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Distribution of *Vibrio vulnificus* in the Chesapeake Bay

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***Vibrio vulnificus* is a potentially lethal human pathogen capable of producing septicemia in susceptible persons. Disease is almost always associated with consumption of seafood, particularly raw oysters, or with exposure of wounds to seawater. An oligonucleotide DNA probe (*V. vulnificus* alkaline phosphatase-labeled DNA probe [VVAP]), previously shown to be highly specific for *V. vulnificus*, was used to enumerate this species in environmental samples collected from the Chesapeake Bay between April 1991 and December 1992. Total aerobic, heterotrophic, culturable bacteria were enumerated by plate counts on nonselective medium. The number of *V. vulnificus* organisms was determined by colony lifts of spread plates for subsequent hybridization with VVAP. *V. vulnificus* was not detected in any samples collected during February and March (water temperature of <8°C) but was found in 80% of the water samples collected during May, July, September, and December (water temperature of >8°C), with concentrations ranging from 3.0×10^1 to 2.1×10^2/ml (ca. 8% of the total culturable heterotrophic bacteria). In a multiple regression analysis, increased *V. vulnificus* concentrations were correlated with lower salinities and with isolation from samples collected closer to the bottom. Isolation from oysters was demonstrable when water temperatures were 7.6°C, with concentrations ranging from 1.0×10^3 to 4.7×10^4/g (ca. 12% of total culturable bacteria). In samples collected in May and July, *V. vulnificus* was identified in seven of seven plankton samples and four of nine sediment samples. Our data demonstrate that *V. vulnificus* is a widespread and important component of the bacterial population of the Chesapeake Bay, with counts that are comparable to those reported from the Gulf of Mexico.**

Vibrio vulnificus is an opportunistic human pathogen common to the estuarine environment, possibly existing as a symbiont of oysters (5, 8, 13, 24, 35). Disease usually follows the ingestion of raw oysters and can result in a septicemia that is rapidly and frequently fatal, with mortality exceeding 50%. Nearly all systemic infections (95%) occur in persons with underlying chronic diseases, including liver disease, hemochromatosis, or conditions which lead to immunosuppression (18, 21, 24, 31, 34). In addition, wound infections and gastroenteritis have been seen in both healthy individuals and persons with underlying illness.

One factor that may influence the incidence of disease is the prevalence of *V. vulnificus* in the environment. The majority of cases occur during the summer months as a result of the consumption of oysters harvested from the Gulf of Mexico (18, 21, 31, 34). In this region, *V. vulnificus* concentrations are commonly reported as ca. 10^3 organisms per g of oyster meat and generally range from 0 to 10^4 organisms per g (3, 4, 37). In Gulf Coast states, both the incidence of *V. vulnificus* disease and the concentration of this pathogen in the environment appear to decrease with colder temperatures during the winter months (16, 18, 21, 34, 37). However, the distribution of *V. vulnificus* is not limited to the Gulf of Mexico, and a serological survey of persons in the Chesapeake Bay area provided immunological evidence for *V. vulnificus* exposure in this region (19). *V. vulnificus* has also been recovered from oysters harvested in geographical areas with a relatively low incidence of disease, such as the colder waters of New England (27–29), the Pacific Coast (14), and the Chesapeake Bay (39). Concentrations in these oysters sometimes exceeded 10^3 organisms per g, and *V. vulnificus* cells were detectable in New England samples from water at a temperature (11.1°C) colder than that reported in the Gulf (29).

Environmental parameters that correlate with recovery of *V. vulnificus* from seawater generally correspond to estuarine conditions of relatively lower salinity (7 to 16 ppt) and higher temperature (exceeding 20°C) (14, 17, 28, 37). In a rich medium, this halophilic bacterium grows optimally at NaCl concentrations ranging from 0.5 to 2.0% and at temperatures between 25 and 37°C (13). However, survival in sterile seawater was observed to be optimal at ca. 13°C in seawater with a salinity of 10 ppt after 6 days, and *V. vulnificus* was recovered from oysters and seawater incubated at a low temperature, e.g., as low as 0°C for several weeks (13, 15). Like other vibrios (30, 42), this species may also persist in a viable but nonculturable state for even longer periods of time (1, 20, 23, 25, 38). Linder and Oliver (20) demonstrated entry of *V. vulnificus* into a nonculturable state, a phenomenon which was rapidly induced by a low temperature (5°C) but eliminated by prestarvation in *V. vulnificus* (23, 38). It has been suggested that survival of *V. vulnificus* in oysters and seawater during conditions of cold temperatures may be explained by the persistence of viable but nonculturable cells of *V. vulnificus* in the environment (23).

In order to gain an improved understanding of the ecology of *V. vulnificus*, we systematically examined the distribution of this bacterium in samples obtained from multiple sites in the Chesapeake Bay. In the past, enumeration of *V. vulnificus* has been hampered by the multiple, time-consuming assays required for the identification of this species. Recently, the use of species-specific monoclonal antibody or nucleic acid probes has permitted rapid, accurate identification of this pathogen (3–6, 12, 22, 32, 33, 36, 40). In the present study, we used a DNA oligonucleotide probe that has been shown previously to be highly specific for the species (22, 40) and enumerated *V. vulnificus* populations from the water column, plankton, oys-

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ters, and sediment by direct colony counts on nonselective media, using a variety of media for recovery.

MATERIALS AND METHODS

Bacterial strains and media. Control strains for DNA probing experiments included *V. vulnificus* MO6-24/O (41), *V. fluvialis* 807 (obtained from S. Richardson), and *V. cholerae* 569B (obtained from J. Kaper). Isolates were stored at −70°C in 50% (vol/vol) glycerol or 10% (vol/vol) dimethyl sulfoxide in L broth (LB) prepared with tryptone (10 g), yeast extract (5 g), and NaCl (10 g) in 1 liter of distilled water and autoclaved. The following media were used for bacterial enumeration: (i) LB agar (LA), LB plus agar (1.5%); (ii) LA plus artificial seawater (LASW), LA prepared with artificial seawater (ASW) (Instant Ocean; Aquarium Systems, Mentor, Ohio) instead of distilled water; (iii) LA plus 50% ASW, LA prepared with a 50% (vol/vol) solution of ASW in distilled water; (iv) thiosulfate citrate bile salts agar (TCBS); and (v) *V. vulnificus* agar as previously described (40). All ingredients for media were purchased from Difco (Detroit, Mich.), and other reagents were obtained from Sigma (St. Louis, Mo.). Selected environmental isolates were identified as *V. vulnificus* by API 20E (bioMérieux Vitek Inc., Hazelwood, Mo.).

Sampling methods. Sampling sites in the Chesapeake Bay employed in this study are shown in Fig. 1. Water samples were collected 1 m below the surface and 1 m above the bottom at all stations, except at Tolly Point and Hog Point, where samples were collected 1 m above the bottom. Samples were collected in April, June, August, and October 1991 and in February, March, May, July, September, and December 1992. Niskin bottles (10 liters) attached to a rosette sampler (General Oceanics, Miami, Fla.) were used to collect water samples. Vertical profiles of temperature, salinity, dissolved oxygen, and fluorescence were determined with a Neal Brown NKIII CTD system (EG & G, Calaumet, Mass.). Dissolved oxygen was measured with a polarographic oxygen probe (Beckman Instruments, Palo Alto, Calif.). Fluorescence and attenuation were measured with a SeaTech (Corvallis, Oreg.) fluorometer and a 25-cm-path-length transmissometer (SeaTech). Samples were processed within 30 min of collection. Seawater was serially diluted in phosphate-buffered saline (PBS), 100 or 200 μl was spread on solid medium, and the inoculated plates were incubated at room temperature. Oysters (6 to 12) were collected by dredging, blended in a Waring blender for 90 s with or without an equal volume of PBS, and plated as described above. Plankton samples were obtained by towing a 64-μm-mesh plankton net, fitted with a 250-ml bucket, 1 to 2 m below the surface for 5 min. The plankton samples were rinsed from the collection bucket, resuspended in approximately 10 ml of sterile PBS, homogenized in a sterile tissue grinder, and plated as described above. Sediment samples were collected by using a Smith-MacIntyre sediment grab sampler. Sediment (1.0 ml) was removed aseptically from the surface of the grab sampler with a syringe modified by the removal of the end flange to form an open cylinder and plated as described above. Enumeration of the total number of culturable heterotrophic bacteria per milliliter was expressed as the average counts from two to four LA plates at the appropriate dilution.

Detection of *V. vulnificus* by oligonucleotide probe. The 24-base sequence for the *V. vulnificus* alkaline phosphatase-labeled DNA probe (VVAP) was derived from the gene sequence for *V. vulnificus* hemolysin, as previously described (40). Filter preparation, hybridization, and probe development conditions have also been described elsewhere (39, 40). Briefly, probing involved colony lifts from plates with 1 to 300 colonies, by using Whatman no. 541 filters. Filters were processed by being microwaved in alkaline lysis buffer, by neutralization in ammonium acetate, and, for the degradation of native enzymatic activity, by incubation in proteinase K at 42°C. Hybridization was done at 56°C for 1 h, and filters were washed twice in sodium citrate with 1% (wt/vol) sodium dodecyl sulfate at 56°C for 10 min. Alkaline phosphatase-positive colonies were visualized by incubation in a solution of substrate prepared with nitroblue tetrazolium-BCIP (5-bromo-4-chloro-3-indolylphosphate toluidinium) tablets, according to the manufacturer's instructions (Sigma). Enumeration of *V. vulnificus* organisms was determined as the average of probe-positive colonies from two to four filters at the appropriate dilution. Probe-positive colonies were recovered by aligning positive colonies on filters with spread plates that had been reincubated after colony lifts were performed with the sterile filters.

Enrichment and recovery. Enrichment studies with alkaline peptone water (APW) were conducted by incubating a 1:10 dilution of sample (0.5 ml of sample in 4.5 ml of APW) or concentrated sample (20×; or 0.5 ml of retentate from 200 ml of filtered [0.2-μm-pore-size filter] seawater resuspended in 4.5 ml of APW) without shaking at 35°C for 6 h. Cultures were serially diluted and plated on LA. For recovery studies, samples were incubated at room temperature for 6 h prior to processing.

Statistical methods. A multiple regression model was used to evaluate the relative impact of salinity, temperature, and site of collection (the surface versus the bottom of the water column) on *V. vulnificus* concentrations in water samples for May, July, September, and December 1992. Bacterial concentrations were log transformed, and statistical analysis was performed by using the Stata3 statistical package (Stata Corporation, College Station, Tex.).

RESULTS

Distribution of *V. vulnificus* in Chesapeake Bay water. *V. vulnificus* was readily detectable in surface water samples collected throughout the Chesapeake Bay and constituted a significant proportion of the total culturable heterotrophic bacterial population in samples collected during warmer months of the year. The heterotrophic population in seawater, enumerated on nonselective medium, ranged from 1.0×10^1 to 3.0×10^3 organisms per ml of water (Fig. 2). When detectable, the number of *V. vulnificus* organisms in surface water ranged from 3.0×10^1 to 2.1×10^2/ml and averaged between 0.6 and 17.4% of the total bacterial population, depending on the sampling station. Overall, the mean number of *V. vulnificus* organisms for the entire set of Chesapeake Bay water samples examined between May and December 1992 constituted 8.0% of the total culturable bacteria. The mean number of *V. vulnificus* organisms in bottom water samples at all stations during warmer months (May, July, September, and December) was 5.3×10^2 organisms per ml, while the surface water samples averaged 2.4×10^1.

V. vulnificus organisms were enumerated in Chesapeake Bay water samples collected from areas with a wide range of temperatures (8 to 26°C), as shown in Fig. 2. However, during February and March 1992, when water temperatures ranged from 1.8 to 7.2°C, *V. vulnificus* was not recovered from any site ($P < 0.001$, Fisher's exact test). In contrast to *V. vulnificus*, the number of culturable, heterotrophic bacteria in Chesapeake Bay water samples did not vary greatly with temperature, with some of the largest numbers of total bacteria found in water samples collected in February and March 1992. Decreases in the number of *V. vulnificus* organisms in surface water also correlated with increasing salinity from the Upper Bay to the Lower Bay. During warmer months (May to December 1992), *V. vulnificus* was isolated from all water samples collected in the Upper Bay (stations 1 to 4), but the organism was recovered less frequently in samples from the Lower Bay in a transect seaward to the Atlantic Ocean (stations 5 to 8).

When data for salinity and location in the water column were entered into a multiple regression model, both variables were independent and significant predictors of *V. vulnificus* counts ($P < 0.001$). During warmer months (excluding February and March) there was not a significant correlation between water temperature and *V. vulnificus* concentrations.

Enumeration of *V. vulnificus* organisms in oysters. The number of *V. vulnificus* organisms was determined in oysters collected from beds located at the mouth of the Severn River (Tolly Point) and the Patuxent River (Hog Point) (Fig. 1). As in the water samples, *V. vulnificus* constituted a significant proportion of the bacterial population, ranging from 0.9 to 29.4% of the culturable heterotrophic bacteria, with a mean of 12.1% for all positive samples at all locations sampled. As for the water samples, *V. vulnificus* was not detected in oysters collected at either location during February and March 1992 (Fig. 3). At other times of the year, *V. vulnificus* was always present in oysters at both locations in numbers ranging from 1.0×10^3 to 4.7×10^4 organisms per g of oyster. Enumeration of the total heterotrophic bacterial populations in oysters ranged from 8.5×10^2 to 4.6×10^5 cells per g of oyster (Fig. 3). Unlike the bacterial counts in water samples, a decrease in the total number of bacteria enumerated in oysters occurred during the coldest months.

Enumeration of *V. vulnificus* organisms in plankton and sediment. Plankton samples obtained during May and July were positive for *V. vulnificus* at all locations sampled, as indicated by the VVAP probe (Table 1). However, *V. vulnificus*

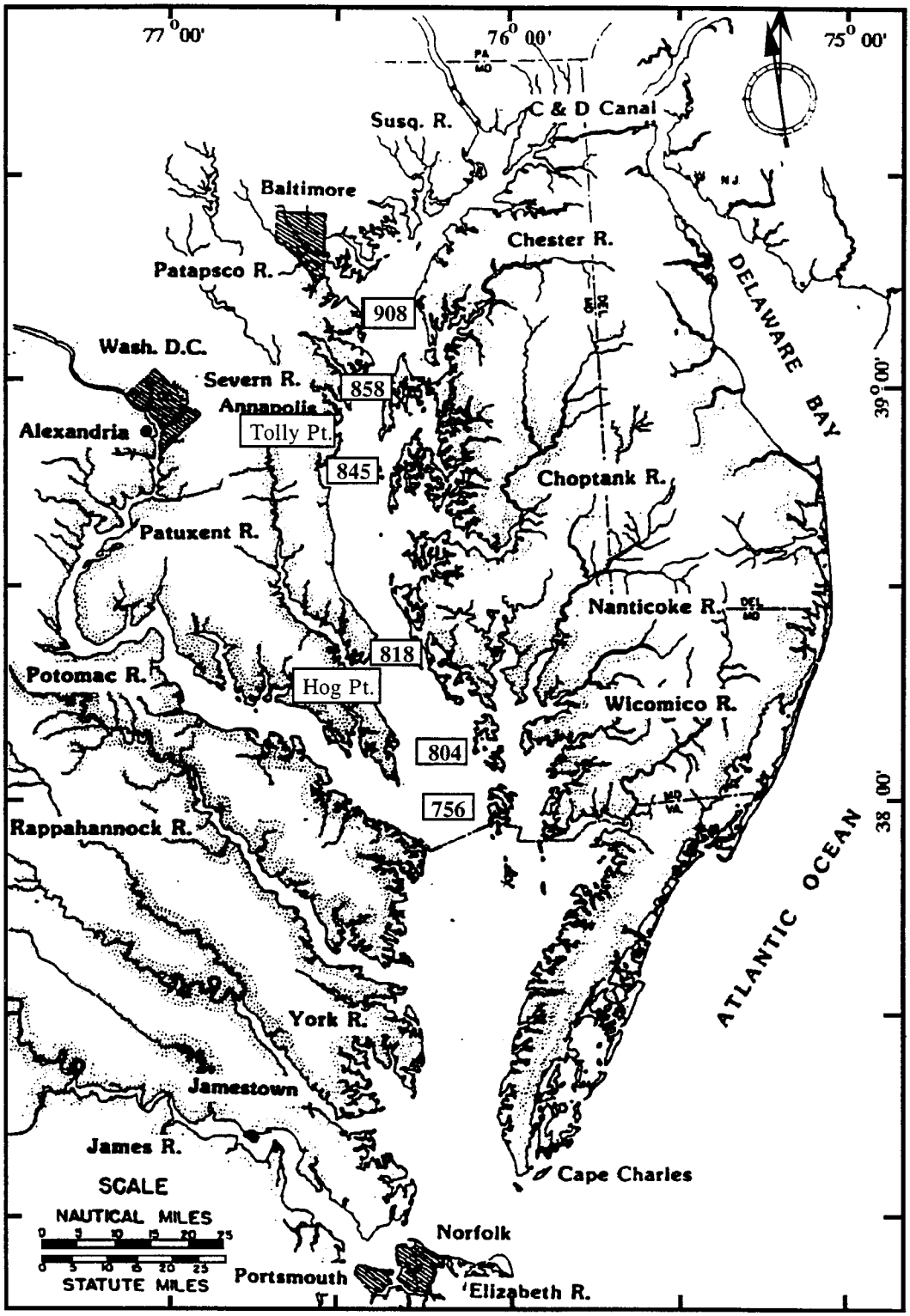
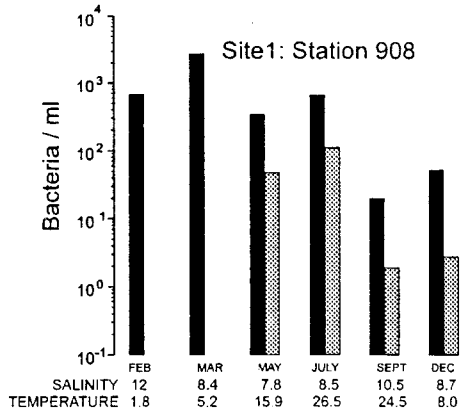
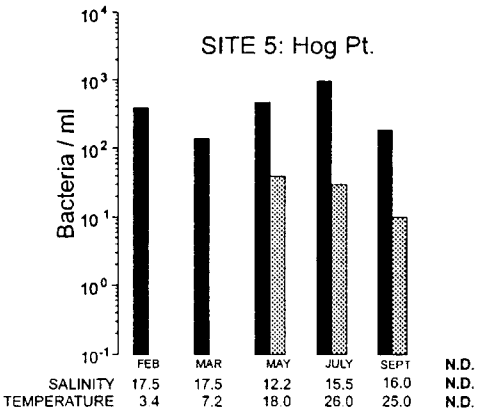


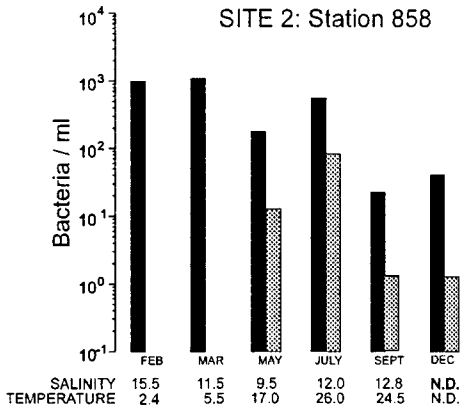
FIG. 1. Sampling stations in the Chesapeake Bay employed in this study.

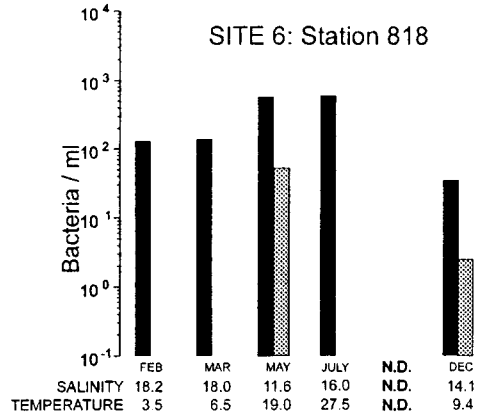
was detected in only four of nine sediment samples, despite the larger total number of bacteria in the sediment samples (usually exceeding 10^4 organisms per ml), compared with the number in water and plankton samples. Nevertheless, *V. vulnificus* was isolated consistently from sediment samples collected at locations of oyster beds (sites 3 and 5) or immediately downstream (site 6) from an oyster bed at numbers between 1.2×10^3 to 3.3×10^5 cells per ml.

Effects of enrichment and plating medium on recovery of *V. vulnificus* and total bacteria. Attempts to recover *V. vulnificus*

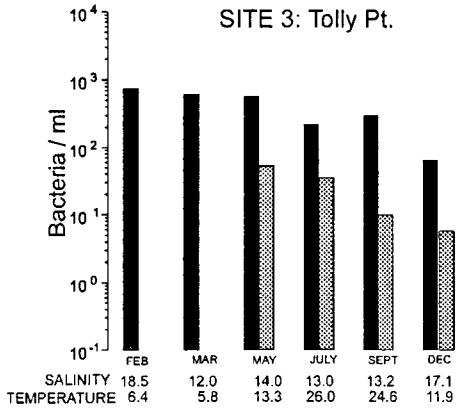

Site1: Station 908
Bacteria / ml
FEB MAR MAY JULY SEPT DEC
SALINITY 12 8.4 7.8 8.5 10.5 8.7
TEMPERATURE 1.8 5.2 15.9 26.5 24.5 8.0

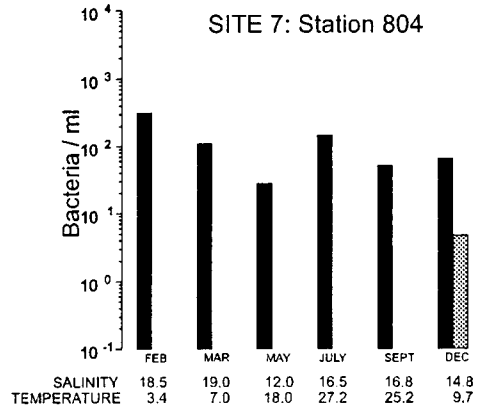

SITE 5: Hog Pt.
Bacteria / ml
FEB MAR MAY JULY SEPT N.D.
SALINITY 17.5 17.5 12.2 15.5 16.0 N.D.
TEMPERATURE 3.4 7.2 18.0 26.0 25.0 N.D.

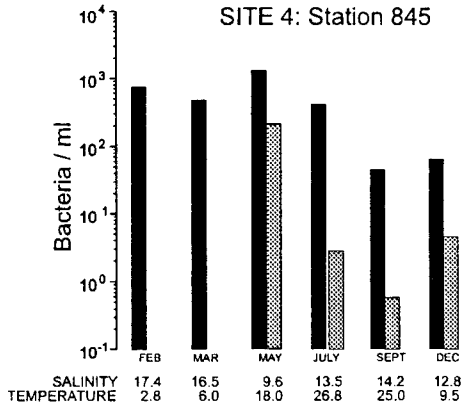

SITE 2: Station 858
Bacteria / ml
FEB MAR MAY JULY SEPT DEC
SALINITY 15.5 11.5 9.5 12.0 12.8 N.D.
TEMPERATURE 2.4 5.5 17.0 26.0 24.5 N.D.

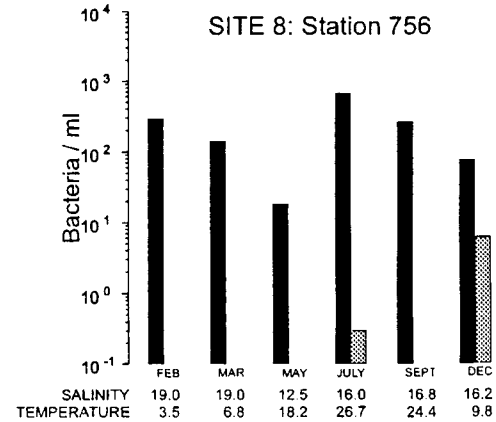

SITE 6: Station 818
Bacteria / ml
FEB MAR MAY JULY N.D. DEC
SALINITY 18.2 18.0 11.6 16.0 N.D. 14.1
TEMPERATURE 3.5 6.5 19.0 27.5 N.D. 9.4


TOTAL
VVAP


SITE 3: Tolly Pt.
Bacteria / ml
FEB MAR MAY JULY SEPT DEC
SALINITY 18.5 12.0 14.0 13.0 13.2 17.1
TEMPERATURE 6.4 5.8 13.3 26.0 24.6 11.9


SITE 7: Station 804
Bacteria / ml
FEB MAR MAY JULY SEPT DEC
SALINITY 18.5 19.0 12.0 16.5 16.8 14.8
TEMPERATURE 3.4 7.0 18.0 27.2 25.2 9.7


SITE 4: Station 845
Bacteria / ml
FEB MAR MAY JULY SEPT DEC
SALINITY 17.4 16.5 9.6 13.5 14.2 12.8
TEMPERATURE 2.8 6.0 18.0 26.8 25.0 9.5


SITE 8: Station 756
Bacteria / ml
FEB MAR MAY JULY SEPT DEC
SALINITY 19.0 19.0 12.5 16.0 16.8 16.2
TEMPERATURE 3.5 6.8 18.2 26.7 24.4 9.8

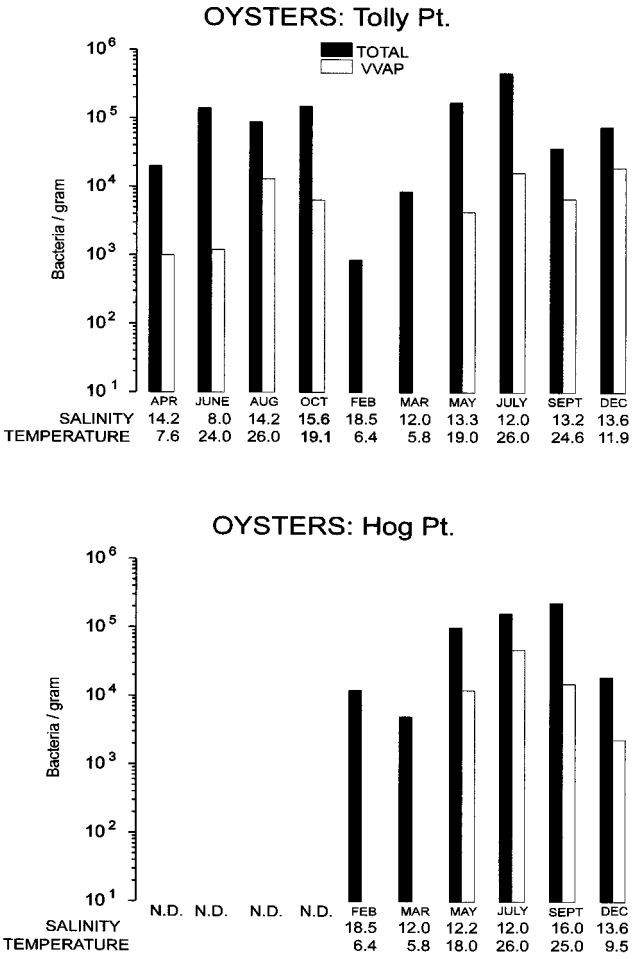


FIG. 3. *V. vulnificus* (VVAP) and total bacteria (total) in oysters. The total number of heterotrophic aerobic bacteria per milliliter was determined by growth on LA medium, and the number of *V. vulnificus* organisms per milliliter was determined by VVAP. See Materials and Methods for details. Samples were collected from April 1991 through December 1992. Temperature (°C) and salinity (ppt) are given for each sample. N.D., no data collected; Pt., Point.

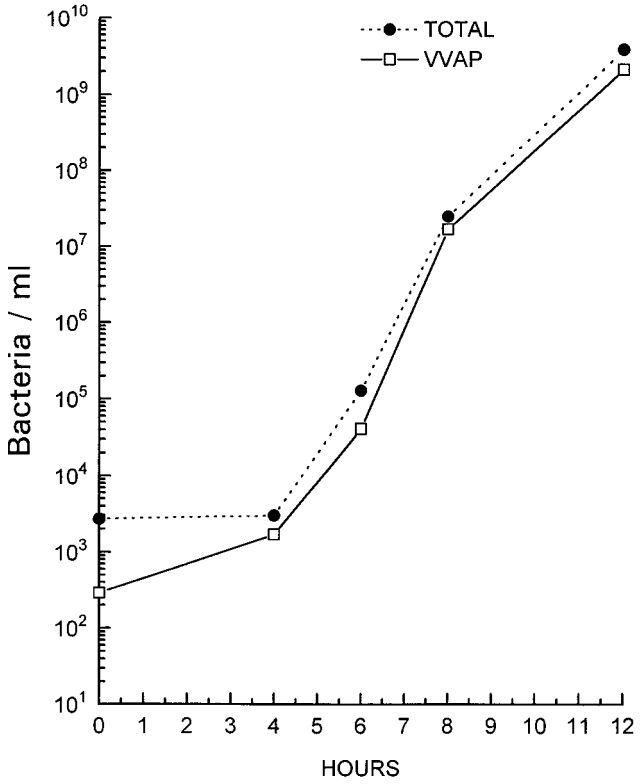


FIG. 4. Growth in enrichment medium. The total number of aerobic heterotrophic bacteria per milliliter was determined by growth on LA medium, and the number of *V. vulnificus* organisms per milliliter was determined by VVAP. See Materials and Methods for details.

during colder months by using enrichment in APW or incubation of water or oyster samples at 25°C prior to plating were unsuccessful even for samples showing a >10-fold increase in overall bacterial counts following treatment (data not given). As shown in Fig. 4, APW enrichment of water samples collected during warmer months of the year showed increased numbers of *V. vulnificus* organisms as well as an increase in the overall proportion relative to the total bacterial population (from 11 to 55%) after incubation for 12 h. Although the recovery of *V. vulnificus* grown on a nonselective medium (LA) was better than that grown on selective medium (TCBS or *V. vulnificus* agar [Table 2]), the addition of artificial seawater to LA did not consistently improve detection (Table 3).

Characterization of environmental isolates. The use of a DNA-based probe in a detection method independent of growth on selective media and without the need to determine a set of phenotypic characteristics resulted in the isolation of a number of *V. vulnificus* strains which did not have the standard biochemical characteristics associated with *V. vulnificus*. Of 67 VVAP-positive strains that grew on TCBS, 6 were sucrose positive. A sample of 20 isolates, randomly picked by selection for luminescence, yielded a VVAP-positive strain. These atypical strains resembled *V. vulnificus* in all other phenotypic characteristics, as determined by API 20E, and are consistent with strain variability for *Vibrio* species (26). All environmental *V. vulnificus* strains isolated from the Chesapeake Bay showed an opaque colony morphology, typical of encapsulation.

DISCUSSION

The Chesapeake Bay is a diverse, well-characterized estuary, with respect to the range of physical and chemical parameters as well as its microbial communities. In the study reported here, *V. vulnificus* was found to be one of the dominant species composing the culturable heterotrophic bacterial population of the Chesapeake Bay. Other investigators have shown that *V. vulnificus* can be readily isolated from water samples collected at locations from New England to Florida on the East Coast (27–29, 40) as well as areas of the Pacific (14) and Gulf Coast (3, 4, 15, 16, 37). Using an oligonucleotide DNA probe specific

FIG. 2. Enumeration of *V. vulnificus* (VVAP) and total bacterial populations (total) in seawater samples. The total number of culturable, heterotrophic, aerobic bacteria per milliliter was determined by growth on LA medium, and the number of *V. vulnificus* organisms per milliliter was determined by VVAP. See Materials and Methods for details. Samples were collected from February through December 1992. Temperature (°C) and salinity (ppt) are given for each sample. N.D., no data collected.

TABLE 1. Numbers of total heterotrophic bacteria and *V. vulnificus* organisms in plankton and sediment samples

Site	No. of organisms in indicated sample[a]							
	Plankton				Sediment			
	Total bacteria		*V. vulnificus*		Total bacteria		*V. vulnificus*	
	May	July	May	July	May	July	May	July
1	410	>10,000	30	2	ND	4,000	ND	0
2	590	610	60	40	ND	840	ND	0
3	450	ND	ND	ND	48,000	11,100	9,900	600
4		>10,000		3		1,230		0
5		ND		ND		360		30
6		ND		ND		1,200		60
7		2,900		3		720		0
8		10,000		5		360		0

[a] Total number of culturable heterotrophic bacteria as determined by plating on LA medium and number of *V. vulnificus* organisms as determined by the VVAP method, expressed as the number of bacteria (10^1) per gram of plankton (wet weight) or sediment (dry weight). See Materials and Methods for details. The sites were sampled during May and July 1992. ND, no data collected.

for *V. vulnificus*, we surveyed the culturable bacterial populations in the Chesapeake Bay and found *V. vulnificus* to be ubiquitous in the estuarine environment, composing ca. 8 to 10% of the total heterotrophic bacterial populations in water and oyster samples collected during warmer months of the year.

A decline in the numbers of *V. vulnificus* organisms during the winter months was observed, suggesting a seasonality for this species in the Chesapeake Bay, as previously reported for *V. parahaemolyticus* (9–11). However, we were able to detect *V. vulnificus* in seawater and oyster samples when water temperatures (8.0 and 7.6°C, respectively) were colder than previously reported; i.e., *V. vulnificus* could not be detected in Gulf of Mexico water samples at temperatures of <12.5°C (16) and, in one study, even at <18°C (33). Environmental surveys contrast with experiments showing that *V. vulnificus* can be maintained for extended periods in oysters at temperatures of <10°C (3, 13, 15). In the study reported here, detection of *V. vulnificus* at lower temperatures may be a function of the sensitivity of the method employed or of adaptation of *V. vulnificus* strains to the colder temperatures found in the Chesapeake Bay environment. O'Neill et al. (29) were able to detect *V. vulnificus* in oysters collected in Maine from water samples at temperatures of ca. 11°C, suggesting that strains of *V. vulnificus* can adapt to the cold.

In response to decreasing temperatures, *Vibrio* species, including *V. cholerae* 01 (41) and *V. vulnificus* (1, 20), have been shown to become nonculturable on solid media while still maintaining viability. It has not yet been demonstrated that *V. vulnificus* populations enter a viable but nonculturable state during winter months in the environment. The results of laboratory studies have suggested that cold-stressed *V. vulnificus* can be induced to revert to a culturable form simply by increasing the temperature to ambient (23). On the other hand, the results of studies with *V. cholerae* 01, by employing dilutions of cold-stressed cells recovered to the same magnitude, suggest the growth of a few culturable cells rather than resuscitation of the entire population on transfer to an elevated temperature (30). Oliver et al. (25) demonstrated that the nonculturable response to a cold temperature is eliminated by prestarvation. Our attempts to resuscitate natural populations by incubation for 6 h at an elevated temperature, with or without the addition of nutrients, were unsuccessful. Further investigations of the viable but nonculturable state are ongoing, and gene probe identification offers a method for assessment of recovery on a variety of media and within a wide range of simulated environmental conditions.

In the multiple regression model, *V. vulnificus* counts in Chesapeake Bay water samples correlated inversely with salinity, a finding in keeping with studies from other estuarine areas. At the same time, *V. vulnificus* has been detected in areas with high salinity in the Atlantic Ocean (3.4×10^2 *V. vulnificus* organisms per ml in one open-ocean sample [38a])

TABLE 2. Enumeration of *V. vulnificus* organisms on different media

Sample[a]	Avg no. of *V. vulnificus* organisms/ml, (10^1) on[b]:		
	LA	TCBS	VVA
Oysters	1,020	117	58
Water	64	4	5
Plankton	34	4	5

[a] Samples were collected in May and July 1992 and processed as described in Materials and Methods.
[b] Results were determined by VVAP (n = 2 to 4). VVA, *V. vulnificus* agar.

TABLE 3. Effect of addition of ASW to medium on bacterial recovery

Sample and site[a]	Avg no. of *V. vulnificus* organisms/total bacteria on[b]:		
	LA	L1/2ASW	LASW
Water			
Site1a	0/230	30/269	20/260
Site1b	10/430	10/840	10/530
Site3a	35/5,900	5/6,800	10/4,200
Site3b	25/4,800	10/5,700	10/4,900
Oysters	6,400/77,000	6,200/46,000	ND/27,200

[a] Samples were collected in October 1991 and processed as described in Materials and Methods.
[b] The number of *V. vulnificus* organisms per milliliter was determined by VVAP (n = 2); the total number of bacteria was determined by plate count on LA, LA plus 50% ASW (L1/2ASW), or LASW as described in Materials and Methods. ND, no data collected.

and in the Gulf of Mexico (3a). Whether these anomalous data are the result of a defined subset within the species or a reflection of the weakness of the correlation of *V. vulnificus* concentrations with salinity is unclear. It has been suggested that salinity and temperature are interdependent and that lower temperatures may increase the tolerance of *V. vulnificus* to higher salinities (13). Additional factors, e.g., sunlight, pH, nutrient concentration, and the presence of lytic phage and/or competing bacterial populations, may also affect survival and distribution of this bacterium in the environment.

As shown for *V. cholerae* 01 (2), *V. vulnificus* is hypothesized to be autochthonous to the estuarine environment, but its ecology remains to be more fully elucidated. *V. cholerae* and *V. parahaemolyticus* colonize planktonic copepods in the environment (7, 11); however, the association of *V. vulnificus* with plankton has not been established, although occasional isolates from plankton samples have been reported (27). As *V. vulnificus* is usually found in seawater in relatively high numbers, it is difficult to determine whether these isolates truly reflect planktonic colonization or seawater contamination. In this study we found that *V. vulnificus* was readily isolated from plankton in the Chesapeake Bay in numbers that generally exceeded those found in water samples, indicating the attachment of *V. vulnificus* to plankton. The results of studies with *V. cholerae* have shown specific association of vibrios with live but not dead copepod populations, suggesting expression of a specific attractant or receptor for colonization (7). While no attempt was made in this study to correlate the association of *V. vulnificus* with specific planktonic species, future surveys will include specific planktonic populations, e.g., zooplankton species and oyster larvae.

The relationship of *V. vulnificus* with oysters in the environment has been previously demonstrated (8, 13, 35), notably within hemocytes of oyster tissue (5). *V. vulnificus* also has been shown to be resistant to depuration, a procedure that is effective in eliminating other bacterial species from oysters (8). The distribution of *V. vulnificus* globally is not clear-cut, since oysters harvested from warm waters of the Gulf of Mexico and assayed for *V. vulnificus* by a most-probable-number procedure were occasionally negative for this organism, even when $>10^2$ *V. vulnificus* organisms per ml could be detected in water samples at sites from which oysters were collected (37). In the present study, *V. vulnificus* was detected in all oyster samples collected at both locations during warmer months. At temperatures as low as 7.6°C, *V. vulnificus* could still be enumerated at concentrations similar to those commonly reported for Gulf of Mexico shellstock (13). On the basis of the small number of samples examined, sediment within or around oyster beds was more likely to contain *V. vulnificus* than those areas not associated with oysters. The exact nature of the relationship between *V. vulnificus* and the Eastern oyster, *Crassostrea virginica*, in the Chesapeake Bay has yet to be determined.

Data on population sizes of *V. vulnificus* are few, especially outside the Gulf of Mexico. Bacterial populations are difficult to characterize by conventional microbiological methods, especially because of problems inherent in the use of selective or enrichment media. Thus, a DNA probe was chosen for identification and its use permitted direct enumeration of *V. vulnificus* organisms, without enrichment or cultivation on selective media. Selective and/or enrichment media are known to yield decreased sensitivity of detection for natural populations for a variety of reasons, including poor or no growth on selective media (15, 39), as well as competition with other bacteria composing the microbial community of the natural environment when an enrichment broth is employed (36). In this study, it was found that during the summer months, selective media yielded about 50% fewer detectable *V. vulnificus* organisms. Other data (15) suggest that when *V. vulnificus* is cold stressed, recovery on selective media may be even further reduced. Additional studies are underway to extend the application of the VVAP gene probe method under different environmental conditions and for other bacterial communities. In conclusion, *V. vulnificus* has been considered primarily a problem for the Gulf Coast oyster industry; however, the results from in this study demonstrate that it is widely distributed in the Chesapeake Bay water and present at concentrations in oysters comparable to those found in the Gulf of Mexico.

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