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Effect of Fecal Pollution on *Vibrio parahaemolyticus* Densities in an Estuarine Environment

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Vibrio parahaemolyticus **densities in the Narragansett Bay, R.I., estuary were found to significantly correlate with the level of fecal pollution in the water. Results of field investigations showed a definite association between the levels of this organism and those of *Escherichia coli*, *Clostridium perfringens*, and enterococci. Densities of *V. parahaemolyticus* were greatest in the near-surface waters of contaminated areas and decreased sharply with both the distance from the sources of fecal pollution and the depth of the water column. A positive association with the amount of particulate matter in the water and specifically with its zooplankton content also was found. No association was obtained with any of the physical or chemical parameters examined except dissolved oxygen. The results of laboratory studies on the growth of *V. parahaemolyticus* in collected estuarine waters were consistent with the field observations in that nutrients in sewage did not directly produce any increase in natural or inoculated levels of the organism. Of several particulates added to the water, only chitin and net zooplankters (live or dead) supported the growth of *V. parahaemolyticus*. The addition of sewage to the water had no measurable effect, whether or not the various particulates also were present. The data show that wastewater effluents have an effect on *V. parahaemolyticus* densities in this estuary and indicate that the effect is indirect, probably mediated by biostimulation of the food chain and manifested at the level of the microfauna.**

Vibrio parahaemolyticus was first identified as a human enteric pathogen in 1951 when Fujino et al. (8) reported on a "shirasu" food poisoning outbreak in Japan. Since that time many investigators have examined the distribution of this species in natural environments, and their results clearly show that this gram-negative, halophilic species, unlike most enteric bacterial pathogens, derives from the marine environment (4, 9, 10, 14). Although the organism has been recovered from coastal and estuarine waters along the Atlantic, Pacific, and Gulf coasts of the United States, the reported densities vary widely. The numbers found in bottom sediments varied from less than 1 to more than 10^4/g (10, 11), and reported densities in the water column ranged from undetectable levels to as many as 10^4/100 ml (21).

The use of different methods and media to enumerate *V. parahaemolyticus* probably contributed to some of the differences in the reported densities, as did the seasonal or temperature-dependent occurrence (10) of these organisms. Indeed, during winter months *V. parahaemolyticus* has been detected only in bottom sediments, and then only at very low levels (10). However, differing methodologies and seasonal variability do not account entirely for the observed differences in the density of the organisms. Other environmental factors must also be operative. Several authors have noted that although this species tends to be prevalent in coastal and estuarine areas, it is infrequently isolated from open-coast environments (11). Furthermore, it has been observed that marine vibrios are favored in waters rich in organic nutrients (2), such as might be expected in areas heavily impacted by land runoff and wastewater discharges. However, results also have been reported which contradict the correlation of high densities of *Vibrio* spp. with the presence of organically enriched water (22, 24). Therefore, the role of the organic content of the water, in particular that derived from wastewater discharges, in the density of *V. parahaemolyticus* in estuarine environments remains unresolved.

V. parahaemolyticus is able to utilize a relatively large number of substrates for growth (6), and in the laboratory this chemoheterotroph has one of the fastest bacterial growth rates (26). Thus, the response to nutrient stimulation in the environment would be expected to be rapid if, in fact, the organism responds directly to nutrients. In a survey of certain recreational areas in Narragansett Bay, R.I., Roland (20) detected *V. parahaemolyticus* cells in only 2 of 75 samples collected in June and July 1972. However, a much greater incidence of this organism in this estuary was suggested by results from previous work performed during the development of a membrane filtration procedure (mVP) to quantify this species in the water column (28). Moreover, *V. parahaemolyticus* densities were found to be at least 10 times greater in the upper Providence River, an estuarine area receiving large volumes of sewage treatment plant effluents, than in the lower bay. The objective of the field and laboratory studies described below was to determine whether *V. parahaemolyticus* responds to nutrient loading, specifically that from municipal sewage treatment plant effluents. If so, the secondary objectives were to determine (i) whether it responds directly or indirectly via planktonic or benthic microflora and microfauna, and (ii) whether multiplication occurs in the sediments or the overlying waters.

MATERIALS AND METHODS

Sampling network and procedures. Water samples were collected periodically during the summer over the network of stations throughout the bay as shown in Fig. 1. In addition, year-round sampling was conducted at station PR 4 to examine the seasonal incidence of *V. parahaemolyticus* strains.

Surface water samples were collected at 0.5 m below the

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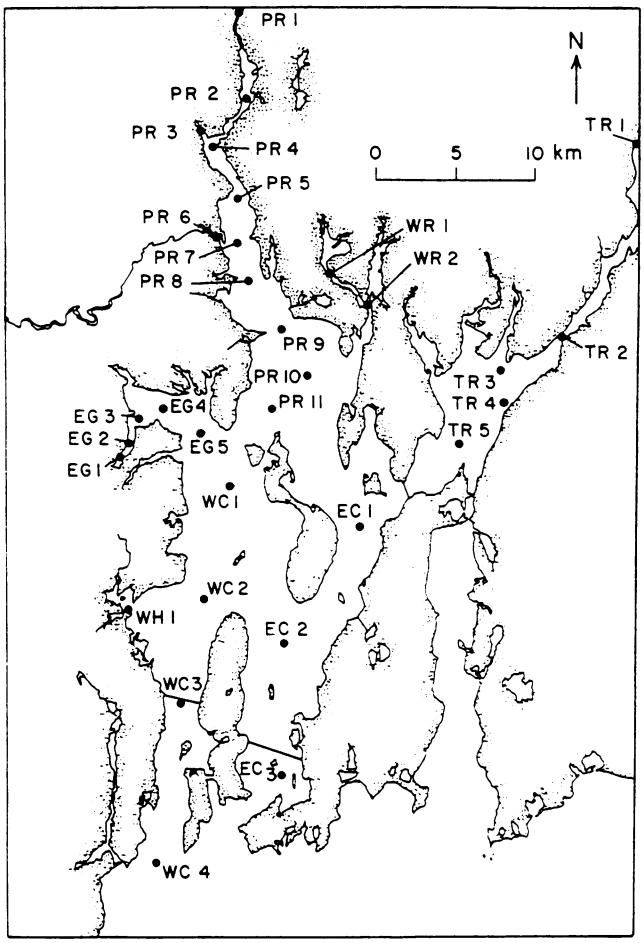

FIG. 1. Sampling station network in the Narragansett Bay estuary, R.I. For station descriptions, see Table 1.

surface of the water in presterilized sample bottles for the microbiological assays and in Cubitainers (Hedwin Corp., Laporte, Ind.) for the chemical determinations. Water from other depths was obtained with a 5-liter Van Dorn sampler (General Oceanics Inc., Miami, Fla). After collection, the samples were transferred to presterilized sample bottles or Cubitainers. Samples for estimating zooplankton populations and for laboratory studies were obtained by passing 10-liter volumes or more of the water through a no. 20 plankton net with a 77-μm mesh. Approximately 2 liters of the filtrate was used to flush the zooplankters retained on the mesh into collection bottles.

Physical parameters. Salinity and water temperature were determined with a YS1 model 33 S-C-T Meter (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio), and dissolved oxygen was determined with a YS1 model 54 Oxygen Meter. Transparency and pH were determined in the laboratory with a Turbidimeter 2100 (Hach Chemical Co., Ames, Iowa) and a Beckman pHase I (Beckman Instruments, Inc., Fullerton, Calif.), respectively.

Plankton analyses. Phytoplankton densities were determined as follows. The water samples were gently mixed and, if required, diluted in filtered bay water; 1.0-ml volumes of the appropriate dilutions were allowed to settle for 30 min in Sedwick-Rafter chambers, and the phytoplankton in the chambers were then counted microscopically at ×450 magnification. When required, the water samples for phytoplankton analyses were stored at 4°C after addition of 0.1% (vol/vol) Lugol iodine; storage never exceeded 48 h. Chlorophyll *a* was determined by the procedure of Strickland and Parsons (23).

The concentrated zooplankton samples used to estimate net zooplankton populations were preserved in a 10% buffered Formalin solution containing 100 ml of formaldehyde (Fisher Scientific Co., Pittsburgh, Pa.), 3.24 g of sodium phosphate dibasic, 5.26 g of sodium phosphate monobasic, and 900 ml of distilled water. Net zooplankton densities were determined within 96 h of collection from total counts made in gridded tissue culture dishes (60 by 14 mm) with a dissecting microscope (Bausch & Lomb, Inc., Rochester, N.Y.) and indirect lighting.

Chemical analyses. Water samples were examined for total Kjeldahl nitrogen, ammonia (distillation followed by nesslerization), nitrates (cadmium reduction method) and nitrites (diazotization), total phosphorus (persulfate digestion followed by ascorbic acid colorimetry) and P_i (ascorbic acid colorimetry), and dissolved organic carbon (filtration followed by combustion-infrared method) by procedures described previously (1).

Bacterial assays. Water samples were assayed bacteriologically within 6 h of their collection. *Escherichia coli* (7), enterococcus (13), and *Clostridium perfringens* (5) densities were determined by the methods described in each reference. Estimates of viable aerobic, heterotrophic bacteria were made from spread plates on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) supplemented with NaCl to a final concentration of 1%. The plates were incubated at 23°C for 1 week. *V. parahaemolyticus* densities were determined by the mVP (28).

***V. parahaemolyticus* isolates.** A portion of the colonies presumptively identified as *V. parahaemolyticus* by the mVP were confirmed as such by the tests and procedures described previously (28). In addition, isolates were tested for toxigenicity as seen from the Kanagawa phenomenon (19) by the following procedure. After the sucrose-negative colonies were scored in the mVP, filters were placed on petri plates (50 by 12 mm) containing 4 ml each of modified Wagatsuma agar (19). After incubation for 2 h at 37°C, the filters were removed from the plates, which were then examined for the hemolysis characteristic of Kanagawa-positive strains.

Susceptibilities of the isolates to novobiocin (10-μg disks; Difco) and 0/129 (2,4-diamino-6,7-diisopropyl pteridine phosphate; Sigma Chemical Co., St. Louis, Mo.) were determined by standard antibiotic disk susceptibility procedures (16), with the exception that Mueller-Hinton agar (Difco) was supplemented with NaCl to a final concentration of 0.85%. This concentration of NaCl supported the growth of halophilic *Vibrio* spp. without affecting their sensitivities to 0/129 (17). The 0/129 disks were prepared by saturating sterile blank disks (Difco) with an aqueous solution of the compound (10 μg/ml) and drying them at room temperature in sterile petri dishes.

Growth studies. The laboratory studies performed to determine the effect of sewage on the growth of *V. parahaemolyticus* were conducted as follows. Approximately 60-liter samples were collected in sterile polypropylene carboys from just below the surface of the water at stations PR 1, PR 4, and PR 11. Qualitative assays on 1-liter volumes of the water and 500-ml quantities of sewage were performed for the presence of bacteriophages (3) and bdellovibrios (18) against *V. parahaemolyticus*. Aliquants of water (4 liters)

then were delivered into 6-liter Erlenmeyer flasks. Various concentrations (0.01, 0.1, 1.0, and 10%, vol/vol) of raw or secondary treated sewage from a given treatment plant were added to all but one of the flasks, which was retained as a control. The flasks were incubated at 23°C with gentle agitation. *V. parahaemolyticus* densities were determined at 0, 3, 6, 12, and 24 h and then daily for a 2-week period. In these experiments the water was or was not membrane filtered (pore size, 0.22 μm; Millipore Corp., Bedford, Mass.), supplemented with sewage, and seeded with *V. parahaemolyticus* cells to initial densities of about 500/100 ml. The *V. parahaemolyticus* strain used in each experiment was isolated from the station which was the source of the water. The inocula were prepared from Trypticase broth (1% Trypticase [BBL Microbiology Systems, Cockeysville, Md.], 0.2% yeast extract, 3.0% NaCl in 100 ml of distilled water) cultures grown overnight at 23°C and washed three times in phosphate-buffered saline (28). Seeded and unseeded experiments were also performed with raw sewage (1.0% vol/vol) from two additional plants, one of which had little industrial input at the time.

The effect of several different particulates on the growth of *V. parahaemolyticus* strains was examined both in the presence and absence of sewage. Kaneko and Colwell (12), in their study on the attachment of bacterial strains to chitin and copepods, reported that adsorption of *V. parahaemolyticus* cells onto these materials is adversely affected by high concentrations of salt and certain cations, salinities, and pH. Accordingly, estuarine water whose salinity was 10 ppt was collected from an area near station PR 1. The water was filtered, adjusted to pH 7.4, and divided into 200-ml portions which were supplemented with the various particulates and seeded with *V. parahaemolyticus* cells to initial densities between 10^3 and 10^4/ml.

The particulate materials tested were obtained, prepared, and added as follows. (i) Chitin (Sigma) particles between 110 and 1,000 μm in diameter were obtained by grinding the raw material with a mortar and pestle and then sieving it. (ii) Net zooplankton, collected as described above, were obtained from the same area as the water. The resuspended zooplankton were decanted into sterile estuarine water three times at 12-h intervals to remove sediment, detritus, and other inert particulates and then reconcentrated by filtration. (iii) "Dead" zooplankton were obtained by cold shocking the suspensions prepared in stage (ii) at 4°C for 8 h. (iv) The source of the suspension of phytoplankton, *Skeletonema costatum*, was a mass culture obtained from the Environmental Research Laboratory, Narragansett, R.I. (v) Sewage particles in secondary treated sewage were obtained by settling secondary treated sewage in Imhoff cones for 30 min and discarding the supernatants. (vi) Bottom sediments were obtained from grab samples collected at stations PR 2 and PR 4. (vii) Silica gel, 100 to 200 μm in size (Nutritional Biochemicals Corp., Cleveland, Ohio), was included as an adsorbent, insoluble platform for attachment. Each of the above particle suspensions except the zooplankton was washed three times in sterile estuarine water by preparing about 10% suspensions of the particles in the water, centrifuging the suspensions at 8,000 × *g* for 15 min, and resuspending the particles to the same volume. The washed suspensions were then added at about 5% (wet weight) (wt/vol) to a series of flasks in which the presence of sewage (1%, vol/vol) and seeding with *V. parahaemolyticus* were treated as variables. The flasks were then incubated at 23°C with gentle agitation. Small volumes (1 to 10 ml) of the test suspensions were removed periodically over a 10- to 14-day period and passed through a 77-μm mesh net. The particle-free filtrates were assayed for *V. parahaemolyticus* as described earlier.

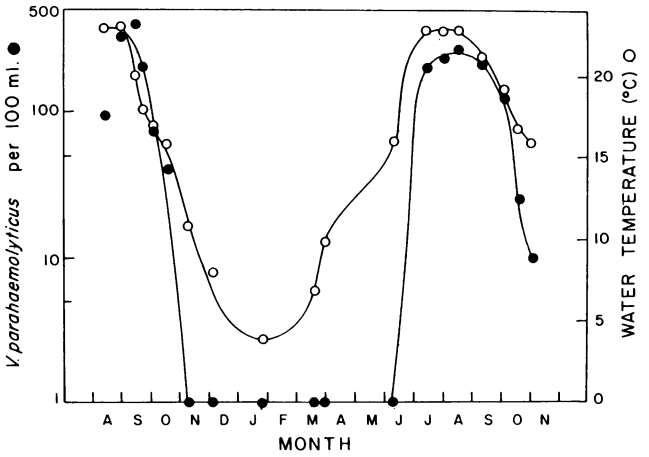

FIG. 2. Effect of water temperature on the incidence of *V. parahaemolyticus* at station PR 5.

RESULTS

Assays for *V. parahaemolyticus* at station PR 5 confirmed that the densities of this species in the water column are directly related to water temperature (Fig. 2). The levels of *V. parahaemolyticus* were maximal during a 2-month period from mid-July to mid-September, after which they declined steadily, as did the temperature. They were not detected in the water column from November, when the water temperature had fallen to 11°C, until the following July, when the temperature had risen to 21°C. Accordingly, bay surveys and other sampling conducted in this study were performed during the summer period of peak *V. parahaemolyticus* densities.

A baywide survey revealed *V. parahaemolyticus* to be distributed throughout the Narragansett Bay estuary (Table 1). A portion of the colonies presumptively identified as *V. parahaemolyticus* by the mVP were picked, streaked for isolation, and tested to verify their identities (28); of these, 96% (339/352) were confirmed as *V. parahaemolyticus*. However, no Kanagawa-positive strains were detected, although two such isolates were obtained from estuarine waters elsewhere in Rhode Island.

In general, densities of *V. parahaemolyticus* decreased with increasing distance from the head of the bay, its major tributaries, and known sources of sewage pollution. The highest levels were found in the upper Providence River (stations PR 4 and PR 5), an area of major fluvial input heavily impacted by wastewater effluents. The lowest densities, less than 1/100 ml, were found near the mouth of the bay (station WC 4). Higher *V. parahaemolyticus* levels were observed in the areas subject to sewage or fecal pollution, that is, the upper Providence River (PR 1 through PR 8), East Greenwich Cove (EG 2), the Warren (WR 2) and Taunton (TR 1 to TR 4) Rivers, and Wickford Harbor (WH 1). Moreover, the densities of *V. parahaemolyticus*, particularly those in the Providence River (Fig. 3), were found to correlate significantly with those of two fecal indicators, *E. coli* and *C. perfringens*, highly with the third (enterococci), and only slightly with the numbers of viable heterotrophs (Tables 1 and 2).

The seemingly poor correlation of *V. parahaemolyticus*

TABLE 1. Bacterial densities determined during baywide surveys

Station[a]	Densities (per 100 ml) of:				
	V. parahaemolyticus	*E. coli*	Enterococci	*C. perfringens*	VHC[b] ($\times 10^4$)
PR 1	52	280	98	740	120
PR 2	52	198	90	600	80
PR 3	140	1,500	18	290	450
PR 4	495	2,300	130	1,700	110
PR 5	280	1,500	200	1,500	190
PR 6	70	160	2	420	550
PR 7	65	40	28	140	63
PR 8	25	21	11	50	70
PR 9	10	16	<1	20	8.6
PR 10	12	2	<1	16	9.0
PR 11	8	<1	2	22	2.0
WR 1	5	<1	ND[c]	3	ND
WR 2	135	74	1	21	66
EG 1	4	<1	<1	560	0.6
EG 2	120	<1	<1	58	13
EG 3	10	<1	<1	4	6.0
EG 4	10	<1	<1	1	1.2
EG 5	9	<1	<1	1	1.5
WH 1	29	110	15	49	37
WC 1	6	<1	<1	2	5.3
WC 2	11	<1	<1	3	8.1
WC 3	3	<1	<1	1	2.2
WC 4	1	<1	<1	2	4.1
TR 1	116	124	55	ND	2.3
TR 2	55	20	<1	ND	1.1
TR 3	80	60	42	ND	29
TR 4	38	1,440	81	ND	190
TR 5	12	120	1	ND	110
EC 1	4	1	2	8	8.1
EC 2	10	1	<1	1	6.0
EC 3	19	11	2	8	8.1
Correlation coefficient[d]		0.81[e]	0.70[e]	0.85[e]	0.30

[a] Abbreviations: PR, Providence River and Upper Narragassett Bay; WR, Warren River; EG, East Greenwich Cove; WH, Wickford Harbor; WC, West channel, lower bay; TR, Taunton River and Mount Hope Bay; EC, East channel, lower bay.
[b] VHC, Viable heterotrophs.
[c] ND, Not determined.
[d] Dependent variable as correlated to *V. parahaemolyticus* densities.
[e] $P < 0.05$.

densities with *E. coli* and *C. perfringens* densities in East Greenwich Cove (Table 1; Fig. 4) is explained by the conditions of sewage discharge into the area (a highly chlorinated, tertiary effluent discharged into a relatively small body of water). Since *E. coli* but not *C. perfringens* organisms were killed by chlorination of the effluent, *E. coli* strains were not detected in the cove, whereas the densities of *C. perfringens* strains decreased with increasing distance from the outfall. Furthermore, the chlorine levels in the immediate vicinity of the outfall (EG 1) were so high that both the *V. parahaemolyticus* and viable heterotroph densities were depressed. Less than 1 km seaward (station EG 2), densities of both *V. parahaemolyticus* and heterotrophic bacteria were elevated, presumably because the chlorine was diluted and dissipated in the intervening distance.

V. parahaemolyticus densities correlated highly with the turbidity of the water and moderately with its dissolved oxygen, dissolved organic carbon, and zooplankton content (Table 2). Poor to slight correlations with the nitrogen, phosphorous, and phytoplankton parameters and with the depth, salinity, and pH of the water were obtained. No

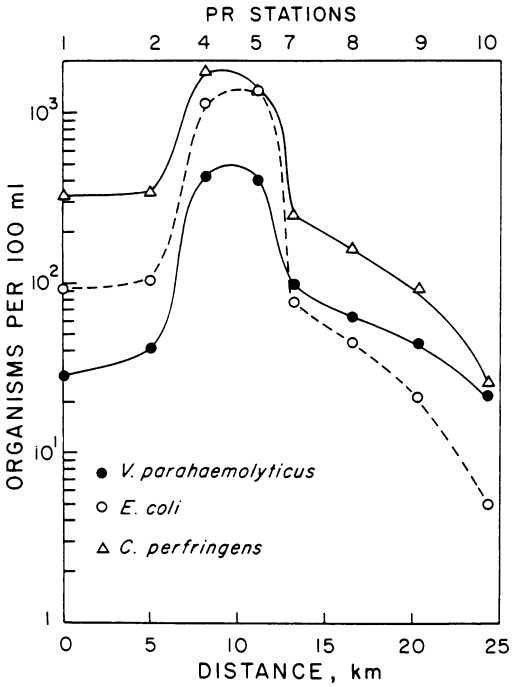

FIG. 3. Relationship of *V. parahaemolyticus* densities to those of *E. coli* and *C. perfringens* at selected Providence River stations.

correlation with temperature was expected since sampling was conducted by design over a restricted range.

Since *V. parahaemolyticus* is thought by some to overwinter in the sediments (10) and because of the correlations obtained with turbidity and zooplankton, samples obtained from several stations at various depths were assayed to determine the distribution of *V. parahaemolyticus* strains in the water column. Densities of *V. parahaemolyticus* were lowest in waters at or near the benthic region and greatest in the upper layers of the water column (Table 3).

Some additional support for the premise that *V. parahaemolyticus* multiplies in the water column was obtained by comparing its density with that of the conservative tracer, *C. perfringens* spores. *V. parahaemolyticus*/*C. perfringens* and *E. coli*/*C. perfringens* ratios were calculated from density data presented in Table 1, and these ratios were plotted

TABLE 2. Correlation coefficients for *V. parahaemolyticus* density versus physical, chemical, and biological parameters

Parameter[a]	r^b	Parameter[c]	r
E. coli density	0.81[d]	Kjeldahl N	0.09
Enterococcus density	0.70[d]	NH_3	0.11
C. perfringens density	0.85[d]	$NO_3^- + NO_2^-$	0.06
Viable heterotrophs	0.30	Total phosphorus	0.13
Station depth	0.09	P_i	0.20
Water temp	0.01	Dissolved organic carbon	0.53[d]
Salinity	0.27	Chlorophyll *a*	0.12
Dissolved oxygen	0.54[d]	Phytoplankton	0.22
pH	0.12	Net zooplankton	0.55[d]
Transparency	0.80[d]		

[a] For entire bay.
[b] Correlation coefficients from regression analyses.
[c] For Providence River and Upper Narragansett Bay stations.
[d] Probability values, $P \leq 0.05$.

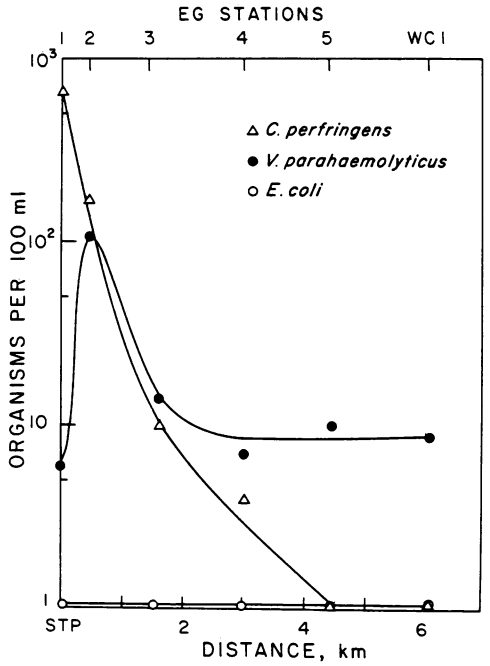

FIG. 4. Relationship of *V. parahaemolyticus* densities to those of *E. coli* and *C. perfringens* at East Greenwich stations and WC 1.

against the distance seaward from station PR 1 (Fig. 5). The *E. coli*/*C. perfringens* ratios increased sharply from stations PR 1 to PR 4, reflecting the relationship of the two indicators in the sewage effluents discharged between stations PR 4 and PR 5. Thereafter, the ratio decreased sharply, presumably reflecting the differential die-off of *E. coli*. By contrast, the *V. parahaemolyticus*/*C. perfringens* ratios increased steadily from stations PR 1 through PR 10, suggesting the multiplication of *V. parahaemolyticus* in the water.

The field studies clearly showed an association of *V. parahaemolyticus* densities with sewage pollution and suggested that the association was an indirect one mediated by some particulate in the water column, probably zooplankton. This was pursued further in the laboratory investigations. No increases in the density of native or seeded *V. parahaemolyticus* were observed in water samples collected from stations PR 1, PR 4, or PR 11 whether or not they were supplemented with various concentrations of raw or secondary treated sewage. On the contrary, the *V. parahaemolyticus* densities gradually decreased with time, and the addition of sewage did not appear to reduce the decay rates. Similar results were obtained with the filtered, seeded water samples (Fig. 6); within the limits of the methods used, neither the action of bacteriophages nor that of bdellovibrios accounted for the decreases.

Additional experiments were performed in which various particulates were added to filtered seawater collected near station PR 1 and treated as described above. The particulates added were silica gel, the alga *S. costatum*, sewage particulates, bottom sediments, net zooplankton, cold-shocked (4°C for 8 h) net zooplankton, and chitin. With all

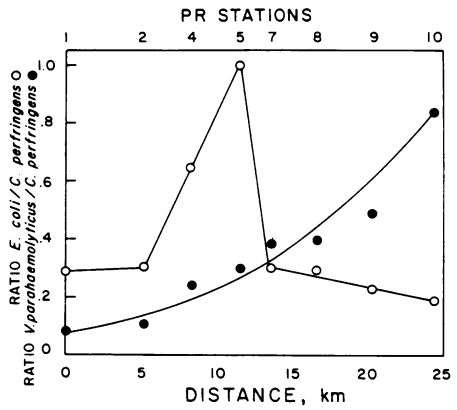

FIG. 5. Relative changes in *V. parahaemolyticus* and *E. coli* densities in the Providence River and Upper Narragansett Bay.

TABLE 3. *V. parahaemolyticus* densities at various depths in the Narragansett Bay estuary

Station	Station depth (m)	Sample depth (m)	*V. parahaemolyticus*/ 100 ml
EG 2	2.7	0.5	120
		2.0	32
EG 4	4.0	0.5	10
		3.0	<1
PR 2	2.7	0.5	30
		2.0	7
PR 4	12.2	0.5	610
		3.0	553
		6.0	180
		9.0	45
		11.0	27
PR 5	12.2	0.5	510
		3.0	523
		6.0	112
		9.0	16
		11.0	20
PR 7	12.2	0.5	315
		6.0	93
		11.5	14
PR 11	10.7	0.5	35
		10.0	<1

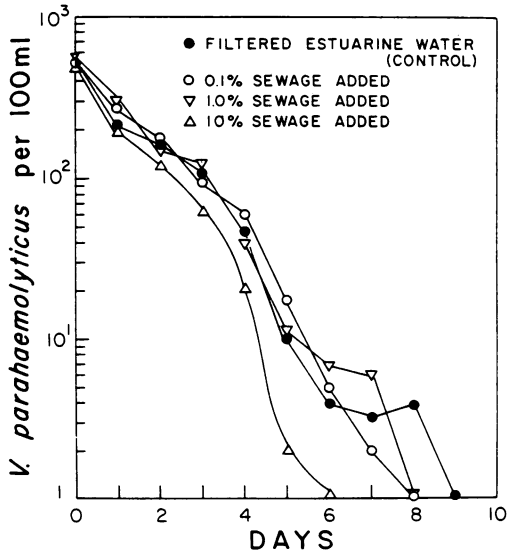

FIG. 6. Decay of seeded *V. parahaemolyticus* densities in filtered estuarine waters (salinity, 8.2 ppt), with and without added secondary sewage.

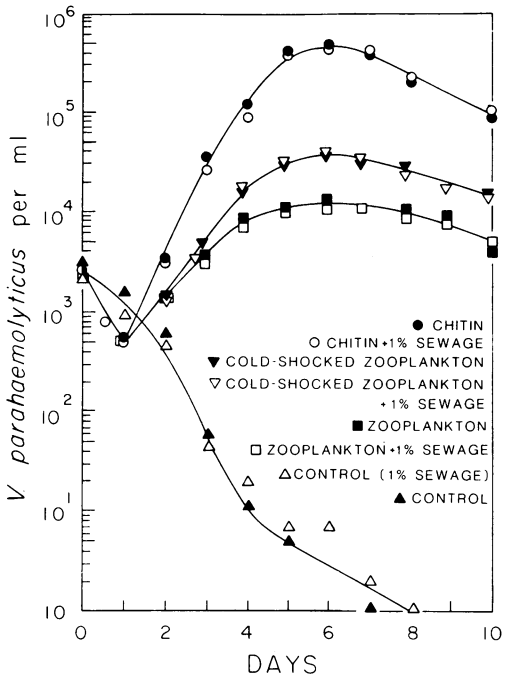

FIG. 7. Effects of zooplankton, cold-shocked zooplankton, and chitin with and without secondary sewage on seeded *V. parahaemolyticus* densities in filtered estuarine water (10 ppt).

but the last three particulates, decreases rather than increases in the particle-free *V. parahaemolyticus* densities were observed. In the flasks containing chitin, net zooplankton, or the cold-shocked zooplankton (Fig. 7), there was an initial drop in the particle-free *V. parahaemolyticus* densities, followed by multiplication to yield a maximum density within 5 to 6 days. There was no evidence that supplementation with sewage influenced the growth of the organism.

DISCUSSION

Results of the field studies categorically show an association of *V. parahaemolyticus* densities with fecal pollution in the Narragansett Bay estuary and suggest an association with the particulate matter in the water. The greater correlation of *V. parahaemolyticus* densities with net zooplankton rather than with phytoplankton levels in the bay is consistent with the findings of other investigators (10) and suggests a more direct role of the zooplankters. This role was confirmed in laboratory studies when the growth of *V. parahaemolyticus* was observed only when zooplankters or chitin was added. Furthermore, neither the decay nor the increase in *V. parahaemolyticus* densities in the laboratory investigations was affected by the addition of sewage. Thus, the data indicate that the effect of sewage on *V. parahaemolyticus* densities in Narragansett Bay is an indirect one, commencing with nutrient loading, probably mediated through the food chain, and manifested at the zooplankton level.

The higher densities of *V. parahaemolyticus* found in the near-surface waters throughout the upper bay suggests a relationship to light. The growth of *V. parahaemolyticus* on at least some portion of the net zooplankton fraction provides an explanation. Generally, a greater incidence of primary producers occurs in this region of the water column, and these constitute the major food sources for certain zooplankters, including copepods (15). Thus, nutrients in wastewater effluents resulting in biostimulation of phytoplankton may indirectly increase the zooplankton levels and, hence, *V. parahaemolyticus* densities.

Neither the design nor the results of this study address the overwintering of *V. parahaemolyticus* in the sediment as proposed by other investigators (10). However, no growth was observed in the laboratory experiments with bottom sediments, and the distribution of *V. parahaemolyticus* found in the water column gave no indication that *V. parahaemolyticus* grows in the benthic region and is carried to the surface along with chitin, detritus, or zooplankters to which it adheres. On the contrary, the data indicate that, during the summer, *V. parahaemolyticus* multiplies in the water column, particularly in the near-surface region, in association with zooplankton and perhaps other suspended substrata. Thus, overwintering may be exactly what happens. That is, there is survival, not multiplication, in the sediments, not only during the winter but also during the summer.

The pathogenicity of *V. parahaemolyticus* for humans is well known, and the waterborne transmission of the organisms to man via shellfish consumption and recreational activities has been documented. From a practical point of view, the data presented in this study might suggest health effects criteria for nutrient loading to restrict the densities of *V. parahaemolyticus* in the water, even though the relationship is an indirect one. However, no enteropathogenic (Kanagawa-positive) isolates of this bacterium were found in the course of this investigation, and relatively few have been detected in other field studies (25–27). Thus, it is evident that the requirement for and usefulness of such criteria are unsubstantiated until such a time as the genetic, ecological, and epidemiological relationships of Kanagawa-positive to Kanagawa-negative *V. parahaemolyticus* strains have been established.

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