APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1983, p. 950–959
0099-2240/83/030950-10$02.00/0

Vol. 45, No. 3

Survival of Natural Sewage Populations of Enteric Bacteria in Diffusion and Batch Chambers in the Marine Environment

EVELYN J. LESSARD* AND JOHN McN. SIEBURTH

Department of Microbiology and Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02881

Received 27 May 1982/Accepted 8 December 1982

The survival of natural populations of *Escherichia coli* and enterococci in sewage was measured in large-volume diffusion chambers in an estuary and a salt marsh. The 5-liter chambers, with polycarbonate membrane sidewalls, were found to be suitable for up to week-long experiments. Decay rates, measured monthly from February to August 1978, ranged from 0.042 to 0.088 h^{-1} (time for 90% of the population to die = 25 to 55 h) for *E. coli* and 0.019 to 0.083 h^{-1} (time for 90% of the population to die = 29 to 122 h) for enterococci and were significantly correlated with temperature. In contrast to the diffusion culture experiments, the decay of *E. coli* in batch culture did not correlate with temperature. Enterococci survived longer than *E. coli* in the Narragansett Bay (estuary) experiments, but survived less well in the more eutrophic salt marsh. The effect of light on survival was examined with light/dark experiments and sampling at frequent intervals over the diel cycle. Diel changes in survival were not evident in the Narragansett Bay experiments. *E. coli*, however, exhibited a diel pattern of growth during the day and death at night in the salt marsh. There was no significant difference in decay rates between light and dark diffusion chambers, nor were decay rates correlated with light intensity. In concurrent batch experiments, survival was significantly greater in the dark for both organisms. These results suggest that the effect of light is complex and that conditions in batch culture may modify the survival of enteric bacteria. Observations made in diffusion chambers may more closely follow the in situ survival of enteric microorganisms.

As long as the concentration of enteric bacteria is used as an index of fecal pollution in seawater, factors affecting the mortality of enteric bacteria will be of concern. There is considerable disagreement, however, over the factors responsible for die-off in seawater. Among the factors that have been implicated are antibiosis (1, 35), predation (8, 30), nutrient deficiencies (4, 25, 26), heavy metal toxicity (18, 19), solar radiation (5, 13, 34), flocculation and sedimentation (44), and temperature (28). The lack of consensus about the major causes of bacterial disappearance in seawater probably arises out of both the complexity of habitat and the artificiality of the experimental methods used. Most investigations have been laboratory studies of pure cultures in batch containers. Little attention has been given to methods for in situ observations.

Some attempts have been made to apply diffusion (dialysis) chambers (32) to in situ observations of fecal coliform decay (22, 23, 42). With two exceptions (9, 14), these studies have dealt with freshwater systems or laboratory aquariums. The diffusion chambers used in these studies were usually limited by one or more of the following: small volume, unsuitable membrane material, a lack of agitation, and slow equilibration with the outside environment. In chambers with volumes of less than 200 ml, significant portions of the original population may be removed in a sampling regime lasting several days (10). Dialysis chambers made with regenerated cellulose have been shown to foul rapidly in seawater (36, 41) and become perforated by cellulose-digesting bacteria (D. Lavoie, M.S. Thesis, University of Rhode Island, Kingston, 1975). Cellulosic membranes and filters are now replaced with Nuclepore filters made of inert polycarbonate (42; D. Lavoie, M.S. Thesis) that pass and reject particles as a function of porosity (33). The lack of adequate agitation in most diffusion chambers allows for the settling of cells and a buildup of solutes at the membrane surfaces and encourages fouling.

In an attempt to overcome these design problems, a 5-liter, actively stirred chamber with Nuclepore membrane sidewalls was designed

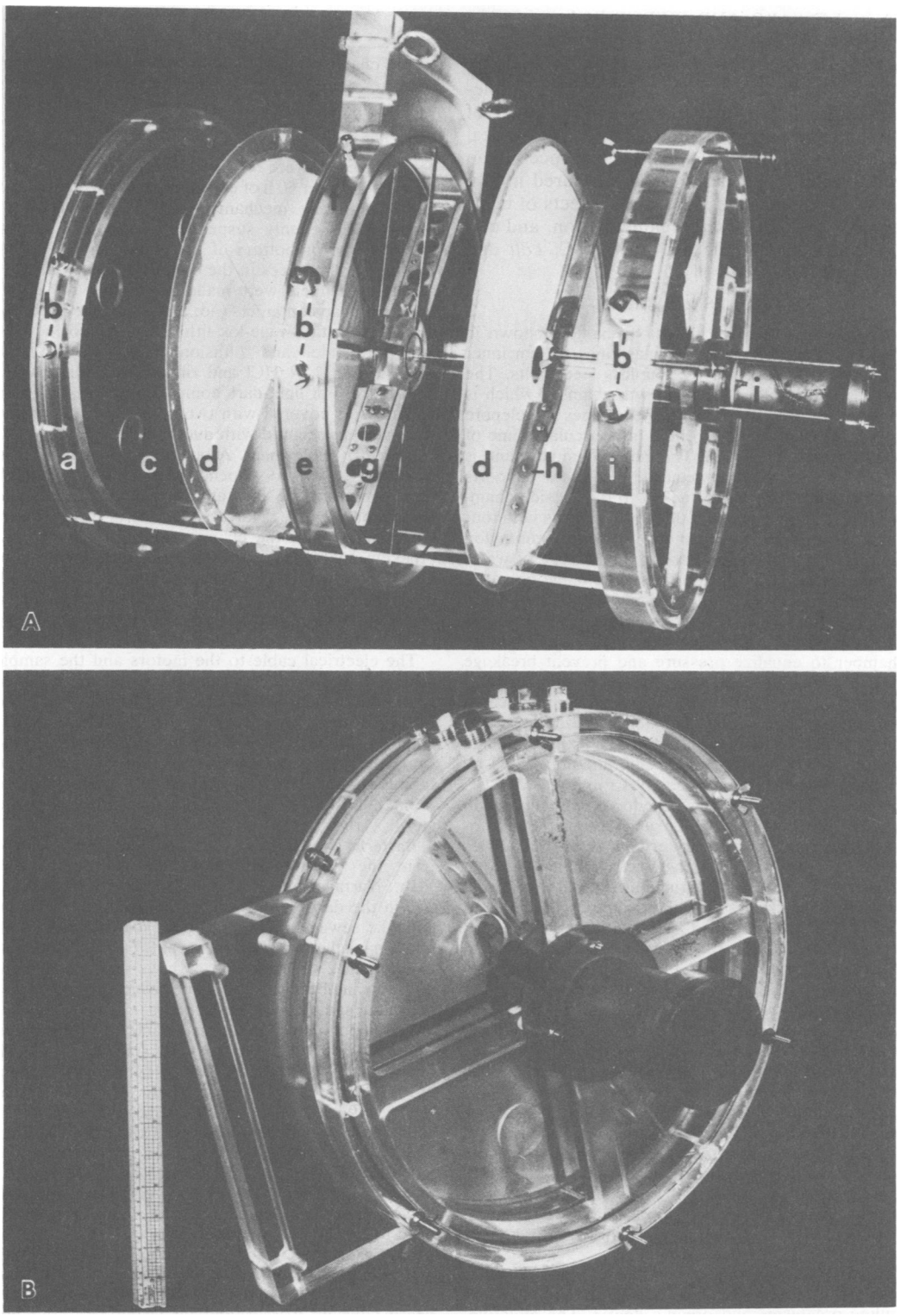

FIG. 1. The 5-liter diffusion chamber for environmental deployment. (A) An exploded view showing (a) outer chamber ring, (b) filling ports with stainless steel fittings, (c) Plexiglas baffle with screened holes, (d) Nuclepore membrane attached to Plexiglas support, (e) inner chamber ring with attachment flange, (f) sampling port with Swage-lok fitting, (g) inner stirring blade, (h) outer stirring blade, (i) outer chamber ring with motor mount, (j) 12-V DC motor. (B) Assembled chamber with improved Lexan motor housing. Rule, 31 cm.

and constructed (37). The present study successfully applied these chambers to in situ multiple-day experiments investigating the survival of natural sewage populations of two types of fecal bacteria (*Escherichia coli* and enterococci) in an estuary and a salt marsh. We measured in situ decay rates and investigated the effects of light, temperature, dissolved organic carbon, and culture mode on the decay rates of *E. coli* and enterococci.

MATERIALS AND METHODS

Chamber design. The diffusion chamber shown in Fig. 1 is made of three Plexiglas rings (47-cm inner diameter) held together by stainless steel bolts. The inner ring forms the 5-liter growth chamber which is enclosed by 0.4-μm-porosity membranes (Nuclepore Corp., Pleasanton, Calif.) held in a circular frame of 0.16-cm-thick Plexiglas. The inner compartment is sealed by an O-ring between the membrane supports and the chamber ring. Attached to the outside chamber ring is a baffle made of 0.64-cm Plexiglas with four holes (5-cm inner diameter) covered with 1-mm nylon screen. This baffle allows for free exchange of water while protecting the membranes. The baffle openings are sealed with duct tape or plugged with no. 10 stoppers to create temporary outer compartments which are filled at the same time as the inner culture chamber to equalize pressure and prevent breakage during filling, deployment, and recovery. Filling ports for both the central and external chambers are located on the same side of the chamber rings and are fitted with threaded stainless steel plugs. Plexiglas stirring blades on both sides of the membrane are turned by the motor shaft at approximately 1 rpm by a 12-V DC motor (M-5M40; TRW Inc., Dayton, Ohio) encased in a waterproof, pressurized Lexan housing. The motor was connected, via underwater connectors and cables (RMG-2-FS and 6SG-2-BCL; Vector Cable Co., Sugarland, Tex.), to a 12-V marine battery in a waterproof case on the dock or shore. Sampling ports located on top of the chamber had stainless steel Swage-lok fittings (no. SS-200; Cambridge Valve and Fitting, Billerica, Mass.) fitted to 0.3-cm (⅛-in.) opaque Impolene tubing (no. 22-p; Imperial Eastman Corp., Chicago, Ill.) used to withdraw the samples. A plastic three-way stopcock valve (no. 5851-10; Ace Glass Co., Vineland, N.J.) was placed on the shore end of the sample tubing, and 20-ml sterile syringes were used to withdraw the samples.

The chambers were designed with a large surface area to volume ratio to minimize the time for equilibration of diffusable substances. The equilibration time was estimated by measuring, at 0.5-h intervals, salinity and dissolved organic carbon inside and outside of an in situ chamber filled with a sewage-seawater mixture. Both parameters reached equilibrium with the outside environment within 2 h.

Stirring blades were incorporated in the chamber design to keep cells suspended and surfaces clean of fouling organisms and organic debris. The effectiveness of the stirring action to retard fouling was determined by examining the Nuclepore membrane from several experimental chambers with a scanning electron microscope. Membranes from stirred and unstirred chambers held in situ at several different temperatures were examined. Micrographs of membranes from unstirred chambers, exposed for 120 h at 0°C, showed an accumulation of fouling organisms and debris (Fig. 2A). The membranes from stirred chambers, however, were relatively free of attaching organisms, even after 50 h of exposure at 24°C (Fig. 2B and C). The stirring mechanism was also effective in keeping cells evenly suspended, as samples drawn from the top or bottom of the chamber showed no significant differences in the concentration of CFU.

Batch chambers were made from 2-liter polyethylene screw-cap containers (no. 2202; Nalgene, Rochester, N.Y.) with Swage-lok fittings and Impolene tubing. Both batch and diffusion chambers were acid washed with 1 N HCl and rinsed in distilled water before use. For light-dark comparisons, the dark containers were covered with two layers of black plastic garbage bags secured with duct tape.

Chamber deployment. A buoyed array was used in Narragansett Bay to suspend the chambers at a depth of 1.5 m from the pier at the U.S. Environmental Protection Agency, Narragansett, R.I. In the Bissel Cove salt marsh, the chambers were fitted with L-brackets and placed on the salt marsh bottom in 1 m of water. To study a diel cycle without tidal influence, all tidal flow in the salt marsh was stopped by blocking a single culvert with an inflatable rubber and wood plug (27).

The electrical cable to the motors and the sample tubing to the chambers were attached to the filled chambers, which were then placed in the water. The baffle closures were removed to permit water exchange with the outside environment. After rinsing the Impolene tubing by withdrawing approximately two tubing volumes (5 to 10 ml, depending on length of tubing), 1 to 10 ml of sample was withdrawn into a sterile syringe.

Bacterial inocula and enumeration. Raw sewage from the South Kingstown Narragansett Sewage Plant, Narragansett, R.I., was stored for about 2 h at 5°C in the dark before dilution. At the experimental site, the sewage was mixed with seawater to a 1:10 dilution in all experiments (except for a 1:25 dilution in experiment 2) in darkened carboys and poured into culture chambers; the chambers were deployed, and the initial samples were taken.

The population of the enteric bacteria after dilution ranged from 0.9×10^3 to 5.3×10^3 CFU/ml for *E. coli* and from 0.5×10^2 to 2.1×10^2 CFU/ml for enterococci. The ratio of enterococci to *E. coli* was similar (mean = 0.05 ± 0.02) for all the experiments. Replicate 1- to 5-ml samples withdrawn from each container with darkened sterile syringes were transported to the laboratory for immediate processing. The mTEC membrane filtration method was used to enumerate *E. coli* (6), and the mE membrane filtration method was used to enumerate enterococci (21).

Survival studies. Experiments conducted at approximately monthly intervals from February to August 1978 consisted of five in Narragansett Bay with a nearly constant 30 ‰ salinity and a temperature range from 0 to 20°C, and one in the Bissel Cove salt marsh, with an average salinity of 26 ‰ and an average temperature of 24°C. Replicability between diffusion chambers was tested in the February (1°C) experi-

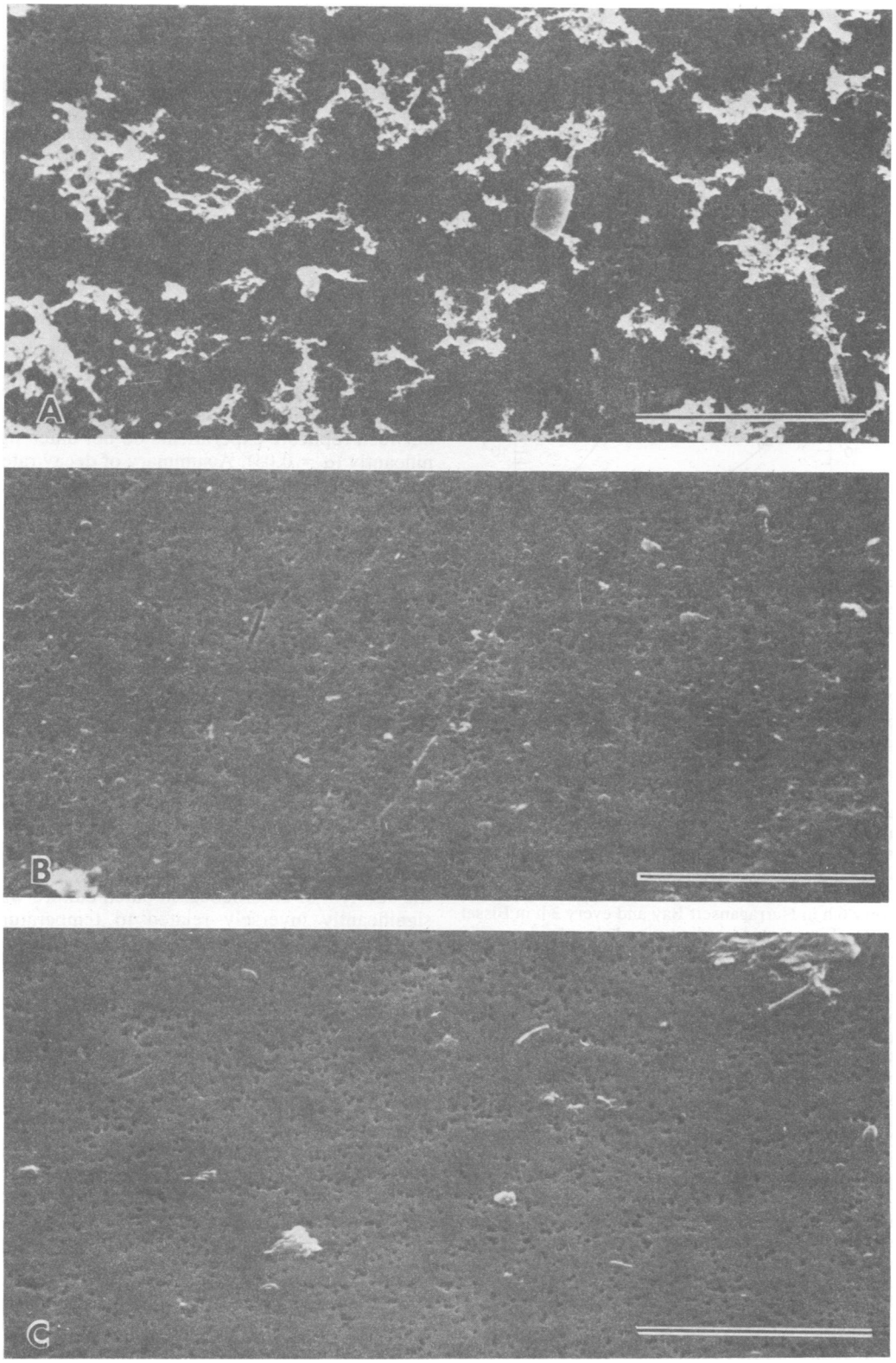

FIG. 2. Effect of stirring on the accumulation of debris and bacteria as seen in scanning electron micrographs of the inside face of an 0.4-μm-porosity Nuclepore membrane held in the 5-liter diffusion chambers. (A) Unstirred, 0°C, 120-h exposure (Narragansett Bay). (B) Stirred, 1°C, 120 h (Narragansett Bay). (C) Stirred, 24°C, 50 h (Bissel Cove). Bars, 10 μm.

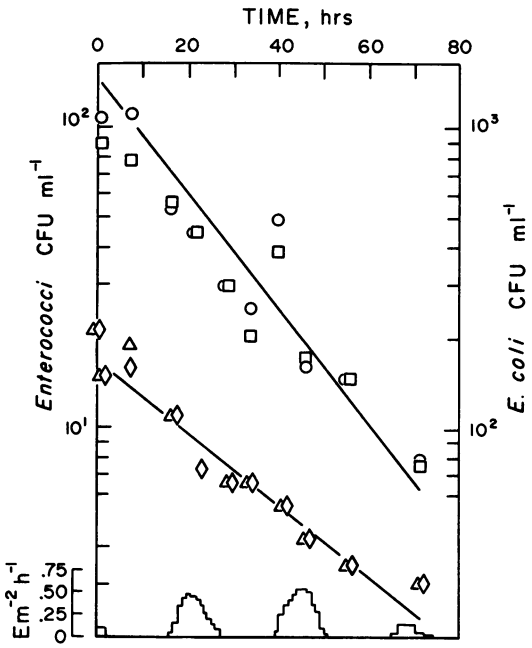

FIG. 3. Survival of *E. coli* and enterococci in replicate diffusion chambers held in Narragansett Bay at 1°C in February. Symbols: ○, □, *E. coli*, replicate chambers; △, ◇, enterococci, replicate chambers; E, light intensity in einsteins.

ment. To test the direct effect of light on survival, three experiments (April, July, and August, at 8.5, 20, and 24°C, respectively) included both light and dark diffusion and batch chambers. To detect any diel patterns in survival, frequent sampling was performed (every 6 h in Narragansett Bay and every 3 h in Bissel Cove). Temperature, salinity, and dissolved organic carbon (DOC) measurements were made in all experiments. DOC was measured by the method of Menzel and Vaccaro (24) as modified by Kerr and Quinn (20). Triplicate samples were sparged and sealed in ampoules immediately after filtration and oxidized within 24 h. Determinations were made with a Beckman 315 Infra-red analyzer, with potassium hydrogen pthalate as a standard and correcting for reagent carbon. In the Bissel Cove experiment, assays for polysaccharides (2), monosaccharides (17), and dissolved oxygen by the Winkler method (40) were also performed. Hourly light intensity data were obtained from Eppley Laboratories (Newport, R.I.). Extinction coefficients were determined at the experimental site with a quantum sensor and meter (Lambda Instruments Corp., Lincoln, Nebr.; models LI 190S and LI 185S) and were used to determine ambient light at the depth of the chambers. Direct, continuous, in situ light measurements were taken during the Bissel Cove experiment with a quantum sensor and integrator (Lambda Instruments Corp.; model LI 510).

Decay rates (k) per hour were determined from the slope of the linear regression of log cell number versus time; the time for 90% of the population to die (T_{90} in hours) was calculated from the relationship $T_{90} = \ln 10/k$ (13). T_{90} is the most common expression used in past literature to express survival time and is included here for comparative purposes. An extreme departure from exponential decay in the Bissel Cove experiment precluded a linear regression, and these data are reported with a graphically estimated T_{90} value only. A statistical comparison of the slopes of the decay curves was performed by using an analysis of covariance procedure (39).

RESULTS

A representative decay curve for the enteric bacteria held in replicate diffusion chambers in Narragansett Bay is shown in Fig. 3 (experiment 2; 1°C). The slopes of the lines (decay rates) between replicate experiments do not differ significantly ($\alpha = 0.05$). A summary of decay rates and T_{90} survival times for *E. coli* and enterococci is presented in Table 1. Also included in Table 1 are environmental parameters (temperature, light intensity, and DOC) measured during each experiment.

The relationship between survival times (T_{90}) in all the light experiments and temperature is illustrated in Fig. 4. There was a significant inverse relationship between T_{90} and temperature in the diffusion chamber experiments (Fig. 4a). For *E. coli* in light diffusion chambers, this relationship is $T_{90} = 54.2 - 1.06T$ (°C) ($r = 0.94$), and that for enterococci in light diffusion chambers is $T_{90} = 104.3 - 3.16T$ (°C) ($r = 0.94$). The survival of *E. coli* in batch containers did not show a strong relationship to temperature (the slope of the regression line was not significantly different from zero; $\alpha = 0.05$), whereas the survival of enterococci in batch culture was significantly inversely related to temperature (Fig. 4b): $T_{90} = 155.5 - 5.4T$ (°C) ($r = 0.96$).

Enterococci survived significantly longer than *E. coli* in both culture modes in the light, except in the Bissel Cove (24°C) experiment, in which enterococci survived somewhat less well (Fig. 4a). This also held true in the dark diffusion chambers, but no consistent relationship was seen in the dark batch containers (Fig. 5).

Several aspects of the effects of solar radiation on survival were considered, including (i) the relationship between the average hourly light intensity over the first 2 h and the first day of exposure and survival time, (ii) survival in light versus dark control containers, and (iii) day/night differences in survival.

Although incident solar radiation increases from February to August, no such trend was seen in the in situ light intensities due to changes in the extinction coefficient and cloud cover (Table 1). There was no significant ($\alpha = 0.05$) relationship between the average hourly light intensity during the first 2 h of exposure and survival times of the enteric bacteria in either

TABLE 1. Decay rates (k) and T_{90} for sewage populations of *E. coli* and enterococci in both diffusion and batch chambers held in natural sunlight and in the dark in Narragansett Bay and Bissel Cove

Expt[a]	Temp (°C)	Description[b]	*E. coli*		Enterococci		I_z[c]	I_z[d]	DOC (mg liter^{-1})
			k (h^{-1})	T_{90} (h)	k (h^{-1})	T_{90} (h)			
1	0	Diffusion-lt	0.042	55.2	0.019	122	25	24	
		Batch-lt	0.036	63.8	0.014	167			
2	1	Diffusion-lt	0.043	53.0	0.027	83.1	11	8	1.65
		Diffusion-lt	0.044	52.3	0.027	83.1			
3	8	Diffusion-lt	0.049	46.9	0.029	79.4	11[e]	15[e]	2.32
		Diffusion-dk	0.044	52.3	0.024	95.9			
		Batch-lt	0.044	52.3	0.025	100.0			
		Batch-dk	0.027	80.0	0.016	140.0			
4	16	Diffusion-lt	0.063	36.7	0.044	52.5	23	16	2.05
		Diffusion-lt	0.060	38.2	0.044	52.5			
		Batch-lt	0.050	46.0	0.049	47.4			
		Batch-lt	0.047	48.6	0.044	52.3			
5	20	Diffusion-lt	0.088	26.2	0.054	42.6	29	15	2.09
		Diffusion-lt	0.075	30.8	0.050	45.3			
		Batch-lt	0.043	53.9	0.036	63.6			
		Batch-dk	0.023	99.3	0.026	87.9			
6	24	Diffusion-lt		33.0		29.0	7[e]	4[e]	5.06
		Diffusion-dk		33.0		39.0			
		Batch-lt		34.0		25.0			
		Batch-dk		124.0		120.0			

[a] Experiments 1 to 5, Narragansett Bay; experiment 6, Bissel Cove.
[b] lt, Light; dk, dark.
[c] I_z, Average light intensity (ly h^{-1}) at the depth of the chambers over the first 2 h.
[d] Average light intensity at the depth of the chambers over the first day of exposure.
[e] I_z determined by converting the in situ light measurements (E m^{-2} h^{-1}) to langleys (g-cal cm^{-2}) per hour, using the conversion 1 einstein = 52×10^3 g-cal (29). All other light intensity measures were determined with hourly records from Eppley Laboratories, Newport, R.I.

culture mode. If the average light intensity over the first day of exposure is considered, there was a positive relationship between light intensity and survival times in the batch culture mode but not in diffusion culture. No relationship was found between ambient in situ DOC and survival in the diffusion chambers.

T_{90} values for survival in experiments with dark controls are compared in Fig. 5. For *E. coli* in diffusion chambers, survival was slightly better in the dark at 8 and 20°C and the same in the light or dark at 24°C (Fig. 5a). Enterococci survival was somewhat greater in the dark in all three experiments (Fig. 5b). None of the differences, however, were significant ($\alpha = 0.05$). Survival in the dark in batch culture for both organisms was always significantly greater than survival in the light (Fig. 5). Survival in dark batch culture was also significantly greater than in either light or dark diffusion culture in all experiments.

Diel periodicity in survival was not observed for either organism in the Narragansett Bay experiments (see, e.g., Fig. 3). However, a diel pattern in survival was apparent for *E. coli* in the Bissel Cove salt marsh experiment (Fig. 6). *E. coli* survival was nearly identical in the light and dark diffusion chambers as well as the light batch container, in which there appeared to be growth during the day followed by a decline at night. Diel patterns were also seen in temperature, total DOC, and carbohydrate concentrations. Temperature and *E. coli* cell number in all but the dark batch container were positively correlated ($\alpha = 0.05$; $r = 0.95$) over the experimental period. After the first 10 h, there was a correlation between cell numbers and both DOC and carbohydrates ($r = 0.88$ and 0.91 for DOC and polysaccharides, respectively; significant at $\alpha = 0.05$). A reverse diel response may be indicated for enterococci, with an increased decay rate during the daylight hours (Fig. 6).

DISCUSSION

There was a marked decrease in the survival of enteric bacteria during the period from February to August in Narragansett Bay. Such a trend could be the result of seasonal changes in physical parameters, such as temperature and solar radiation, as well as changes in biological parameters, such as DOC concentrations, populations of predators, or the production of inhibitory substances.

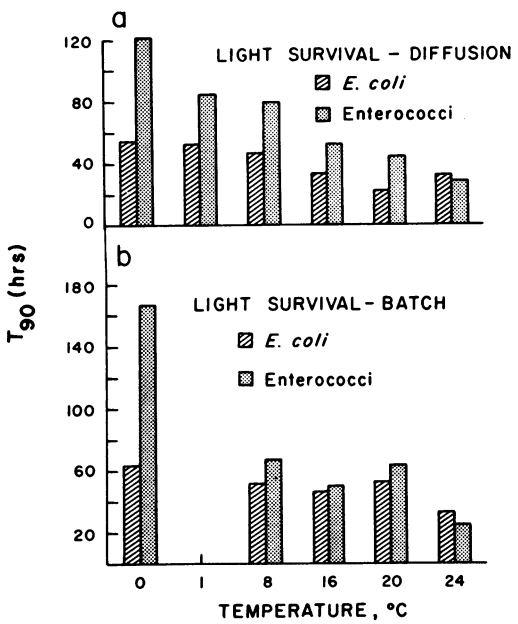

FIG. 4. T_{90} of *E. coli* and enterococci as a function of temperature in light diffusion experiments (a) and batch experiments (b).

In laboratory studies (4, 15, 28, 43), the survival time of pure cultures of coliform bacteria in seawater or freshwater has been shown to decrease with increasing temperature. Two studies (9, 42) have also reported this temperature effect on pure cultures of *E. coli* when held in small-volume diffusion chambers suspended in outdoor incubators. The present study confirms and extends these studies with pure cultures to natural sewage populations of both *E. coli* and enterococci under in situ conditions. Our results suggest that temperature may exert a major control on the magnitude of decay rates of wild coliform populations in relatively low-nutrient estuarine waters.

The effect of temperature on decay rate may be related to its effect on metabolism. At low nutrient levels and low temperatures, toxic materials may be metabolized at slower rates and survival prolonged. Graham and Sieburth (14) found that increasing the incubation temperature from 15 to 25°C without added nutrients led to the decline of *Salmonella typhimurium* and *E. coli* in artificial seawater. However, with the addition of Trypticase (BBL Microbiology Systems) both organisms grew. Thus, with adequate nutrients, increasing temperature results in the growth of enteric bacteria rather than decay (see also reference 28). This phenomenon may have occurred in our experiment in the eutrophic Bissel Cove salt marsh, in which periods of *E. coli* growth were also seen which roughly corresponded to increases in temperature, DOC, and polysaccharides (Fig. 6).

That temperature may have a predictable effect on survival in estuarine environments is important, as this information could be used for estimating survival rates of enteric bacteria at different times of the year. Note that the survival of *E. coli* in the batch culture experiments reported here did not show a predictable relationship to temperature. At least for *E. coli*, diffusion culture may be a more useful tool for establishing temperature-survival relationships.

One desirable attribute of an indicator organism is persistence (3). The results of this study indicate that natural sewage populations of enterococci survive longer than *E. coli* in an estuarine environment, suggesting that enterococci may be a better indicator than the more commonly used *E. coli*. In contrast to the Narragansett Bay experiments, *E. coli* survived somewhat longer than enterococci in the salt marsh (Fig. 4a and 6). This latter result may have been due to the richer nutrient regime in the salt marsh. Although no comparable study with natural populations of enteric bacteria from sewage has been made, other studies have reported that fecal streptococci are hardier than coliforms (22, 42). In contrast, Slanetz and Bartley (38) found that fecal streptococci survived less well than fecal coliforms in dialysis bags suspended in

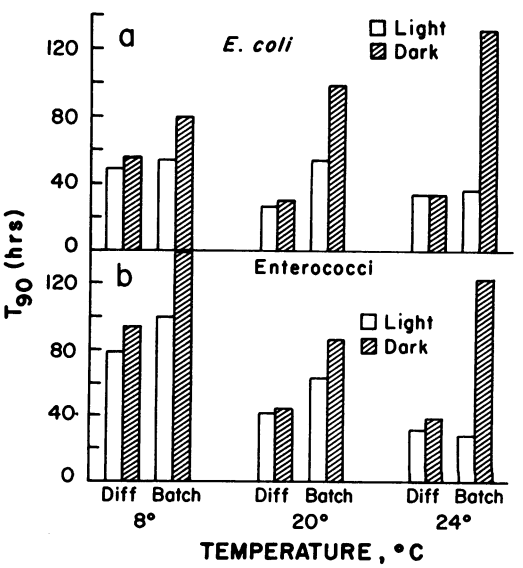

FIG. 5. T_{90} of *E. coli* (a) and enterococci (b) in light and dark diffusion and batch experiments at three different temperatures.

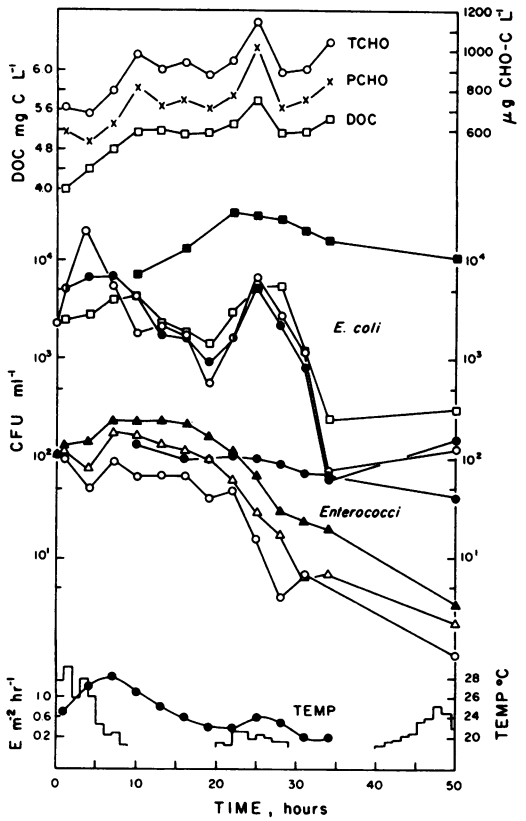

FIG. 6. Diel pattern in temperature, DOC, polysaccharides (PCHO), total carbohydrates (TCHO), light intensity (E [einsteins]) and *E. coli* and enterococci cell numbers in the light (open symbols) and in the dark (closed symbols) in both batch and diffusion chambers in the Bissel Cove salt marsh, August 1978.

seawater. It is possible that their results were due to leakage of organic matter from the dialysis bags (C. M. Burney, M.S. Thesis, University of Rhode Island, Kingston, 1976).

Numerous laboratory studies have demonstrated that near-UV light can be lethal or growth inhibitory to a great variety of bacteria and that exogenous or endogenous photosensitizers (e.g., fluorescent dyes, chlorophylls, cytochromes, and NADH) may be involved (7, 16). The ecological implications of the harmful action of sunlight on enteric bacteria in seawater has largely been ignored until recently due to the prevalent assumption that the turbidity of coastal waters would preclude a strong effect of light. Several workers, however, present evidence that visible light can decrease the survival of enteric bacteria in seawater (12, 13, 34). Based on tracer studies (11, 12), it has been proposed that light is the major mechanism of coliform decay in seawater, and a decay model based on light intensity has been developed (5; C. Chamberlain, Ph.D. Thesis, Harvard University, Cambridge, Mass., 1977). No inverse relationship between light intensity and survival was found in the present in situ study. In fact, a positive relationship between hourly light intensity over the first day of exposure and T_{90} can be found in the batch culture experiments (Table 1). This was also seen in the salt marsh experiment, in which *E. coli* numbers increased during the daylight hours (Fig. 6). Such a positive effect could be the result of organic matter release during photosynthesis.

Light also has some detrimental effect, as can be seen by the prolonged survival in many of the dark control containers. This prolonged survival was slight in the diffusion chambers (8 to 30% higher T_{90} in the dark for enterococci and 0 to 18% higher T_{90} for *E. coli*) and was not significant in any single experiment. In the batch culture experiments, however, survival was significantly longer in the dark (54 to 265% higher T_{90} for *E. coli* and 40 to 380% higher for enterococci). These results suggest that light inhibition, when it occurs, is indirect and dependent on diffusable exogenous materials in the environment, which would affect both light and dark diffusion chambers, but not the dark batch containers. If there was a direct inhibiting effect of light (via endogenous photosensitizers), increased survival would be expected in both dark batch and dark diffusion chambers. The lack of evidence for direct light inhibition in the diffusion chambers could be due to the selective exclusion of wavelengths below 340 nm by the Plexiglas chambers (the polyethylene batch bottles transmit 1 to 10% of light from 300 to 340 nm). However, if the batch containers were selectively passing more lethal wavelengths of light, one might expect a decreased survival time in the light batch chambers relative to the light diffusion chambers. In general, however, survival in the light batch containers was greater than in the light diffusion chambers. There is, possibly, an ameliorating effect of increased nutrient concentrations in the batch containers. Further experiments are needed to determine light and nutrient interactions.

Inhibitory effects of light might also be manifested in diel changes in survival. Increased decay rates during the day were not noted for enterococci or *E. coli* in Narragansett Bay. A reverse trend occurred in the salt marsh experiment, in which *E. coli* cell numbers increased during daylight hours and decreased at night and were closely correlated with DOC and polysaccharides after the first 10 h of incubation. This supports the idea that increasing nutrients may overcome the bactericidal effect of seawater in situ, a phenomenon often seen in laboratory

studies (25, 26, 31, 34). It would have been interesting to see whether this diel trend continued past the 33-h study period. A single sample taken at 50 h showed that the population may again have recovered on day 3. These results suggest that the survival response of *E. coli* is quite different in a high-nutrient salt marsh than in a low-nutrient estuary. This phenomenon deserves further study, as wastewater contamination of coastal marshes is not uncommon.

The goal of many investigations of the decay of fecal bacteria is to relate some easily measured environmental parameter with survival so that a general predictive model can be developed. The results of this study, as well as others, suggest that the decay phenomenon is probably due to complex interactions among factors peculiar to each environment, season, and part of the diel cycle, which may be better studied with in situ diffusion chambers.

ACKNOWLEDGMENTS

This research was supported in part by grants R804866010 and R804866012 from the U.S. Environmental Protection Agency and grant OCE-7681779 from the Biological Oceanography Program of the National Science Foundation.

We thank Al Dufour and Victor Cabelli for the opportunity to fabricate and test these chambers, Wayne Brandt for his role in the design and fabrication of the chambers, and the Engineering Instrument Shop of the University of Rhode Island for making necessary modifications. We also thank Gary Hammond, Ken Hinga, Curtis Burney, and Ken Johnson for their help in the field and laboratory.

LITERATURE CITED

1. **Aubert, M., S. Pesando, and M. Gauthier.** 1975. Effects of antibiosis in a marine environment, p. 191–196. *In* A. L. H. Gameson (ed.), Discharge of sewage from sea outfalls. Pergamon Press, New York.
2. **Burney, C. M., and J. McN. Sieburth.** 1977. Dissolved carbohydrates in seawater. II. Spectrophotometric procedure for total carbohydrate analysis and polysaccharide estimation. Mar. Chem. **5**:15–28.
3. **Cabelli, V.** 1978. New standards for enteric bacteria, p. 233–271. *In* R. Mitchell (ed.), Water pollution microbiology. John Wiley & Sons, New York.
4. **Carlucci, A. F., and D. Pramer.** 1960. An evaluation of factors affecting survival of *Escherichia coli* in seawater. II. Salinity, pH, and nutrients. Appl. Microbiol. **8**:247–250.
5. **Chamberlain, C., and R. Mitchell.** 1978. A decay model for enteric bacteria in natural waters, p. 325–348. *In* R. Mitchell (ed.), Water pollution microbiology, vol 2. John Wiley & Sons, New York.
6. **Dufour, A. P., E. T. Strickland, and V. J. Cabelli.** 1975. Enumeration method for thermotolerant *Escherichia coli*, p. 79–81. *In* D. S. Wilt (ed.), Proceedings of the 9th Shellfish Sanitation Workshop. U.S. Food and Drug Administration, Charleston, S.C.
7. **Eisenstark, A.** 1971. Mutagenic and lethal effects of visible and near-U.V. light on bacterial cells. Adv. Genet. **16**:167–198.
8. **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.
9. **Faust, M., A. E. Aotaky, and M. T. Hargadon.** 1975. Effects of physical parameters on the in situ survival of *Escherichia coli* from estuarine waters. Appl. Environ. Microbiol. **30**:800–806.
10. **Fliermans, C. B., and T. W. Gordon.** 1977. Modification of membrane diffusion chambers for deep water studies. Appl. Environ. Microbiol. **33**:207–210.
11. **Foxworthy, J. E., and G. R. Kneeling.** 1969. Eddy diffusion and bacterial reduction in waste fields in the ocean. University of California, Los Angeles.
12. **Gameson, A. L. H., and D. J. Gould.** 1975. Effects of solar radiation on the mortality of some terrestrial bacteria in seawater, p. 209–219. *In* A. L. H. Gameson (ed.), Discharge of sewage from sea outfalls. Pergamon Press, New York.
13. **Gameson, A. L. H., and J. R. Saxon.** 1967. Field studies on the effect of daylight on mortality of coliform bacteria. Water Res. **1**:279–295.
14. **Graham, J. J., and J. McN. Sieburth.** 1973. Survival of *Salmonella typhimurium* in artificial and coastal seawater. Rev. Intern. Ocean. Med. **29**:5–29.
15. **Hanes, N. G., G. A. Rohlick, and W. B. Sarles.** 1965. Effects of temperature on survival of indicator bacteria in water. J. N. Engl. Water Works Assoc. **80**:13–16.
16. **Jagger, J.** 1976. Effects of near-ultraviolet radiation on microorganisms. Photochem. Photobiol. **23**:451–454.
17. **Johnson, K. M., and J. McN. Sieburth.** 1977. Dissolved carbohydrates in seawater. I. A precise spectrophotometric analysis for monosaccharides. Mar. Chem. **5**:1–13.
18. **Jones, G. E.** 1967. Precipitates from autoclaved seawater. Limnol. Oceanogr. **12**:165–167.
19. **Jones, G. E.** 1967. Growth of *Escherichia coli* in heat and copper-treated synthetic seawater. Limnol. Oceanogr. **12**:165–167.
20. **Kerr, R. A., and J. G. Quinn.** 1975. Chemical studies on the dissolved organic matter in seawater. Isolation and fractionation. Deep Sea Res. **22**:107–116.
21. **Levin, M. A., J. R. Fischer, and V. J. Cabelli.** 1975. Membrane filter technique for enumeration of enterococci in marine waters. Appl. Microbiol. **30**:66–71.
22. **McFeters, G. A., G. K. Bissonette, J. J. Jezenski, C. A. Thomas, and D. G. Stuart.** 1974. Comparative survival of indicator bacteria and enteric pathogens in well water. Appl. Microbiol. **27**:823–829.
23. **McFeters, G. A., and D. G. Stuart.** 1972. Survival of coliform bacteria in natural waters: field and lab studies with membrane filter chambers. Appl. Microbiol. **24**:805–811.
24. **Menzel, D. W., and R. F. Vaccaro.** 1964. The measurement of dissolved organic and particulate carbon in seawater. Limnol. Oceanogr. **9**:138–142.
25. **Moebus, K.** 1972. Seasonal changes in antibacterial activity of North Sea water. Mar. Biol. **13**:1–13.
26. **Moebus, K.** 1972. Bactericidal properties of natural and synthetic seawater as influenced by addition of low amounts of organic matter. Mar. Biol. **15**:81–88.
27. **Nixon, S., C. Oviatt, J. Garber, and V. E. Lee.** 1976. Diel metabolism and nutrient dynamics in a salt marsh embayment. Ecology **57**:740–750.
28. **Orlob, G. T.** 1956. Viability of sewage bacteria in seawater. Sewage Ind. Wastes **28**:1147–1167.
29. **Parsons, T. H., M. Takahashi, and B. Hargraves.** 1977. Biological oceanographic processes. Pergamon Press, New York.
30. **Roper, M. M., and K. C. Marshall.** 1978. Biological control agents of sewage bacteria in marine habitats. Aust. J. Mar. Freshwater Res. **29**:335–343.
31. **Scarpino, P. V., and D. Pramer.** 1962. Evaluation of factors affecting the survival of *Escherichia coli* in seawater. VI. Cysteine. Appl. Microbiol. **10**:436–440.
32. **Schultz, J. S., and P. Gerhardt.** 1969. Dialysis culture of microorganisms: design, theory, results. Bacteriol. Rev. **33**:1–47.
33. **Sheldon, R. W.** 1972. Size separation of marine seston by membrane and glass-fiber filters. Limnol. Oceanogr. **17**:494–498.
34. **Sieburth, J. McN.** 1964. Role of algae in controlling bacterial populations in estuarine waters, p. 217–233. Comm. Int. Explor. Sci. Mer. Medit., Symp. Pollut. Mar.

Par. Microorgan. Prod. Petrol. Monaco, Avril. 1964.
35. **Sieburth, J. McN.** 1968. The influence of algal antibiosis on the ecology of marine microorganisms, p. 63–94. *In* M. R. Droop and E. J. Ferguson Wood (ed.), Advances in the microbiology of the sea. Academic Press, Inc., New York.
36. **Sieburth, J. McN.** 1975. Microbial seascapes. University Park Press, Baltimore.
37. **Sieburth, J. McN.** 1979. Seamicrobes. Oxford University Press, New York.
38. **Slanetz, L. W., and C. H. Bartley.** 1965. Survival of fecal streptococci in seawater. Health Lab. Sci. **2**:142–148.
39. **Snedacor, G. W., and W. Cochran.** 1967. Statistical methods. Iowa State University, Ames.
40. **Strickland, J. D., and T. R. Parsons.** 1972. A practical handbook of seawater analysis, 2nd ed. Bull. J. Fish. Res. Bd. Can. 167.
41. **Vargo, G., P. Hargraves, and P. Johnson.** 1975. Scanning electron micrographs of dialysis tubes incubated in flowing seawater. Mar. Biol. **31**:113–120.
42. **Vasconcelos, G. J., and R. G. Swartz.** 1976. Survival of bacteria in seawater using a diffusion chamber apparatus in situ. Appl. Environ. Microbiol. **31**:913–920.
43. **Verstraete, W., and J. Voets.** 1976. Comparative study of *Escherichia coli* survival in two aquatic ecosystems. Water Res. **10**:129–136.
44. **Weiss, C. M.** 1951. Adsorption of *Escherichia coli* on river and estuarine silts. Sewage Ind. Wastes **23**:227–237.