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Incidence of *Vibrio parahaemolyticus* in Chesapeake Bay

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A Bay-wide survey of the distribution of *Vibrio parahaemolyticus* was carried out in Chesapeake Bay during May 1972, to determine whether the annual cycle of *V. parahaemolyticus* which was observed to occur in the Rhode River subestuary of Chesapeake Bay took place in other parts of Chesapeake Bay. In an earlier study, April to early June, when the water temperature rises from 14 to 19 C, was found to be a critical period in the annual cycle of the organism in the Rhode River, since this is the time period when the annual cycle is initiated. Results of this study, however, revealed that *V. parahaemolyticus* could not be found in the water column during May 1972. Nevertheless, several samples of sediment and plankton yielded *V. parahaemolyticus* isolates. Comparison of data with those for the Rhode River area examined in the earlier studies of the annual cycle of *V. parahaemolyticus* suggests that the time of initiation of the annual cycle of *V. parahaemolyticus* in the open Bay proper may be influenced by various factors such as temperature and salinity, i.e., deeper water locations may show initiation of the *V. parahaemolyticus* annual cycle later than shallow areas. Confirmation of the presence of the organism in the samples studied was accomplished using numerical taxonomy with 19 reference strains also included in the analyses.

In an earlier study of the ecology of *Vibrio parahaemolyticus*, the annual cycle of this organism was established for the Rhode River subestuary of Chesapeake Bay (4). In the Rhode River, organisms surviving in the sediment during the winter are released into the water column, thereby becoming associated with the zooplankton, i.e., the copepod populations. These events take place from April to early June, when the water temperature rises to between 14 and 19 C. The total numbers of *V. parahaemolyticus* in the water column during this period are below detectable levels, but once the water temperature reaches 19 to 20 C, the organism reaches detectable levels as a result of its association with the zooplankton populations. Thus, the initial events in the annual cycle of *V. parahaemolyticus* are particularly important in the Rhode River area. In this study, a Bay-wide survey of the distribution of *V. parahaemolyticus* was undertaken in May 1972. The water temperature at the time of the sampling operation was ca. 16 to 22 C. The main purpose of the study was to find out whether the initiation of the annual cycle of the organism observed in the Rhode River subestuary occurred simultaneously in all other parts of the Bay.

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To confirm the identification of the *V. parahaemolyticus* isolates, numerical taxonomy was employed using a set of 19 reference strains, including *V. parahaemolyticus* strains isolated from various sources.

MATERIALS AND METHODS

Sampling. Bay-wide sampling to study the distribution of *V. parahaemolyticus* was accomplished 15 to 18 May 1972 aboard the R/V *Ridgely Warfield*, the Johns Hopkins University research vessel. A total of eleven stations were sampled (Fig. 1). The latitude and longitude for each station are cited by Kaneko (Ph.D. thesis, Georgetown Univ., Washington, D.C., 1973).

Sampling procedures. Water samples were collected with the Niskin sampler at 2 to 5 m below the surface and were transferred to presterilized glass bottles (250 to 500 ml).

Sediment samples were collected using a Shipek grab and were transferred to presterilized wide-mouth glass bottles (300 ml).

Plankton samples were collected using a #20 nylon plankton net with a 77-μm opening (Wildlife Supply Co., Saginaw, Mich.). The plankton net was towed through water just below the surface for 15 to 20 min. All plankton samples were transferred to sterile, wide-mouth glass tubes (300 ml).

Temperature and salinity were measured at both the surface and the bottom throughout the samplings, using a conductivity and temperature instrument developed by Johns Hopkins University (6). The pH and dissolved oxygen measurements were taken using

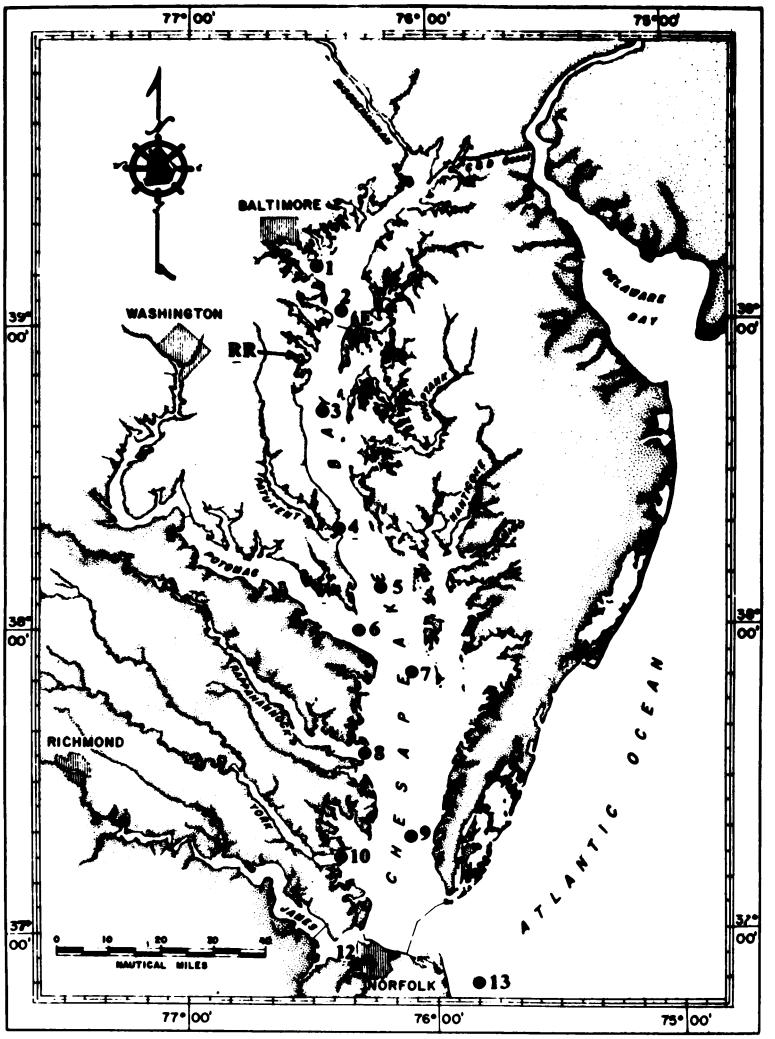

FIG. 1. *Stations, i.e., locations, sampled in Chesapeake Bay. Stations 1 to 13 comprised the R/V Ridgely Warfield cruise (May 1972). Areas: RR, Rhode River, E, Eastern Bay, C, Chester River, and W, Wicomico River. Stations were as follows: 1, Sparrow Point in Baltimore Harbor; 2, Sandy Point Light; 3, off Chesapeake Beach; 5, Point No Point Light; 6, mouth of Potomac River; 7, off Tangier Island; 8, mouth of Rappahannock River; 9, off Cape Charles City; 10, mouth of York River; 12, Elizabeth River (Norfolk Harbor); and 13, Cape Henry.*

a Corning portable pH meter, model 6, and the YSI model (Yellow Springs Instrument Co., Yellow Springs, Ohio) dissolved oxygen meter, respectively. Turbidity was measured using a Secchi disk.

Bacteriological analyses. All bacterial analyses were carried out on board as soon as possible after collection of the samples. Procedures for the determination of bacterial counts and the isolation and identification of *V. parahaemolyticus* have been described in a previous paper (4). However, in this study, the following criteria were employed in the identification of colonies appearing on TCBS agar, as a pragmatic approach to the problem. Colonies appearing on TCBS agar were regarded as presumptive vibrios (PV), and colonies which appeared as typical colonies of *V. parahaemolyticus* were regarded as presumptive *V. parahaemolyticus* (PVP). Thus, PV and PVP correspond to the terms "*Vibrio*-like organisms" (VLO) and "*V. parahaemolyticus*-like organisms" (VPLO) used in the previous paper (4). The end result, therefore, is that PV and PVP are used in the same sense as the "presumptive" test for *Escherichia coli*. Therefore, in a strict sense, this terminology has no taxonomic validity, but, for the working bacteriologist, offers the practical value of indicating those bacteria categorized into certain groups.

Numerical taxonomy. Substrate utilization tests

were employed and were basically as described by Stanier et al. (7). The basal medium employed in this study was composed of: NaCl, 2.4%; $MgSO_4 \cdot 7H_2O$, 0.7%; $MgCl_2 \cdot 6H_2O$, 0.53%; KCl, 0.07%; $NH_4H_2PO_4$, 0.05%; K_2HPO_4, 0.05%; and refined Ionagar (Difco), 1.5%. The pH of the medium was adjusted with tris(hydroxymethyl)aminomethane buffer to 7.2. A total of 154 organic compounds were tested as sole carbon source (Kaneko, Ph.D. thesis, Georgetown Univ., Washington, D.C., 1973). The number and kinds of substrate compounds employed were not exactly the same as those of Stanier et al. (7).

Sixteen strains isolated and identified as *V. parahaemolyticus* and 19 reference strains were employed in the numerical analyses. The reference strains were: *V. parahaemolyticus* SAK-7, SAK-23, FC 1011, Bainbridge 4203 and 10734; *V. ichthyodermis* NCMB 407; *V. anguillarum* ATCC 14181; *V. alginolyticus* ATCC 17749; *V. cholerae* ATCC 14035; *V. marinoprasens* ATCC 19648; *V. marinofulvus* ATCC 14395; *V. ponticus* ATCC 14391; *V. haloplanktis* ATCC 14393, *Beneckea nereida* ATCC 25917; *B. pelagia* ATCC 25916; *B. neptuna* ATCC 25919; *B. campbellii* ATCC 25920; *Photobacterium pierantonii* ATCC 14546; and *Lucibacterium harveyi* ATCC 14126.

Numerical analysis was done using the IBM 360/40 system, with disk and tape drives, located at the Georgetown University Computation Center. The programs used were GTP-1, -2, -3, -4, and -5 (Georgetown Taxonomy Programs 1-5), written by R. D'Amico and R. R. Colwell.

RESULTS

The environmental parameters included in the study are given in Table 1. The surface water temperature at all stations was between 16.2 and 21.6 C; the average temperature was 19.8 C. The bottom water temperature at all stations was between 11.9 to 20.4 C; the average was 14.1 C.

Salinity of surface and bottom water varied at the stations. The lowest salinity for surface and bottom water was recorded at station 1 in Baltimore Harbor, 1.6 and 6.5‰, respectively. The highest salinity, >23‰, was recorded at station 13, outside the Bay. In most cases, the difference in salinity between surface and bottom water was >4‰.

The bacterial counts of the water and sediment samples are given in Table 2. The total viable heterotrophic aerobic bacterial counts (TVC) at all stations were between 10^3 and 10^7 per 100 cc of water. Presumptive vibrio counts (PV) were relatively constant at ca. 10^3, with the exceptions noted at stations 8 and 12, where higher counts were recorded. In the case of PVP, four stations, 1, 8, 10, and 13, showed positive PVP counts. However, further work on the identification of these isolates showed that *V. parahaemolyticus* was not present. When TVC and PV counts at stations located along the

TABLE 1. *Environmental parameters measured during the R/V Ridgely Warfield cruise, May 1972*

Station	Depth (m)	Temp (C)	Salinity (‰)	pH	Dissolved O_2 (ppm)[a]	Turbidity (m)
1	14.6	S[b] 17.9	1.6	8.0	5.6	0.7
		B[c] 15.0	6.5	6.5	—[d]	
2	15.8	S 16.6	4.0	8.2	5.9	1.4
		B 12.3	12.2	7.0	—	
3	13.7	S 17.1	8.9	8.6	9.4	1.7
		B 16.1	9.4	8.2	—	
5	12.8	S 16.3	9.7	8.4	9.2	2.6
		B 13.9	13.8	7.4	—	
6	13.7	S 18.0	8.5	8.9	—	1.2
		B 14.7	12.5	7.3	—	
7	29.9	S 17.6	10.2	8.9	9.5	2.6
		B 15.3	16.9	7.8	—	
8	10.6	S 20.6	12.0	8.9	—	2.0
		B 16.9	14.9	8.3	—	
9	13.7	S 18.9	14.5	8.8	—	—
		B 16.2	21.3	8.2		
10	12.2	S 20.4	16.2	8.8	9.2	1.9
		B 16.9	20.2	8.5	—	
12	13.7	S 21.6	15.8	8.0	8.6	1.3
		B 20.4	16.0	7.7	—	
13	15.0	S 16.2	<23.0	7.9	9.0	2.3
		B 11.9	—	7.5	—	

[a] ppm = microliter/liter.
[b] S, Surface water.
[c] B, Bottom water.
[d] —, No data.

TABLE 2. *Bacterial counts of water and sediment samples collected in several areas of Chesapeake Bay during the R/V Ridgely Warfield cruise, May 1972*[a]

Station	TVC (SWYE)[b]		PV (25 C)		PVP (25 C)		*V. parahaemolyticus*	
	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
1	1.7×10^5	6.7×10^6	1.1×10^3	2.1×10^4	0.4×10	6.7×10	0.0	0.4×10
2	4.6×10^5	1.1×10^7	1.4×10^3	4.2×10^4	0.0	0.8×10	0.0	0.0
3	1.5×10^5	2.9×10^6	1.4×10^3	9.5×10^3	0.0	5.6×10	0.0	0.0
5	1.0×10^5	6.5×10^6	2.1×10^3	1.3×10^5	0.0	1.0×10^2	0.0	0.2×10
6	1.0×10^5	6.3×10^6	1.0×10^3	1.2×10^4	0.0	2.9×10^3	0.0	1.0×10
7	3.9×10^5	5.8×10^6	1.2×10^3	2.6×10^6	0.0	6.7×10^4	0.0	0.1×10
8	2.1×10^4	5.5×10^6	1.1×10^4	2.5×10^4	0.4×10	7.3×10^2	0.0	0.0
9	2.0×10^4	5.3×10^6	4.4×10^3	2.4×10^6	0.0	1.7×10^4	0.0	0.0
10	1.0×10^4	6.4×10^5	4.5×10^3	2.9×10^5	7.5×10^2	2.0×10^3	0.0	0.1×10
12	4.6×10^6	6.7×10^7	2.4×10^5	1.2×10^5	0.0	0.0	0.0	0.0
13	2.0×10^3	4.9×10^5	1.5×10^3	9.4×10^4	2.1×10	4.0×10^2	0.0	0.0

[a] Numbers given are per 100 cc of water and 10 g (wet weight) of sediment, respectively.

[b] SWYE, Seawater-yeast extract medium.

center line of the Bay are examined, it can be seen that TVC decreased rapidly from station 7 to 13, but the PV counts did not change significantly (Fig. 2).

Results for sediment show that the TVC were between 10^5 and 10^8 per 10 g (wet weight) of sediment samples (Table 2). PV counts were recorded between 10^3 and 10^7 at all stations. PVP counts of the sediment appeared to be station dependent. *V. parahaemolyticus* counts at five stations were positive, although the numbers were less than 10 per 10 g of sediment. Three of the five stations were located at the mouth of rivers. The distribution pattern of the TVC and PV counts for the stations located along the center line of the Bay is shown in Fig. 3. The TVC decreased from station 9 to station 13 and the PV counts were maximum at stations 7 and 9.

Plankton samples were collected at seven stations (Table 3). The TVC for the plankton samples showed that there were large differences between stations. The PV counts, however, were relatively constant from station to station, ca. 10^3 to 10^5. PVP counts were <100 at all stations. Stations 8 and 13 were positive for *V. parahaemolyticus*, although the numbers were low.

Results of the numerical taxonomy analysis of 16 strains isolated during the survey and 19 reference strains are shown in Fig. 4. Organisms isolated during the cruise were clearly in the same cluster as the reference strains of *V. parahaemolyticus* isolated from various sources, including strains from victims of *V. parahaemolyticus* food poisoning.

DISCUSSION

In the Rhode River subestuary, organisms

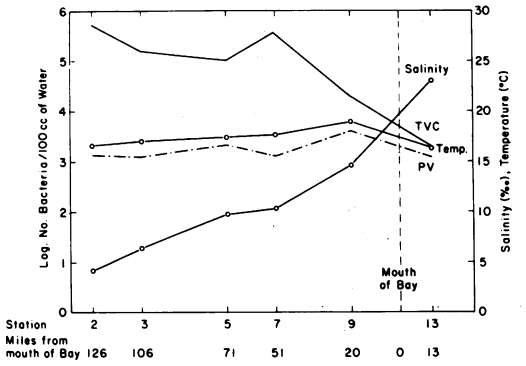

FIG. 2. *Distribution of TVC and PV in water collected from several areas (each station located centrally in the Bay) in Chesapeake Bay, May 1972.*

survive the winter in the sediment, to be released into the water column and become associated with zooplankton, such as copepods, when the water temperature reaches 14 to 15 C in April (4). At this point, the number of *V. parahaemolyticus* in the water remains at a very low, undetectable level. When the water temperature rises to or between 19 and 20 C, the numbers are increased to detectable levels, resulting from the release of *V. parahaemolyticus* from their association with copepods (4). The temperature of the water column in the range of 14 to 19 C, therefore, is particularly important to initiate the annual cycle of *V. parahaemolyticus*. Whether the observations made for the Rhode River apply to other parts of the Bay, including areas where rivers flow into Chesapeake Bay, and areas along the center of the Bay, was the point of this study.

One major difference between the stations in the Rhode River and those employed in this

study was depth and other environmental parameters. The temperature of the surface water was the same as the comparable season in the Rhode River. The Rhode River is a very shallow area, 2 to 3 m deep, and the bottom is very muddy. Transparency, in most cases, was less than 1.0 m throughout the year. The salinity in the Rhode River area varies between 4 to 11‰, and 5.8‰ salinity was recorded from April to early June 1971. Since the depth of the Rhode River stations was very shallow, there was no significant difference between the surface and bottom water, unlike the salinities obtained in this study.

Temperatures recorded at the Rhode River area from April to early June were between 14 and 19 C for surface water and 15 to 20 C for bottom water. It can be seen then that the temperatures recorded for the open Bay in this study showed significant differences between surface and bottom water temperatures, although it should be acknowledged that not all stations in the open Bay showed significant differences between surface and bottom water temperatures. The pH data also revealed lower pH at the bottom as compared with surface water pH, as already shown by other investigators (3). TVC and PV counts in the Rhode River subestuary from April to early June were 10^4 to 10^6, and 10^3 to 10^4, respectively, per 100 cc of Rhode River water with no significant differences from the results obtained during this study, except for TVC at station 13. With respect to the PVP counts, the PVP for Rhode River water showed much higher counts ($>10^3$) when the water temperature was 19 C. On the other hand, PVP counts during this cruise were relatively low; no PVP counts were detected at the stations located on the center line of the Bay. However, *V. parahaemolyticus* was not isolated from the water samples collected at the Rhode River (4) as was the case in this study.

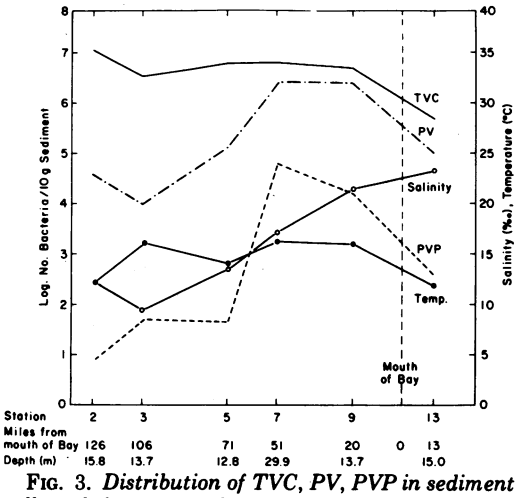

FIG. 3. *Distribution of TVC, PV, PVP in sediment collected from several areas (each station located centrally in the Bay) in Chesapeake Bay, May 1972.*

In the case of the sediment samples, direct comparison of Rhode River and open Bay samples cannot be made since there are significant differences in depth, temperature, and other environmental parameters. Nevertheless, the TVC, PV, and PVP, during April to early June, were ca. 10^6, 10^2 to 10^5, and 10^1 to 10^4, respectively, at the Rhode River area and 10^5 to 10^8, 10^3 to 10^7, and 0 to 10^5, respectively, in the open Bay. In the case of *V. parahaemolyticus*, 8.8×10^2 counts were recorded for Rhode River sediment during this time, but only extremely low numbers of *V. parahaemolyticus* were recorded for the open Bay stations. Furthermore, six of the 12 stations in Chesapeake Bay failed to reveal the presence of *V. parahaemolyticus*. This was the case, in particular, for stations located in the center of the Bay. This observation suggests that *V. parahaemolyticus* may not be evenly distributed or survive throughout the entire Bay during the winter, whereas the organism clearly survives in the sediment in the Rhode River subestuary. One of the possibilities

TABLE 3. *Bacterial counts of plankton samples collected during the R/V Ridgely Warfield cruise, May 1972*[a]

Station	TVC (SWYE)	PV (25 C)	PVP (25 C)	*V. parahaemolyticus*	Characteristics of plankton
1	9.1×10^6	2.5×10^3	0.0	0.0	Mostly copepods
3	1.4×10^9	1.7×10^5	0.0	0.0	Mostly copepods
5	3.8×10^7	4.4×10^4	0.6×10	0.0	Mostly small crustaceans, including copepods
7	2.2×10^8	4.3×10^4	0.7×10	0.0	Phytoplankton and copepods
8	1.8×10^6	5.5×10^4	6.8×10	1.1×10	Phytoplankton and copepods
9	6.7×10^4	2.1×10^4	2.0×10	0.0	Mostly phytoplankton
13	1.6×10^5	4.9×10^4	1.6×10	0.2×10	Mostly phytoplankton with some small crustaceans

[a] Numbers given are per gram (wet weight) of plankton.

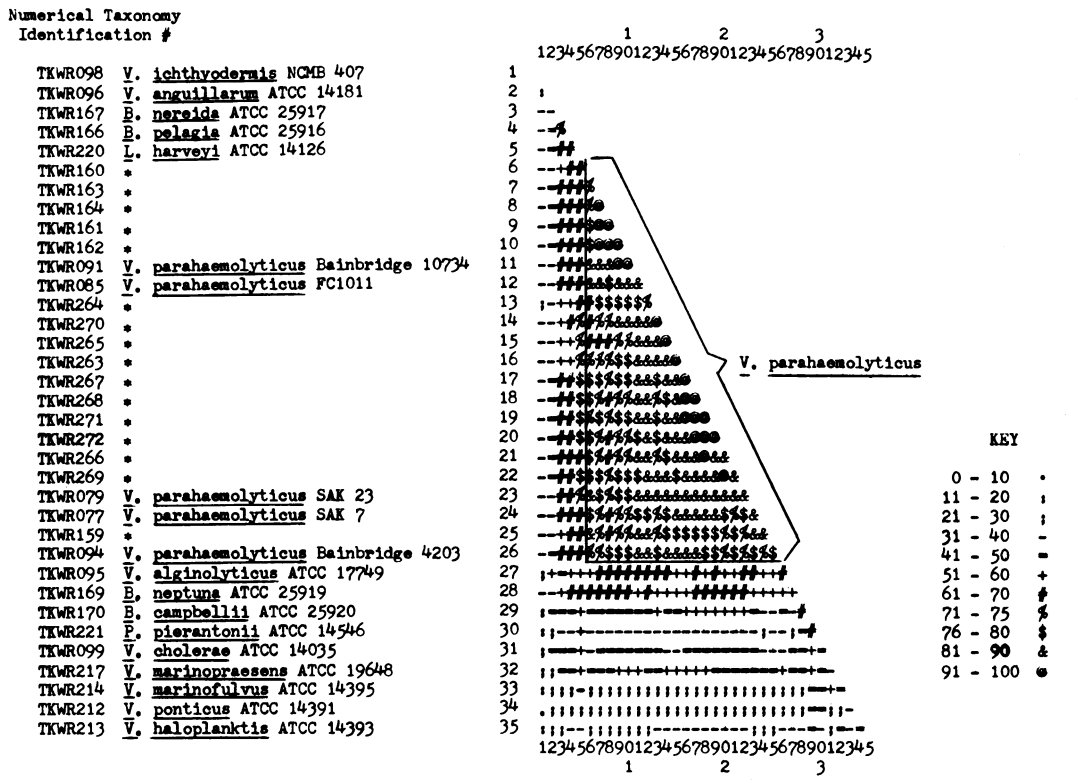

FIG. 4. *Numerical taxonomy analysis of isolates collected during the R/V Ridgely Warfield cruise. *, V. parahaemolyticus isolated during the cruise.*

for this observation may be the relatively low temperatures at the bottom in the open Bay, which may cause a retardation of the initiation of the annual cycle of *V. parahaemolyticus*.

In the case of plankton samples, it is necessary to consider the characteritics of the plankton which were collected in order to interpret the bacterial counts for the plankton samples. In the Rhode River subestuary, the main component of the zooplankton population was copepods throughout the entire year (4). On the contrary, the components of the plankton samples obtained in this study were quite different from station to station. The TVC and PV of the plankton samples were 10^6 to 10^8 and 10^3 to 10^7 per g (wet weight) of plankton in the Rhode River in the period April to early June, respectively (4). In this study, ca. 10^5 to 10^9 and 10^3 to 10^6 per g (wet weight) of the plankton were recorded. Significant differences were observed for PVP and *V. parahaemolyticus* counts. About 10^5 of PVP and 10^3 *V. parahaemolyticus* per g (wet weight) of plankton were recorded in the Rhode River in early June, when the water temperature was 19 C. Less than 10^2 PVP per g of plankton and samples from only two stations with confirmed isolation of *V. parahaemolyticus*, with counts, at the most, of 1.1×10, were recorded in this study. These observations indicate that the proliferation of PVP and *V. parahaemolyticus* had not yet started in the areas sampled at time of this study. There is a distribution pattern for the various components of the plankton (1, 2) which could cause a significant difference in the quantity and quality of the bacterial community associated with the plankton. Also to be considered are the differences in salinities. Since the adsorption of *V. parahaemolyticus* onto copepods occurs more efficiently at lower salinities, the phenomenon of adsorption is an important factor determining the continuation of the annual cycle of *V. parahaemolyticus* (5). Thus higher salinity at the bottom would be less favorable for adsorption. Furthermore, from the viewpoint of adsorption, various factors which influence the vertical and horizontal distribution of *V. parahaemolyticus* and other organisms closely related to *V. parahaemolyticus* will have an effect where depth is an important parameter.

In this study samples were collected in May. Thus, it will be necessary to collect additional

samples in the warmer months in the open Bay to settle the question of the initiation of the annual cycle of *V. parahaemolyticus*. Such data will be presented in a separate communication (Sayler, Nelson, Justice, and Colwell, unpublished data).

Numerical taxonomy used in this study proved to be a useful tool for the identification of *V. parahaemolyticus*. Misidentification of *V. parahaemolyticus* can be avoided if a more complete phenetic analysis as was done in this study is carried out. There are so many closely related *Vibrio* spp. in the estuarine and coastal waters that only a few diagnostic tests will not be sufficient for identification of *V. parahaemolyticus*.

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