Estuaries Vol. 4, No. 4, p. 321–327 December 1981

Distribution of *Vibrio parahaemolyticus* in Chesapeake Bay During the Summer Season

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ABSTRACT: The distribution of *Vibrio parahaemolyticus* in Chesapeake Bay during the warmer weather of the summer months was examined. This species was found throughout the Chesapeake Bay and its tributaries, even in areas of very low salinity. Counts of this species ranged from 0.04 per 100 ml to 46 per 100 ml in the water column and 2.03 to $\geq 2.4 \times 10^3$ per 100 cc of sediment. A variety of physical, chemical and bacteriological properties associated with the incidence and distribution of *V. parahaemolyticus* were examined and salinity was found to be the major influence among the factors examined. Correlation and regression analysis showed that the population size of this species increased with increasing salinity in the estuary.

Introduction

Vibrio parahaemolyticus is probably the most extensively studied bacterial species, with respect to autecology in the estuarine environment (reviewed by Joseph et al. 1982, in press). Indeed, few aquatic bacterial species have been as thoroughly studied in a single environment as has *V. parahaemolyticus* in Chesapeake Bay (Kaneko and Colwell 1973, 1975, 1978). Nevertheless, there are a few aspects of its distribution in nature which bear further investigation. A major question examined in the present study was the distribution of this species during the warm months of the year. A Chesapeake Bay-wide survey undertaken by Kaneko and Colwell (1975) carried out during May, 1972, was at a time of the year when water temperatures were just warming to 15 °C and above, a critical temperature range in the annual cycle of this organism (Kaneko and Colwell 1978). *V. parahaemolyticus* could not be isolated from the water column at that time at stations sampled by Kaneko and Colwell (1975) although several samples of sediment and plankton yielded *V. parahaemolyticus*. It appeared appropriate, therefore, to undertake studies in Chesapeake Bay during the months of June, July, and August, 1978, in order to determine the distribution of this species during what should be the optimal season for its growth and distribution in the estuary. Environmental factors influencing growth, survival and distribution of *V. parahaemolyticus* were also examined.

Materials and Methods

Sampling Sites

A total of 21 stations throughout the Chesapeake Bay and its tributaries, including the Potomac and James rivers, were sampled for the presence of *V. parahaemolyticus* (Fig. 1). Stations were selected to provide sites representative of varying degrees of pollution, and of a wide salinity range. Stations along the length of the Chesapeake Bay were sampled during the summer months of 1978, thereby encompassing a significant salinity gradient along a single track line. Stations in the Baltimore harbor and the Potomac River downstream from Washington, D.C. were sampled to assess the impact of pollution on the distribution of *V. parahaemolyticus*.

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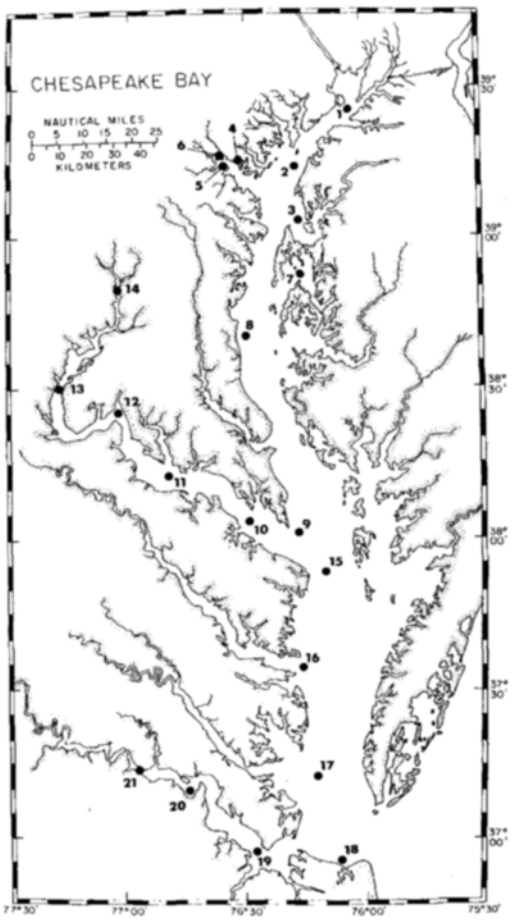

Fig. 1. Location of stations sampled in Chesapeake Bay.

Collection of Samples

Surface water samples were collected with a 2-liter sterile Niskin Bag sampler (General Oceanics, Miami, Fla.) or, for larger volumes, with a submersible pump, which was thoroughly flushed with water from the site to be sampled prior to sample collection. Samples were processed aboard ship immediately after collection.

The upper 10 cm of sediment was sampled using a non-aseptic Petite Ponar grab sampler (Wildlife Supply Co., Saginaw, Mich.). Sediment samples were subsampled aseptically from the center of the grab sample for microbiological examination.

Concurrently with sample collection, dissolved oxygen, temperature, salinity, transparency and total suspended matter values were recorded, using methods detailed previously (Kaper et al. 1979).

Bacteriological Analyses

Total viable aerobic heterotrophic counts (TVC) were determined using Upper Bay Yeast extract agar (UBYE) (Sayler et al. 1975). Salinity of the medium was increased for lower Bay stations to correspond to *in situ* salinity, 15–20‰. Replication of subsamples and plating was accomplished using an optimal allocation scheme developed in an earlier study (Kaper et al. 1978) and employing four subsamples and two plates per dilution per subsample, thereby minimizing the variance of the total plate count.

Presumptive total and fecal coliforms were estimated by using lactose and EC broths (Difco Laboratories, Detroit, Mich.), respectively, in a three-tube replication of a most-probable-number (MPN) series (APHA, 1971).

V. parahaemolyticus were enumerated by an MPN precedure whereby 1,000, 100, 10, and 1 ml sample volumes are inoculated into a modified arabinose, ethyl violet broth (Horie et al. 1964) containing (grams per liter): peptone (Difco), 5.0; beef extract (Difco), 3.0; NaCl, 30; bromothymol blue, 0.03; ethyl violet, 0.001; and galactose, 5.0 (pH 9.0). Double-strength broth was used for 10 ml volumes of samples, and single-strength broth for 1, 100 and 1,000 ml volumes, with the 100 and 1,000 ml samples first being concentrated using 0.45 μm membrane filters. After incubation for 24 h at 37 °C, the enrichment broth cultures were streaked onto thiosulfate citrate bile salts agar (TCBS) plates (Difco) which were incubated at 37 °C for 24 h. Colonies were picked and inoculated into a multitest tubed medium (Kaper et al. 1980) which allowed the following biochemical reactions to be recorded in a single tube: fermentation of mannitol, lactose and sucrose; arginine dihydrolase; and production of indole, H_2S, and gas from carbohydrates. Strains yielding the following reactions were recorded as presumptive *V. parahaemolyticus*: cytochrome oxidase (+), growth in 0% NaCl (−), growth at 43 °C (+), acid from mannitol (+), acid from lactose (−), acid from sucrose (−), gas from carbohydrates (−), H_2S

production (−), indole production (+), and argine dihyrolase (−). Samples yielding isolates of presumptive *V. parahaemolyticus* were recorded as MPN values using published tables (APHA 1971; DeMan 1977) or the formula of Thomas (1942).

Statistical Analyses

Physical, chemical and bacteriological data were entered and stored on an IBM 370/168 computer. Multiple linear correlation coefficients were calculated using the BMDP8D computer program of the Health Sciences Computing Facility, University of California, Los Angeles (Dixon 1975). Stepwise regression analyses were performed using the BMDP2R program of this same package of programs. Bacteriological data were transformed by a $\log_{10}$ transformation and calculations were performed on the IBM 370/168 computer. Correlation coefficients (r) and F values were tested for significance at the 95% and 99% confidence levels by comparing computed r and F values with tabulated critical values, as given in Snedecor and Cochran (1967).

Results and Discussion

Results of the Bay-wide survey for *V. parahaemolyticus* are given in Table 1. *V. parahaemolyticus* was found at nearly every station sampled, except stations 11 and 13, both of which are located in the Potomac River, and station 21, in the James River. MPN values of <0.03 for stations 11 and 13 indicate that no *V. parahaemolyticus* was recovered from the 1,000 ml volumes of water sampled at these stations. The value of <0.3 for station 21 indicates that no isolates were recovered from 100 ml volumes sampled. All of the sediment samples examined in this study yielded *V. parahaemolyticus* isolates except those from stations 20 and 21. Counts ranged from 0.04 per 100 ml to 46 per 100 ml in the water column and 2.03 to $\geq 2.4 \times 10^3$ per 100 cc of sediment. Interestingly, *V. parahaemolyticus* was found throughout the Chesapeake Bay, even in essentially freshwater areas of the bay, such as stations 1 and 14, which are located at the head of the bay, and in Alexandria, Va., respectively (salinity = 0.1‰). *V. parahaemolyticus* was also found in samples collected from polluted areas of the Chesapeake Bay, such as Baltimore Harbor. Stations 4, 5 and 6 are located in the inner Baltimore Harbor. Fecal coliform values obtained at the Baltimore Harbor stations were *ca.* 3.5×10^4 per 100 ml of water. In addition to high coliform counts, samples collected from these stations demonstrated high total, viable heterotrophic bacterial counts, *viz.*, 10^5 cells per ml of water. *V. parahaemolyticus* recovered from samples collected at stations 4, 5 and 6 were present at MPN values of 0.4, 0.9, and 3 per 100 ml of water, respectively.

A wide range of physical and chemical properties were measured during the study. Salinity varied from 0.1‰, at the head of the bay (station 1), to 20.1‰ at the mouth of the bay (station 18). Suspended particulate values varied by more than a log, *viz.* 3.4 mg per l (station 9) to 38.9 mg per l (station 20). Dissolved oxygen also varied greatly, from a value of 1.4 mg per l at station 6 in the Baltimore Harbor to 14.1 mg per l at station 11. The water temperature was relatively conservative during the course of the study, varying from 21.2 to 28.9 °C.

Data gathered in this study were subjected to statistical analysis using simple correlation and step-wise multiple linear regression techniques. The correlation matrix resulting from analysis of data for water samples is presented in Table 2. The only property found to be significantly correlated with occurrence of *V. parahaemolyticus* in the water column was salinity. Stepwise multiple linear regression analysis confirmed the importance of salinity and yielded the following:

Step No.	Variable Entered	Coefficient	r^2	Increases in r^2
1	Salinity	0.107	.290	.290
2	Dissolved oxygen	−0.125	.391	.101
Y intercept = 0.205				
F value = 4.486		df = 2.14	p = <.05	

The significance of dissolved oxygen in the stepwise multiple regression analysis and not in the correlation analysis is due to the fact that the correlation analysis is calculated on the basis of a single variable in the equation. Then, salinity, by itself, was

TABLE 1. Physical, chemical and bacteriological properties associated with incidence and distribution of *V. parahaemolyticus* in Chesapeake Bay.

Station No.	Date	Sample[a]	TVC (per ml)	Total Coliforms MPN per 100 ml	Fecal Coliforms MPN per 100 ml	*V. parahaemolyticus* MPN per 100 ml	Salinity (‰)	Temperature (°C)	Transparency (m)	Dissolved O_2 (mg per l)	Suspended Particulates (mg per l)
1	7/20	TW	1.1×10^3	8	<2	0.04	0.1	28.0	0.6	9.4	ND[b]
		Sed	1.4×10^5	1.1×10^4	<20	150					
2	7/20	TW	5.7×10^2	1.7×10^2	21	11	1.9	27.3	0.6	7.8	ND
		Sed	1.9×10^5	1.6×10^5	<20	430					
3	8/2	TW	9.7×10^2	35	0.2	0.04	7.0	25.6	1.3	7.9	9.0
		Sed	5.3×10^5	2.4×10^3	<20	93					
3	7/20	TW	5.4×10^3	49	<2	11	7.4	25.5	1.5	10.1	ND
4	8/2	TW	3.4×10^5	$\geq 2.4 \times 10^3$	1.3×10^2	0.4	5.5	26.3	1.2	3.4	6.4
		Sed	3.0×10^6	1.5×10^4	<20	43					
5	8/2	TW	1.8×10^5	5.4×10^4	3.5×10^4	0.9	5.8	24.2	0.8	5.3	12.6
		Sed	8.4×10^6	2.2×10^6	4.9×10^3	3					
6	8/2	TW	6.0×10^5	1.6×10^5	4.9×10^3	3	4.5	25.8	1.3	1.4	9.2
		Sed	6.0×10^6	$>2.4 \times 10^6$	1.4×10^5	23					
7	8/2	TW	5.3×10^2	17	0.5	0.9	9.4	27.0	1.4	ND	9.6
		Sed	5.8×10^4	3.5×10^3	50	$\geq 2.4 \times 10^3$					
8	8/2	TW	1.7×10^3	2.4×10^2	4	4.3	9.5	27.0	1.3	ND	13.8
		Sed	7.7×10^4	2.4×10^3	20	93					
9	6/1	TW	1.3×10^4	17	<0.2	0.29	7.9	21.2	2.5	10.5	3.4
10	6/1	TW	2.5×10^4	35	0.5	0.75	5.1	22.5	1.5	11.1	5.6
11	6/1	TW	8.1×10^4	46	33	<0.03	2.6	25.0	0.8	14.1	13.4
12	6/1	TW	2.2×10^3	1.1×10^2	0.2	0.23	0.7	22.5	0.3	6.7	29.8
13	6/2	TW	2.5×10^3	9.2×10^2	1.3	<0.03	0.1	23.5	0.5	7.0	23.8
14	6/2	TW	1.0×10^5	2.2×10^2	8	0.93	0.1	24.1	0.8	9.9	12.3
15	6/15	TW	1.5×10^4	7	0.5	15	10.4	25.4	1.6	9.5	6.2
		Sed	7.8×10^6	1.6×10^4	<20	93					
16	6/15	TW	9.6×10^3	1.7	0.5	46	12.5	26.0	1.8	8.8	12.8
		Sed	1.8×10^6	2.7	<20	$\geq 2.4 \times 10^3$					
17	6/15	TW	5.5×10^3	2.3	0.2	0.9	15.4	26.8	2.3	9.3	4.0
		Sed	6.1×10^5	4.9×10^2	<20	1.2×10^2					

TABLE 1. Continued.

Station No	Date	Sample[a]	TVC (per ml)	Total Coliforms MPN per 100 ml	Fecal Coliforms MPN per 100 ml	*V. parahaemolyticus* MPN per 100 ml	Salinity (‰)	Temperature (°C)	Transparency (m)	Dissolved O_2 (mg per l)	Suspended Particulates (mg per l)
18	6/15	TW	2.5×10^3	1.1×10^2	5	4.3	20.1	25.3	1.8	8.0	8.2
		Sed	1.6×10^5	1.7×10^2	20	$\geq 2.4 \times 10^3$					
19	6/15	TW	4.4×10^3	33	13	46	15.8	26.3	1.0	8.4	12.8
		Sed	7.1×10^5	3.5×10^3	<20	2.4×10^3					
20	6/15	TW	5.5×10^2	5.4×10^2	3.3	4.0	0.2	28.4	0.4	7.2	38.9
		Sed	8.5×10^4	9.2×10^3	<20	<0.3					
21	6/15	TW	2.9×10^3	2.4×10^2	1.1	<0.3	0.0	28.9	0.6	6.3	25.7
		Sed	5.3×10^5	2.4×10^4	<20	<0.3					

[a] TW = top water sample, Sed = sediment sample.
[b] ND = not determined.

correlated with incidence of *V. parahaemolyticus,* but dissolved oxygen, by itself, was not significantly correlated. The stepwise multiple regression technique is capable of considering several variables in the equation simultaneously. Thus, salinity is the variable first entered in step 1 (*vide supra*). Once the variation due to salinity is calculated, part of the residual variation can be accounted for by the addition of dissolved oxygen to the equation. No other variables, upon inclusion in the equation, could account for a significant portion of the remaining variance.

Thus, the population size of this species increased with increasing salinity in the Chesapeake Bay. In addition, dissolved oxygen concentration had a smaller negative effect, *viz.* increasing population size with decreasing dissolved oxygen concentration. This influence could result from increased nutrient levels in eutrophic areas of Chesapeake Bay. Sediment samples also demonstrated the same trend, i.e., larger numbers of *V. parahaemolyticus* were isolated from sediment samples with an overlayer of water of higher salinity when compared to areas where the salinity of the water was low (Table 1). This relationship was also statistically significant (data not shown).

In an earlier study of the incidence and distribution of bacterial pathogens in Chesapeake Bay (Kaper et al. 1979), correlations of *V. parahaemolyticus* with TVC and coliform bacteria were observed when salinity was not highly variable among the samples collected during a transect cruise into Baltimore Harbor. Larger numbers of *V. parahaemolyticus* (>240/100 ml) were isolated from samples collected at the more polluted stations than at less polluted sites. The correlation matrix resulting from the analysis exhibited positive correlations between TVC, total coliforms and fecal coliforms (Kaper et al. 1979). Since *V. parahaemolyticus* in the U.S. has not been reported to be excreted in human or animal feces, the increased numbers observed in this study are probably due to higher nutrient concentrations in the water in the areas sampled.

The fact that *V. parahaemolyticus* was isolated from essentially freshwater areas of the bay was somewhat surprising, but not

TABLE 2. Correlation matrix of physical, chemical, and bacteriological conditions associated with *V. parahaemolyticus* in the water column in Chesapeake Bay.

	Salin	Temp	TVC	TC	FC	Vp	Trans	DO	Sus Mat
Salin	1.0								
Temp	0.09	1.0							
TVC	−0.02	−0.14	1.0						
TC	−0.33	0.01	0.53[b]	1.0					
FC	−0.08	0.18	0.66[b]	0.85[b]	1.0				
Vp	0.50[c]	0.21	−0.05	−0.09	0.15	1.0			
Trans	0.61[b]	0.12	0.10	−0.48[c]	−0.23	0.38	1.0		
DO	0.11	−0.19	−0.26	−0.74[b]	−0.54[c]	−0.21	0.22	1.0	
Sus Mat	−0.59[c]	0.22	−0.42	0.25	−0.03	−0.17	−0.69[b]	−0.18	1.0

[a] Abbreviations: Salin = salinity, Temp = temperature, TVC = total viable count, TC = total coliforms, FC = fecal coliforms, Vp = *V. parahaemolyticus*, Trans = transparency, DO = dissolved oxygen, and Sus Mat = total suspended matter.

[b] Significant at the 0.01 level.

[c] Significant at the 0.05 level.

unprecedented. Sayler et al. (1976) examined the upper Chesapeake Bay and recovered several isolates of *V. parahaemolyticus* from water samples of low salinity, including an isolate from suspended sediment, when the water temperature was 4.3 °C and salinity 0‰. The findings of Sayler and co-workers and of the present study can most probably be explained by tidal transport of *V. parahaemolyticus* into areas of lower salinity, accounting for the presence of *V. parahaemolyticus* in the uppermost reaches of the Chesapeake Bay, the James River, and the Potomac River. Interestingly, the same phenomenon has been observed in water and fish taken from the essentially freshwater part of the River Hooghly in India, *ca.* 50 miles upriver from the Bay of Bengal (De et al. 1977). *V. parahaemolyticus* is widely distributed in this area, being found in 40% of pond water samples "having practically no salinity" and fed chiefly by rain water (Sircar et al. 1976). It should be noted that *V. parahaemolyticus* is responsible for about 7–10% of all gastroenteritis cases in Calcutta (Deb et al. 1975). *V. parahaemolyticus,* in England, has also been observed to flourish in freshwater drainage ditches and ponds containing large concentrations of organic matter (J. Lee, pers. commun.). The influence of organic matter on the incidence and population size of *V. parahaemolyticus* in Narragansett Bay was noted by Watkins and Cabelli (1978), who observed that adsorption of *V. parahaemolyticus* to particles is greater in water of lower salinity, a finding consistent with the observations of Sayler and co-workers (1976) in Chesapeake Bay. Thus, when *V. parahaemolyticus* is sought in brackish or freshwater environments, factors such as presence of particulate matter and the concentration of nutrients should be considered, in addition to salinity, temperature, and number of coliforms.

Acknowledgments

This investigation was supported by Public Health Service Grant 1R22AI14242-01. Shiptime aboard the R/V RIDGELY WARFIELD was made available by National Science Foundation Grant GB-35261X. Acknowledgment is made to the captain and crew of the R/V RIDGELY WARFIELD for their excellent field assistance.

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