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Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay

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A study of the ecology of *Vibrio parahaemolyticus* and related vibrios in the Rhode River area of Chesapeake Bay was carried out over the period December 1970 through August 1971. The incidence of *V. parahaemolyticus* and related vibrios was found to be correlated with water temperature. The vibrios could not be detected in the water column during the winter months, although they were present in sediment. From late spring to early summer, when water temperatures were 14 ± 1 C, vibrios over-wintering in sediment were released from the bottom communities and attached to zooplankton, proliferating as the temperature rose. The number of vibrios in and on plankton was reflected in the water column bacterial population densities at water temperatures of ca. 19 C. Thus, temperature of the water column in the range of 14 to 19 C was found to be critical in the annual cycle of the vibrios. Interaction between sediment, water, and zooplankton was found to be essential in the natural estuarine ecosystem. Bacterial counts of zooplankton were found to be temperature dependent. The bacterial population associated with zooplankton was found to be predominantly on external surfaces and was specific, differing from that of the sediment. *Vibrio* spp. and related organisms comprised the total bacterial population associated with zooplankton in summer months. The ecological role of *Vibrio* spp., including *V. parahaemolyticus*, was found to be significant, with respect to their property of chitin digestion and in relation to the population dynamics of zooplankton in Chesapeake Bay.

More than 70% of the cases of food poisoning in Japan are caused by ingestion of seafood, i.e., fish and shellfish, contaminated with *Vibrio parahaemolyticus* (27). The incidence of food poisoning in Japan caused by this organism is restricted to the summer months, very likely because of sensitivity of the organism to low temperatures. *V. parahaemolyticus* was implicated in only a single reported outbreak of food poisoning in the United States until recently, when food poisoning associated with ingestion of infected crab meat occurred in Maryland during the summer of 1971 (Maryland State Department of Health, *personal communication*).

Historically, *V. parahaemolyticus* was first isolated in 1950 from a major food poisoning outbreak traced to ingestion of "Shirasu" (partially boiled juvenile sardines) (7). Despite extensive studies of the distribution of this organism in the marine environment, the natural habitat of *V. parahaemolyticus* is not fully known, and whether *V. parahaemolyticus* is truly of marine origin remains to be determined (12). A number of papers describe the distribution and isolation of *V. parahaemolyticus*, and it is generally accepted that the incidence of *V. parahaemolyticus* is highest in estuarine or coastal areas of the world oceans. Many workers have isolated *V. parahaemolyticus* and related organisms from seawater, sediment, and marine animals, viz., fish, shellfish, and plankton, from coastal or neritic water (8, 10, 11, 13, 21, 22, 23, 24, 25, 31, 33) and from the open sea (1, 2). The first isolation of *V. parahaemolyticus*-like organisms in the United States, from Puget Sound and Washington coast sediments (19), was subsequently confirmed (3). *V. parahaemolyticus* was identified as the causative agent in mortalities of blue crabs in Chesapeake Bay (16) and gulf shrimp (32) and has been isolated from seawater and sediment collected in New Hampshire (5).

Most of the isolations of *V. parahaemolyticus*

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cited above were limited to the months of April through October, with recovery of the organism occurring predominantly during the summer months. Apparently great difficulty is encountered in the isolation of *V. parahaemolyticus* during the winter months, although Shimidu (H. Shimidu and H. Matsuno, Jap. J. Bacteriol. **16**:840, 1961) isolated *V. parahaemolyticus*-like organisms from fish during the winter, and Asakawa (Y. Asakawa, S. Akabane, and M. Noguchi, Jap. J. Pub. Health, **13**:158, 1966) isolated *V. parahaemolyticus* from fish and shellfish, at a very low incidence, also during the winter when water temperatures were ca. 6 to 7 C.

It has been suggested that *V. parahaemolyticus* can survive the winter in sediment, in scavenger fish (24), and shellfish, but the numbers of organisms isolated are small. In general, failure to isolate *V. parahaemolyticus* in winter months is a common experience. Thus, the important questions remain, namely, how does *V. parahaemolyticus* survive over winter and what is its ecological role in nature?

In our study the seasonal incidence of *V. parahaemolyticus* was followed. The goals were to determine the ecological niche of *V. parahaemolyticus* in the natural environment and its habitat and role in the natural cycles of plankton, water, and sediment, and to investigate the mechanism(s) whereby this microorganism survives the winter.

MATERIALS AND METHODS

Sampling. Monthly sampling was carried out from December 1970 through August 1971 at the Rhode River in Chesapeake Bay. Bottom sediment in the Rhode River consists of a thick, black mud at the mud-water interface to 10 to 15 cm. Below 15 cm the sediment is composed of a greenish-black clay. The average depth of the Rhode River area sampled is 2 to 3 m.

A reversing thermometer was used to measure surface and bottom water temperatures. During the coldest months, ice forms over the surface water to a 2- to 3-cm thickness, with the surface water temperature fluctuating from −1 C in February to 31 C in July.

Salinity was determined as described in the *Manual of Seawater Analysis* (30). A 4% minimum salinity was observed in April and May, due to fresh water run-off, and 12% maximum in July 1971.

Water samples were collected at a depth of 50 cm by aseptic techniques. A core sampler fitted with autoclavable core liners (Wildlife Supply Company, Saginaw, Mich.) was used to collect sediment samples.

Dissolved oxygen was determined by using a YSI oxygen meter, and turbidity with a Secchi desk. All samples were brought back to the laboratory for bacteriological examination within 5 hr after collection.

A no. 20 plankton net (77-μm mesh size) was employed in collecting plankton. Plankton populations comprised adult copepods, with low levels of juveniles and eggs, in the winter months, whereas mature adult copepods, juveniles, and eggs were equally represented in samples collected in the spring months. In early summer, the percentage of mature adult forms decreased, with juvenile and egg stages predominant, and in mid-summer the population was composed of juveniles, with only a few small-sized adults observed.

After collection, water and plankton samples were homogenized by blending for 10 min in a Sorvall Omnimixer. Plankton samples were homogenized in a salts solution consisting of 2.4% NaCl, 0.7% $MgSO_4 \cdot 7H_2O$, 0.07% KCl, 0.53% $MgCl_2 \cdot 6H_2O$, *p*H 7.6 ± 0.2. Sediment samples were weighed and diluted with the sterile salts solution and mixed for 10 min with a magnetic stirrer. All bacterial counts were determined by the most probable number method.

For total viable counts, YE medium (0.3% yeast extract, 1.0% peptone, 0.5% sodium chloride, *p*H 7.2 to 7.4) and SWYE medium (0.3% yeast extract, 1.0% peptone, salts solution [*see above*], *p*H 7.2 to 7.4) were employed. Inoculated media were incubated at 25 C for 48 hr. Salts solution was used as diluent in all serial dilutions.

V. parahaemolyticus was isolated on TCBS medium (BBL, Division of Bioquest, Cockeysville, Md.), which contains 0.5% yeast extract, 1% polypeptone peptone, 1% sodium citrate, 1% sodium thiosulfate, 0.5% oxgall, 0.3% sodium cholate, 2% sucrose, 1% sodium chloride, 0.1% iron citrate, 0.004% thymol blue, 0.004% bromothymol blue, and 1.4% agar, *p*H 8.6 ± 0.2. SWYE tubes showing growth were transferred to TCBS agar plates, and the plates were incubated at 37 C for 24 to 48 hr after inoculation. Colonies appearing on TCBS plates were *Vibrio* spp. (VLO), and "typical" green colonies on TCBS agar were *V. parahaemolyticus* (VPLO) in the presumptive identification procedure followed. The "presumptive" identification is as used in coliform counts. *V. parahaemolyticus* is applied to those organisms with the following characteristics: gram negative; positive motility; good growth in media containing 3% and 7% NaCl; no growth or slight growth at 0 and 10% NaCl; growth at 43 C; ctyochrome oxidase positive; acid formation in glucose under aerobic and anaerobic conditions; negative sucrose fermentation; lactose negative; no H_2S produced in triple sugar iron medium; Voges-Proskauer test negative; indole positive; nitrate reduced; gelatin liquefied; starch hydrolyzed under both aerobic and anaerobic conditions; catalase positive; methyl red positive; lysine decarboxylase positive; mannitol positive; beta-hemolysis on human blood agar; and bioluminescence negative.

Lactose broth tubes incubated at 37 C for 48 hr were used for coliform counts, and positive lactose broth (Difco Laboratories, Detroit, Mich.) tubes were transferred to EC broth (Difco) for enumeration of *E. coli* type I. EC broth tubes were incubated at 45.5 ±

0.5 C for 48 hr in a water bath. Numerical taxonomy, deoxyribonucleic acid base composition determinations, serological analyses (K and O typing), and gas chromatography of fatty acids and phospholipids of the *V. parahaemolyticus* isolates have been done, and results of this aspect of the study will be published in a subsequent report from this laboratory.

Since plankton, detritus of plankton, and other particulate matter (greater than 77-μm diameter) were present in the water column, 100-ml water samples were collected and filtered by using a sterilized plankton net. Bacterial counts of the filtrate were compared with counts of unfiltered water. In this set of experiments, water samples were homogenized before inoculation into YE, SWYE, and lactose broth media.

To examine the mechanism of bacterial association with zooplankton, bacteria on the external surface of the zooplankton were killed by treatment for Formalin. Several concentrations and time intervals were used to establish effective concentration. After Formalin treatment, the zooplankton was washed well in sterile salts solution, homogenized, and examined for total viable bacteria by the most probable number method. Bacterial counts obtained after Formalin treatment were considered to derive from bacteria located inside the plankton. Thus, the percentage of bacteria of internal association could be estimated. Standard error for these determinations was calculated, and the bacterial counts reported represent an average for at least three, usually five, analyses per sample.

RESULTS

Total viable counts of the water column were found to fluctuate between 10^4 and 10^6 bacteria per 100 ml throughout the year (Fig. 1). *Vibrio* spp. (VLO), however, were related to water temperature, i.e., in mid-winter ca. 10^2/100 ml were observed at water temperatures to 6 C, the number of vibrios gradually increasing between 14 and 19 C, to stabilize at water temperatures of 20 to 30 C. Maximum *Vibrio* spp. counts, ca. 10^4/100 ml, were obtained in August.

Counts of *V. parahaemolyticus* (VPLO) were below detectable levels until the middle of April, when water temperatures rose to ca. 14 C, with a marked increase noted when the water temperature rose 5 degrees, to 19 C. *V. parahaemolyticus* (VPLO) counts were maximal (6.2×10^3/100 ml) in July when the water temperature was highest (31 C). Isolation of *V. parahaemolyticus* from water samples was difficult until early June, that is, until the temperature of the water column reached 19 C. Increased *V. parahaemolyticus* counts were obtained when water temperatures were greater than 20 C, but even in July, the peak month of incidence, *V. parahaemolyticus* counts were only 3.4×10^2/100 ml. Thus, counts of *Vibrio* spp. and *V. parahaemolyticus* were directly related to water temperature, and a temperature of 14 to 19 C was found to be critical for the appearance of *V. parahaemolyticus* in the water column in late spring or early summer.

If water samples were filtered, with a no. 20 plankton net, a difference in bacterial counts between filtered and unfiltered water was easily detected (Table 1). Most of the water samples, especially those containing *V. parahaemolyticus* (VPLO), yielded low bacterial counts where the bacteria occurred predominantly as free cells in the water column. One exception was noted, in the case of a *V. parahaemolyticus* count obtained for sample no. 2 (see Table 1). More than 80% of *V. parahaemolyticus*, as well as *Vibro* spp. (VLO) and those organisms closely related to *V. parahaemolyticus* (VPLO), were associated with plankton, plankton detritus, or other particulate matter trapped during plankton hauls. Conversely, coliforms were not found to be associated with the plankton or detritus but existed as free cells in the water column.

Sediment samples showed relatively constant bacterial counts (total viable counts) of 10^6/g throughout the year (Fig. 2). However, counts of *Vibrio* spp. (VLO) in the sediment were found to be influenced by temperature. That is, counts of *Vibrio* spp. (VLO) in sediment through the winter were in the range of 10^2/g when the bottom temperature was ca. 6 C. Counts increased until the bottom water temperature reached 20 C; above 20 C, sediment counts were relatively constant, with maximum counts of ca. 7.5×10^4/g obtained in August. Counts of *V. parahaemolyticus* (VPLO) in sediments were relatively constant over the winter (ca 10/g) and represent a minimum overwintering population of this organism. With a sharp rise in the bottom water temperature, VPLO repopulation rate of increase was very rapid, with a leveling off at 20 C. VPLO counts increased slightly over the summer months, with maximum counts noted (6.6×10^4/g) in August at 27.5 C.

Sediment counts (Table 2) of typical *V. parahaemolyticus*, using a restricted definition of this species, were low when compared with total counts of other vibrios (VPLO), particularly during the winter months. For example, although *V. parahaemolyticus* strains can survive in sediments, the numbers of *V. parahaemolyticus* reported for samples taken during the winter months was less than 1/g. At 28.5 C, *V. parahaemolyticus* counts were 5.7×10^3/10 g. The high counts of *V. parahaemolyticus* obtained in the summer months decreased slowly during the fall, in

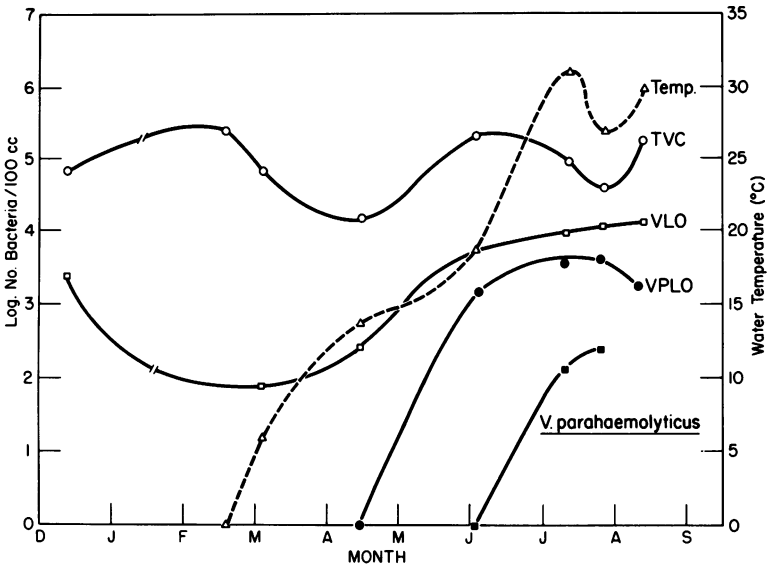

FIG. 1. *Bacterial counts/100 ml of water showing relationship of the incidence of Vibrio spp. (VLO), organisms closely related to Vibrio parahaemolyticus (VPLO), and V. parahaemolyticus strains with temperature. VPLO appeared in the water column at 14 C and V. parahaemolyticus at 19 C.*

TABLE 1. *Effect of filtration on bacterial counts of water samples (100 ml)*

Sample no.[a]	Total viable count		VLO	VPLO	*V. parahaemolyticus*	Coliform	*E. coli*
	YE[b]	SWYE[b]					
1 A[c]	4.6×10^3	4.6×10^3	4.6×10^2	9.3×10	9.1	2.3×10	4.0
B[c]	2.4×10^5	4.6×10^4	2.4×10^3	2.4×10^3	1.5×10^2	2.3×10	4.0
2 A	1.5×10^5	4.6×10^4	4.6×10^3	1.5×10^3	2.0×10^2	2.3×10	0
B	4.6×10^5	2.4×10^5	2.4×10^4	1.1×10^4	1.4×10^2	2.3×10	0
3 A	4.6×10^4	1.1×10^4	1.1×10^3	4.6×10^2	1.1×10	7.5×10	7.5×10
B	2.4×10^5	1.5×10^5	2.4×10^4	1.1×10^4	1.5×10^2	4.3×10	2.3×10

[a] All samples were taken 8 July 1971.
[b] For composition of media, see Materials and Methods.
[c] A, Each water sample was filtered through a plankton net (77-μm opening), and the filtrate was homogenized and counted. B, The water samples were homogenized and counted without filtration.

parallel with dropping temperatures. Thus, *V. parahaemolyticus* survives the winter, but winter conditions are detrimental in terms of total viable population. A rise in temperature (to 15–20 C) was found to correlate with a population increase in Rhode River sediment, with the maximum population achieved during the summer months and the length of the winter also contributing to survival and repopulation.

Distribution with depth of *Vibrio* spp. (VLO), organisms closely related to *V. parahaemolyticus* (VPLO), and *V. parahaemolyticus* in sediment is shown in Table 3. VLO and VPLO decreased sharply with core depth, i.e., 3 to 26% at 3 to 6 cm. Total viable counts and coliform counts did not change with core depth.

Bacterial counts of plankton revealed a striking seasonal change which was quite different from that of the sediment and water column (Fig. 3). Total viable counts of plankton were 2.4×10^5/g between January and early March, when the water temperature was $\leq$ 6 C, and increased as the water temperature went up from 10 to 31 C. *Vibrio* spp. (VLO) counts also increased rapidly with water temperature, reaching a maximum population of 10^9 in July.

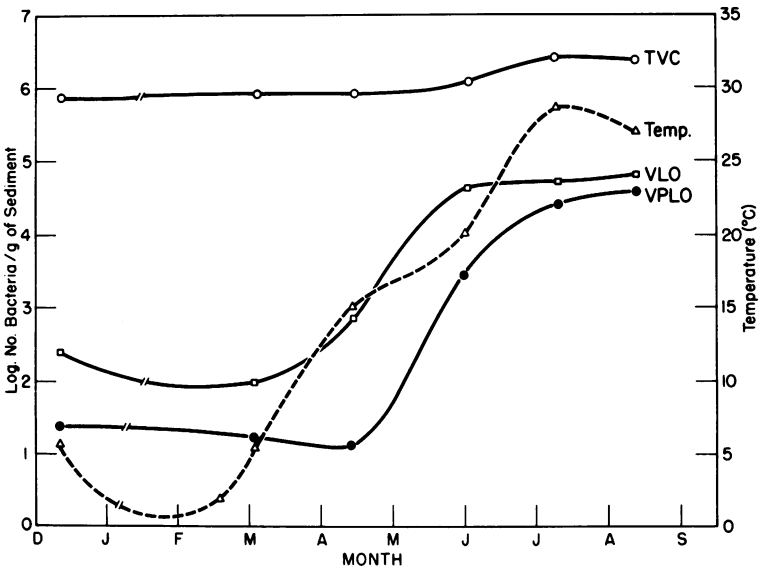

FIG. 2. *Changes in total viable count (TVC), numbers of Vibrio spp. (VLO), and organisms closely related to Vibrio parahaemolyticus (VPLO) and temperature, measured during the period December 1970 through August 1971. Survival of VPLO in sediments during the winter months is shown.*

TABLE 2. *Counts of V. parahaemolyticus in sediments (10 g)*[a]

Sampling date	No. of Samples	Range of counts	Mean of counts	Bottom temp (C)
12-11-70	4	2.0×10–3.3×10^3	7.5×10^2	6.0
3-3-71	3	5.2–6.3	5.8	6.0
4-13-71	2	7.7–9.9	8.8	14.9
6-1-71	3	4.7–6.5×10^2	3.3×10^2	20.4
7-8-71	3	7.9×10^2–1.5×10^4	5.7×10^3	28.5

[a] Sediments were taken from less than 3 cm core depth.

Nearly the entire total viable count of the plankton samples consisted of *Vibrio* spp. during the summer months. Thus, the association of *Vibrio* spp. with plankton was unequivocal.

The appearance of *Vibrio* spp. closely related to *V. parahaemolyticus* (VPLO) in association with plankton was observed in April, and strains of *V. parahaemolyticus* could be isolated at the end of April, when the numbers of *V. parahaemolyticus* increased with rising water temperature.

Results of experiments designed to measure external versus internal bacterial counts of zooplankton are given in Table 4. The number of bacteria located internally in the zooplankton was found to be constant, whereas external bacterial counts varied with temperature. Thus, a specific internal commensal bacterial flora for plankton was detected.

DISCUSSION

V. parahaemolyticus was first isolated during major food poisoning outbreaks associated with ingestion of seafish, and many surveys of its distribution have been done. *V. parahaemolyticus* and related halophilic vibrios were isolated from the marine environment by Nagao (23) and others (21, 22, 24). However, isolation of the typical *V. parahaemolyticus* during winter months is uncommon, and, in those instances where *V. parahaemolyticus* was reported to have been isolated during the winter months, a very low rate of occurrence was noted.

Shimidu and Matsuno (Jap. J. Bacteriol., **16**:840, 1961) isolated *V. parahaemolyticus*-like organisms during the winter and suggested that *V. parahaemolyticus* survives the winter in shellfish and fish (24) despite *V. parahaemolyticus* being mesophilic and not growing below 10 C (9). Factors besides temperature which affect growth of *V. parahaemolyticus* in the natural environment are not yet documented, nor is the cause of the apparent restriction of *V. parahaemolyticus* to coastal waters understood.

During summer months, the incidence of *V. parahaemolyticus* in plankton samples has been found to be higher than in seawater or sediment (8, 15). *V. parahaemolyticus* and vibrios closely related to it are chitinoclastic (6, 33), and the

TABLE 3. *Influence of core depth on bacterial counts*

Sample no. (date)	Core depth (cm)	Total viable count		VLO	VPLO[a]	Coliform
		YE	SWYE			
1	0-3	4.5×10^5	5.0×10^5	1.2×10^3	5.5×10^2	1.2×10^2
(12-11-70)	3-6	3.6×10^5	3.2×10^5	7.2×10	2.8×10	3.3×10^2
2	0-3	1.0×10^4	4.6×10^5	1.9×10^2	4.6×10	1.9×10^2
(3-3-71)	3-6	3.6×10^5	7.9×10^5	4.9×10	7.9	7.9×10
3	0-3	3.4×10^5	7.4×10^5	3.4×10^4	1.4×10^3	1.4×10
(6-1-71)	3-6	1.7×10^6	4.1×10^6	1.7×10^3	4.4×10	8.9×10
	6-9	3.7×10^5	5.4×10^5	3.7×10^2	7.1	3.7×10

[a] Including *V. parahaemolyticus*.

association of chitinoclastic bacteria with zooplankton has been reported (18, 28).

In the Rhode River the heterotrophic, aerobic bacterial flora associated with plankton was found to be specific (Table 5), with *Vibrio* spp. comprising the dominant portion of the total viable count. In midsummer, *Vibrio* spp. comprised 100% of the total viable count; organisms closely related to *V. parahaemolyticus* comprised 6.5% of the *Vibrio* spp. count, and *V. parahaemolyticus* 9.5% of those organisms identified as closely related to *V. parahaemolyticus*. The association of *Vibrio* spp. with plankton suggests that these organisms play an important role in the cycling of elements in the marine environment, most probably via mineralization of the copepod.

Several investigators have shown that the bacterial flora of plankton is not the same as the flora of the environment where the samples are taken (18, 28). Shimidu (29) reported that $\geq$ 70% of the heterotrophic bacteria isolated from plankton were *Vibrio* and *Aeromonas*. Our results show that *Vibrio* spp. comprise nearly 100% of the total viable count of plankton in midsummer months, dropping to $\leq$1.0% in the winter in parallel with seasonal temperature changes. Furthermore, a specific bacterial flora was found to be internally located in the plankton.

Plankton surfaces are coated with a slimy exudate to which bacteria attach and take metabolic products as a ready source of food and energy (35). If, in addition, bacteria associated with plankton are chitinoclastic, they are thereby capable of decomposing plankton and recycling organic matter comprising the plankton. Our studies indicate that *Vibrio* spp., including *V. parahaemolyticus*, occurring as free cells in the water column derive from release of bacteria from plankton during the

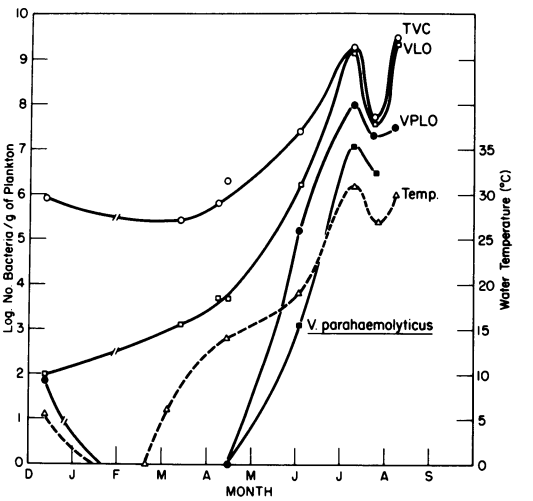

FIG. 3. *Bacterial counts per gram of plankton, showing seasonal changes in the bacterial flora of plankton. Organisms closely related to V. parahaemolyticus (VPLO) and V. parahaemolyticus strains appeared simultaneously in and on plankton when the water temperature reached 14 C.*

mineralization process (see Table 5). *V. parahaemolyticus* reappearing in the water column in early June are released into the water column after decline and decomposition of the plankton bloom.

Estuarine water contains a higher concentration of organic matter than does the open ocean, resulting from high productivity and river flow from land (14, 17, 26). If an estuary is, in addition, heavily polluted with raw sewage, selection of bacterial types will occur. From our studies *V. parahaemolyticus* counts were not found to correlate with *E. coli* counts, contrary to reports of other investigators. Oshiro (25) reported 10 to 20 *V. parahaemolyticus*/ml in a heavily polluted area in the Seto Inland Sea of

TABLE 4. *Enumeration of the bacterial population associated with plankton*

Sample no.	Date	Association	Total viable count		VLO	VPLO	*V. parahaemolyticus*
			YE	SWYE			
1	7-8-71	Internal only	8.8×10^4	2.1×10^6	7.6×10^5	6.9×10^4	1.0×10^2
2	7-8-71	Internal only	5.2×10^4	1.0×10^6	1.0×10^6	1.0×10^6	4.6×10
3	7-22-71	Internal only	4.3×10^4	8.3×10^5	8.3×10^5	8.3×10^5	6.5
4	7-22-71	Internal only	2.4×10^4	2.4×10^6	2.4×10^6	2.4×10^6	2.4×10^2
5	7-8-71	Total count[a]	$>1.9 \times 10^8$	$>1.9 \times 10^9$	1.9×10^9	2.7×10^8	1.3×10^7
6	7-8-71	Total count[a]	$>1.9 \times 10^8$	1.9×10^9	1.9×10^9	1.4×10^7	1.4×10^7
7	7-22-71	Total count[a]	2.6×10^7	4.1×10^7	1.9×10^7	1.9×10^7	3.6×10^6
8	7-22-71	Total count[a]	1.2×10^8	6.0×10^7	6.0×10^7	2.8×10^7	5.3×10^5

[a] Total viable count of the total plankton mass, reported on a per gram (wet weight) basis.

TABLE 5. *Ratio of V. parahaemolyticus to VPLO, VLO, and total viable count (TVC)*

Sampling date	VLO/TVC (%)			VPLO/VLO (%)			*V. parahaemolyticus*/VPLO (%)			*V. parahaemolyticus*/TVC (%)		
	Water	Sediment	Plankton	Water	Sediment	Plankton	Water	Sediment	Plankton	Water	Sediment	Plankton
12-11-70	2.5	<0.1	<0.1	0	0.6	69.5	0	35.7	100.0	0	<0.001	<0.1
3-3-71	<0.1	<0.1	—[a]	0	19.0	—	0	10.5	—	0	<0.01	—
3-12-71	—	—	0.5	—	—	0	—	—	—	—	—	—
4-8-71	—	—	3.7	—	—	0	—	—	—	—	—	—
4-13-71	1.4	0.6	0.2	0	7.1	0	0	3.0	0	0	<0.01	0
6-1-71	3.1	3.2	11.8	16.9	9.0	12.1	2.7	1.5	0.7	0.16	0.01	0.01
7-8-71	10.4	3.1	100.0	47.6	53.0	6.5	3.0	2.3	9.5	0.15	0.04	0.62
7-22-71	30.9	—	73.0	51.3	—	74.0	9.1	—	7.8	1.4	—	4.2
8-11-71	9.3	4.1	100.0	6.4	70.0	3.9	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]

[a] Dashes indicate no data.
[b] In progress.

Japan. Horie (13) reported 20 to 100/ml in Tokyo Bay, and higher counts of *V. parahaemolyticus* in the mouth of the river feeding into Tokyo Bay. Baross and Liston (4) observed ca. 10 to 5,000/ml in Puget Sound. Our data showed relatively low counts of *V. parahaemolyticus*, averaging less than 10/ml, but no correlation with *E. coli* counts.

The bacterial flora of Rhode River sediment was characteristic and differed from the plankton or water column. The sediment total viable count was constant, without seasonal change, although the components of the community did change from season to season. For example, *Vibrio* spp. comprised only a small portion of the sediment bacterial flora (Table 5). Heterotrophic bacteria constituted the major portion of sediment flora and are involved in decomposition and regeneration of organic matter in the upper sediment layers (34). The role of heterotrophic bacteria other than *Vibrio* spp. may be more important in the sediment and should be studied further. The sediment provides an extremely limited ecological niche for *V. parahaemolyticus*.

Overwintering is one consideration in tracing the epidemiology of *V. parahaemolyticus*. *V. parahaemolyticus* survives associated with, or in, shellfish and scavenger fish such as gobies which occur on the bottom, which is not altogether surprising since the benthos is always in contact with bottom microbial flora. Furthermore, shellfish are filter-feeding and concentrate bacteria. The number of bacteria in shellfish has been found to be correlated with ambient temperature (4). From the results of this study it is now clear that *V. parahaemolyticus* survives in sediment at low temperatures. A protective function, as yet undetermined, is provided by sediment which permits survival of *V. parahaemolyticus*. Asakawa observed that *V. parahaemolyticus* sur-

vived in tubes of 3% NaCl peptone water placed in sediment and exposed to seasonal environmental changes. Sensitivity of *V. parahaemolyticus* inoculated into fish homogenate to cold temperatures has been reported by Matches (20). However, sensitivity depends on age of culture and environmental factors including concentration of organic and inorganic matter. Our data and results of other investigators thus show that 10 C is a minimum temperature for growth of *V. parahaemolyticus* in the natural environment. However, at ≤15 C, growth of *V. parahaemolyticus* is markedly retarded (9).

There are several different types of estuaries, and the annual cycle of *V. parahaemolyticus* described herein for Chesapeake Bay may not hold for other estuaries. Nevertheless, the general relationships of bacterial flora, water, plankton, and sediment which have been described may well prove to be valid for the estuarine environment in general.

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