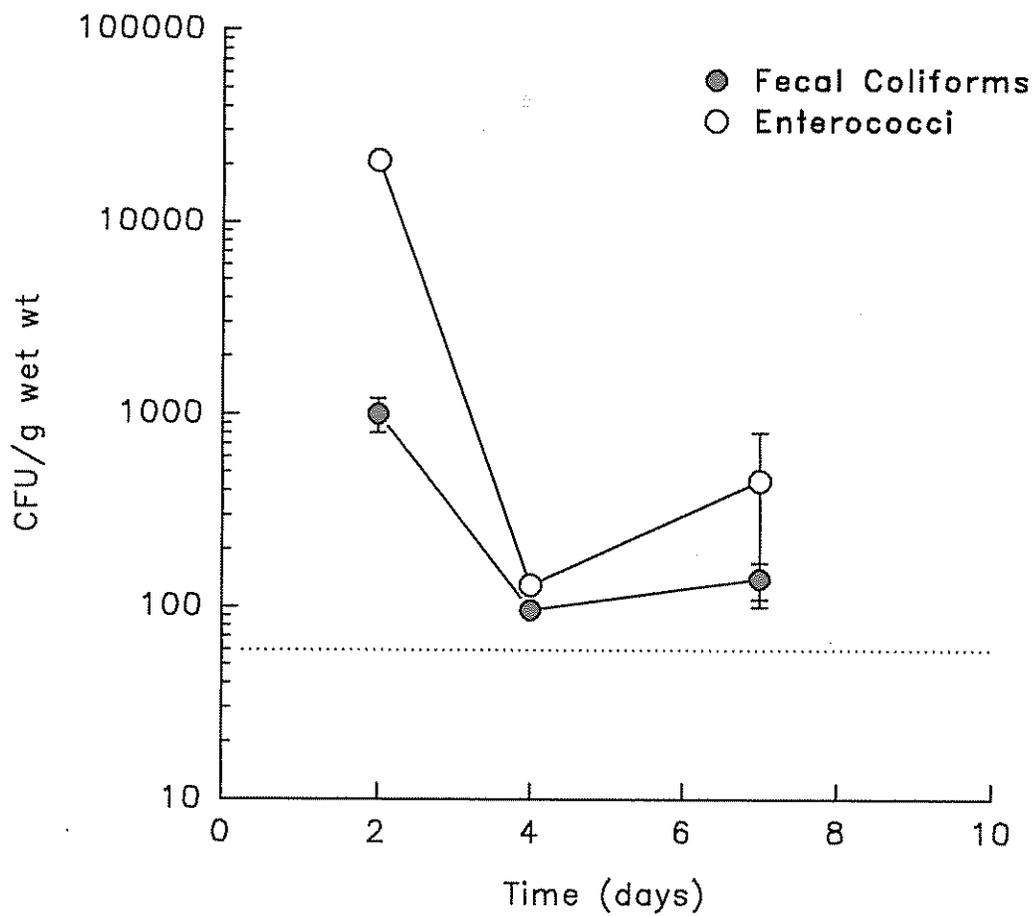


FIG. 6. Survival of seeded fecal coliforms and enterococci in Weir River, WR2, surface sediments in the field - September 1990.

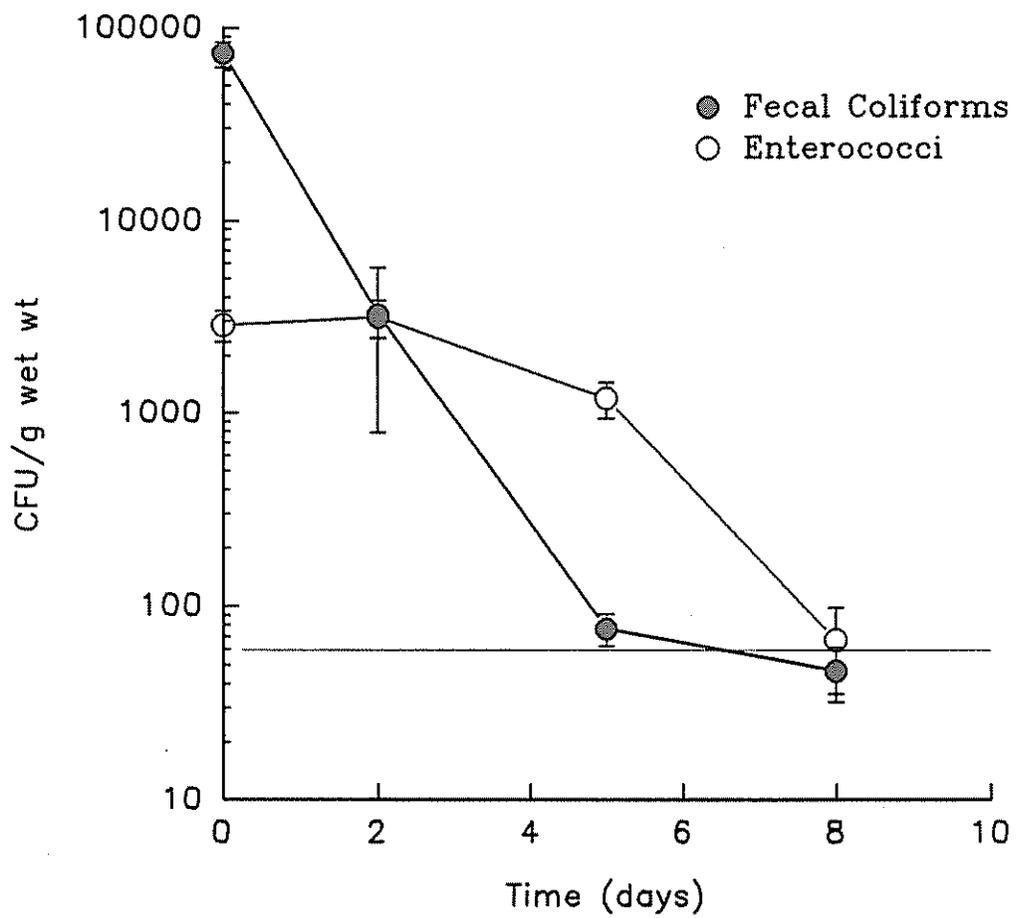


Figure 7. Survival of seeded fecal coliforms and enterococci in Wier River, WR2, surface sediments in the field - December 1990.

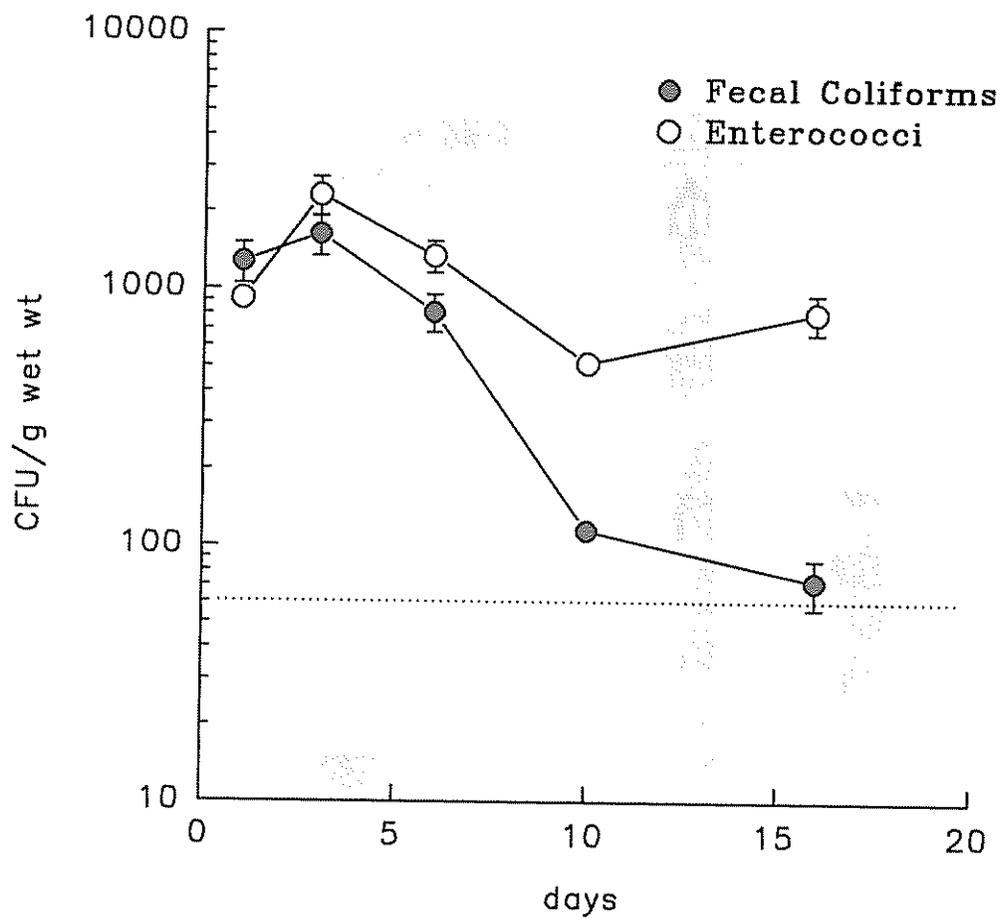


FIG. 8. Survival of seeded fecal coliforms and enterococci in Weir River, WR2, surface sediments in the field - April 1991.

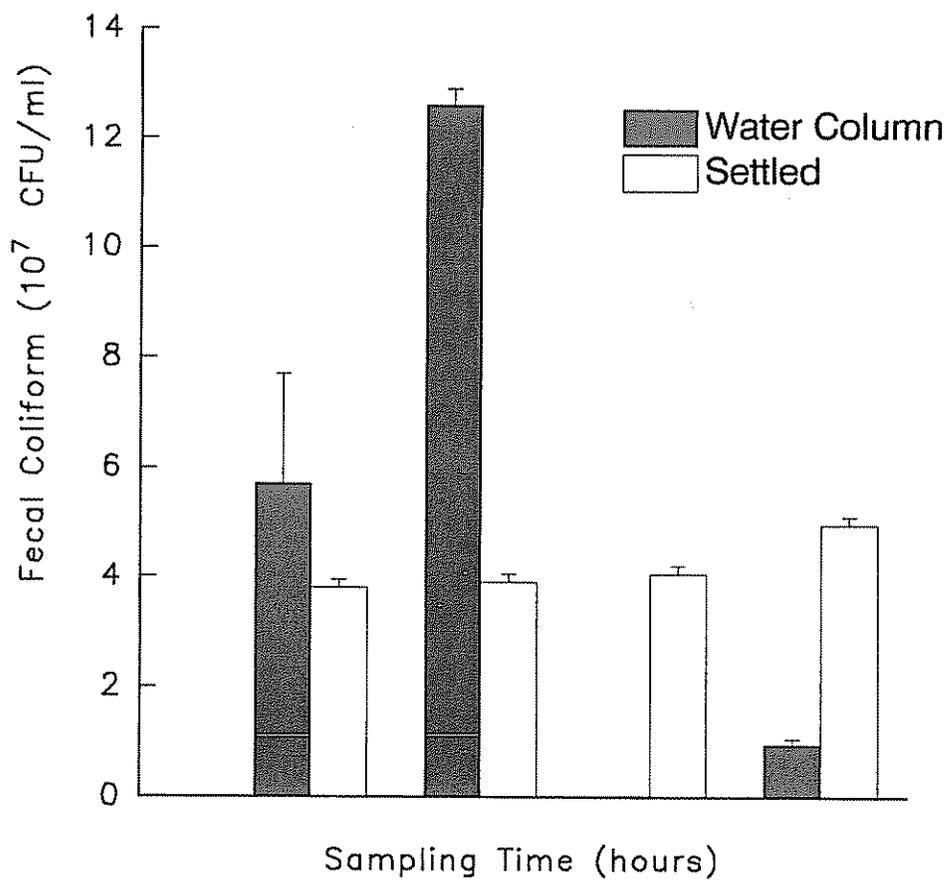


FIG. 9. Settling characteristics of fecal coliforms from raw sewage.

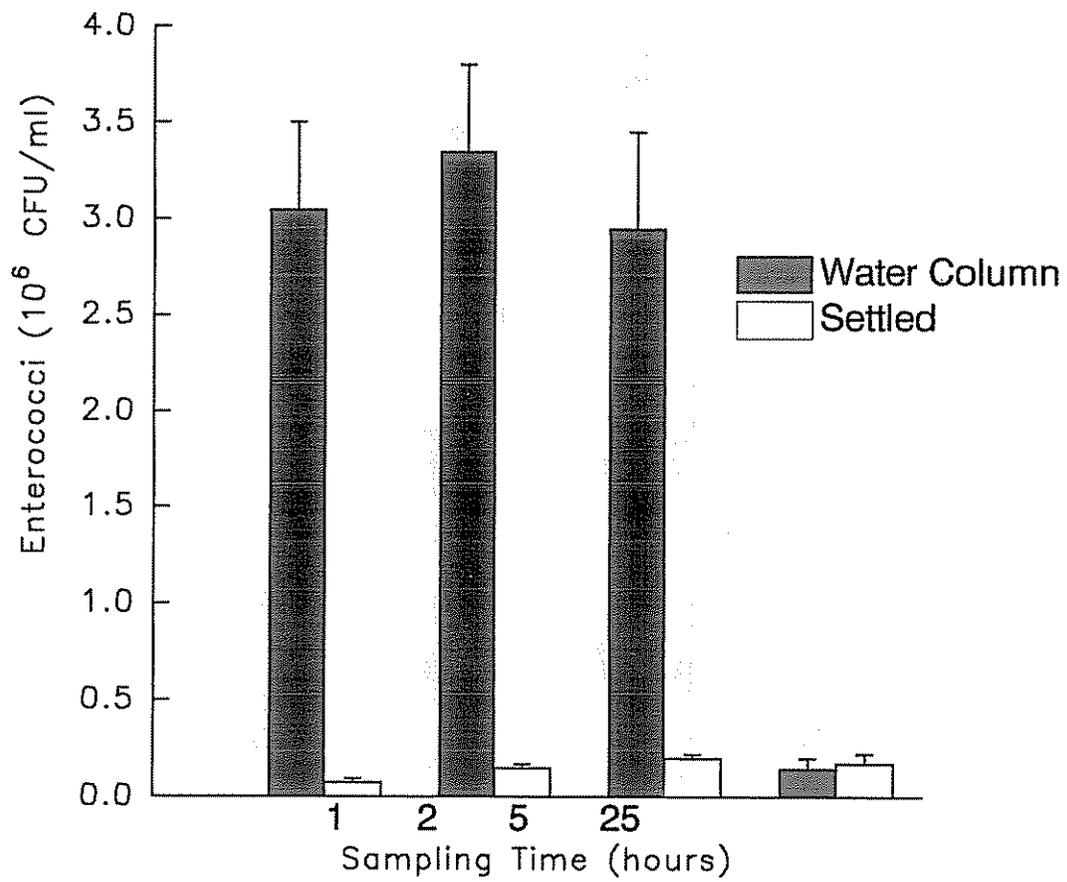


FIG. 10. Settling characteristics of enterococci from raw sewage.

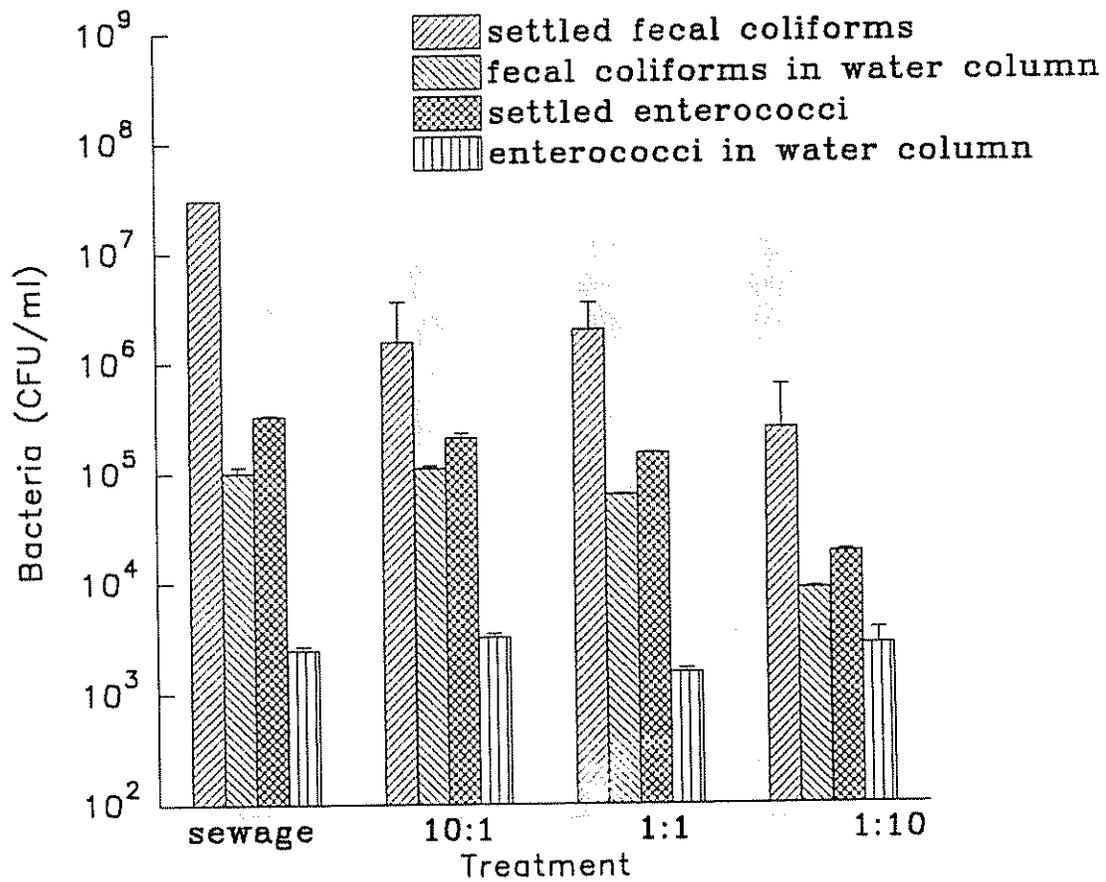


FIG. 11. Effect of seawater and dilution on settling characteristics of fecal coliforms and enterococci from raw sewage.

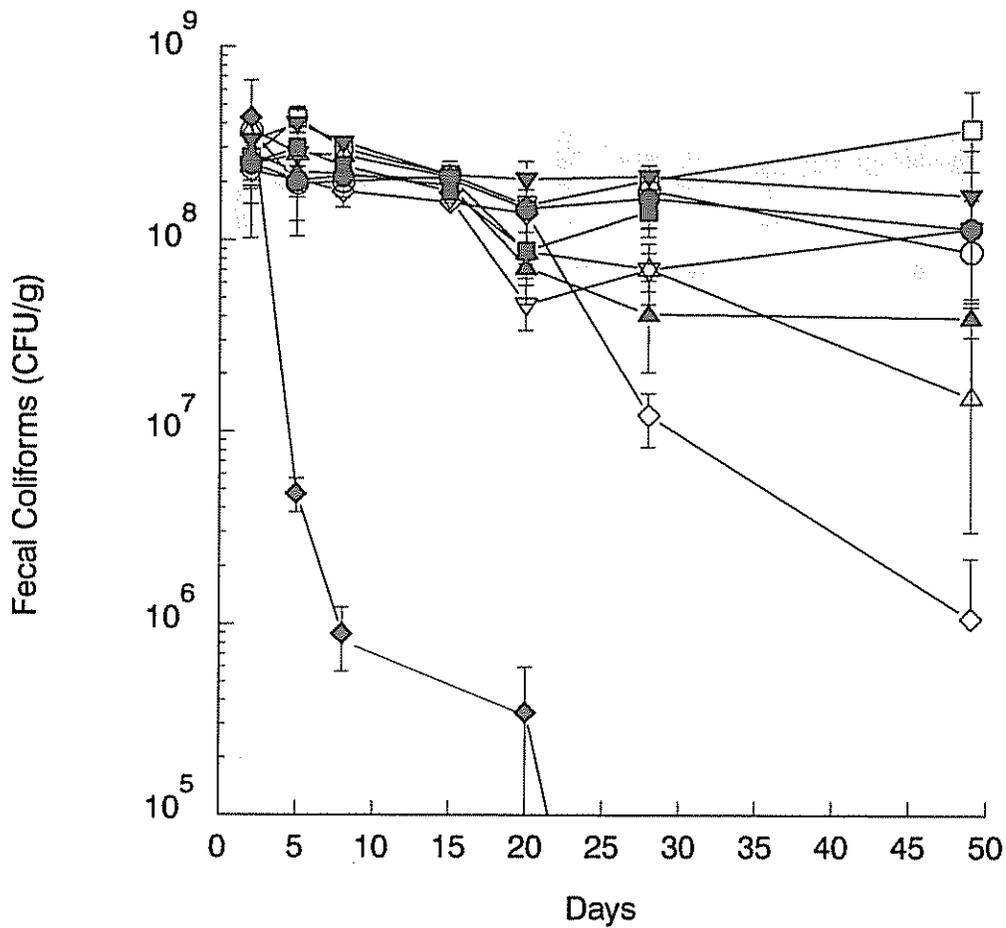


FIG. 12. Effect of temperature and salinity on the survival of fecal coliforms in muddy sediments. Symbols: 4°C, 0‰ (○); 4°C, 15‰ (◐); 4°C, 30‰ (▽); 12°C, 0‰ (∇); 12°C, 15‰ (◻); 12°C, 30‰ (◼); 25°C, 0‰ (△); 25°C, 15‰ (▲); 25°C, 30‰ (◇); Nonsterile control, 25°C, 30‰ (◆).

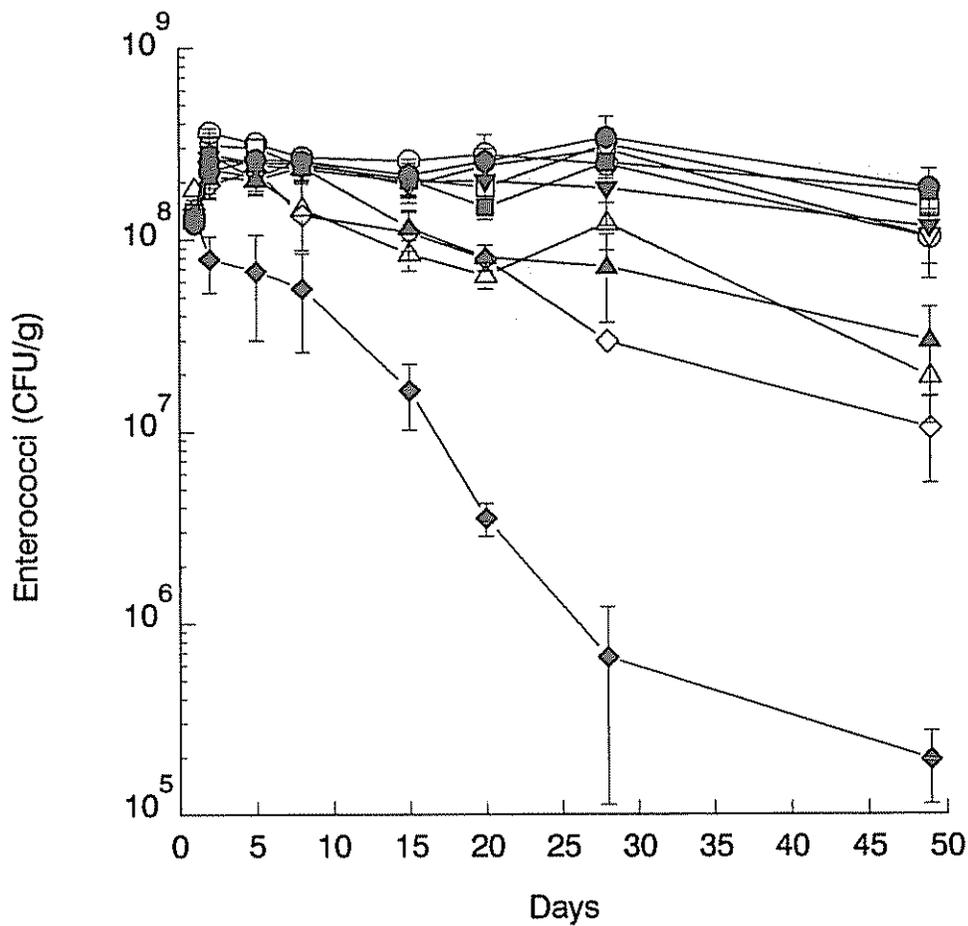


FIG. 13. Effect of temperature and salinity on the survival of enterococci in muddy sediments. Symbols: 4°C, 0‰ (○); 4°C, 15‰ (◐); 4°C, 30‰ (∇); 12°C, 0‰ (▽); 12°C, 15‰ (□); 12°C, 30‰ (■); 25°C, 0‰ (△); 25°C, 15‰ (▲); 25°C, 30‰ (◇); Nonsterile control, 25°C, 30‰ (◆).

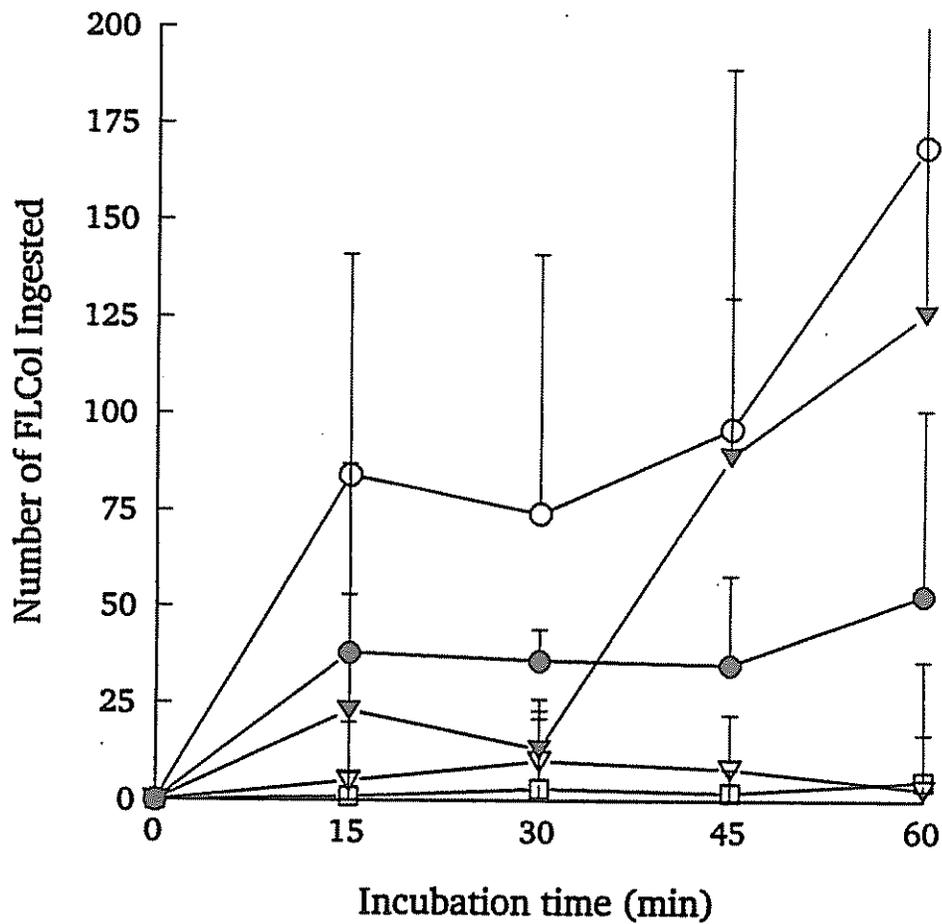


Figure 14. Consumption of fluorescently-labeled coliforms by Prorodon sp. (○), Chlamidodon sp. (●), other ciliates (▽), Metoncholaimus sp. (▼), and other nematodes (□) as a function of the incubation time.