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Isolation and Characterization of *Vibrio vulnificus* from Two Florida Estuaries

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Vibrio vulnificus was enumerated in seawater and shellfish from two Florida estuaries at selected seasonal intervals. There were significant fluctuations in the presence and numbers of *V. vulnificus*. Relatively high seawater temperature and salinity favored the presence of *V. vulnificus* in both seawater and shellfish samples.

Vibrio vulnificus is a lactose-fermenting, halophilic, gram-negative, and potentially pathogenic marine vibrio. Reports of its occurrence in seawater and association in human infections are increasing (2, 3, 5).

It has been suggested that some *Vibrio* spp. may be indigenous to estuarine microflora (4). Kaneko and Colwell (4) have reported high levels of *Vibrio parahaemolyticus* in seawater during the warm months and low levels in cold months, appearing mostly in sediments.

This study reports the seasonal occurrence of *V. vulnificus* in both seawater and shellfish from two Florida estuaries which are closely associated with both shellfish harvesting and recreational activities.

Seawater, oyster, and clam samples were collected from three Tampa Bay and eight Apalachicola Bay stations (Fig. 1–3). Tampa Bay samples were collected in May and October 1980 and in January, April, and July 1981. Apalachicola Bay samples were obtained in April, July, and November 1980 and in February, May, and August 1981. Stations were classified as approved, unapproved, or conditionally approved for shellfish harvesting by the Florida State Department of Natural Resources (Fig. 1 and 3). Seawater was obtained 2 ft (ca. 61 cm) or less below the surface on an outgoing tide. Shellfish were collected with the associated seawater samples, transferred to the laboratory, and prepared for bacteriological analysis by established techniques (1, 6).

Specifically, oysters and clams were cleaned, shucked, weighed, and homogenized in a two-fold dilution (wt/vol) of peptone water. Seawater and homogenized shellfish samples were serially diluted in 10-fold increments of 100 ml of 0.5% peptone water (pH 7.2). Serially diluted sample (1 ml) was transferred immediately into triplicate tubes containing 9 ml of alkaline peptone broth (pH 8.4). After 12 h of incubation at 37°C, these inocula were streaked from alkaline peptone broth onto thiosulfate-citrate-bile salts-sucrose agar (Difco Laboratories). Plates were incubated for 24 h at 37°C. All typical green and gray colonies were inoculated into API (Analytab Products) biochemical strips for species identification. A most-probable-number (MPN) estimate of *V. vulnificus* numbers was then determined from standard MPN tables for a three-tube series.

Seawater temperature and salinity were measured in situ with a Beckman RS5-3 thermister-salinometer. Fecal coliform MPN was determined by standard methods (6).

The levels of *V. vulnificus* and selected environmental data at Apalachicola Bay are shown in Table 1. *V. vulnificus* was isolated more often from both seawater and oyster samples during the months of July and November than at the other sampling times. *V. vulnificus* was not detected during April and February in either seawater or oyster samples. In May, relatively high levels were detected in some seawater samples but not in oysters. In August, *V. vulnificus* was again primarily detected in seawater; however, at one site, oysters contained relatively high counts. Examination of environmental data shows that *V. vulnificus* was isolated more often when the water temperature was greater than 17°C and in a greater proportion of samples over 29°C. In addition, *V. vulnificus* was isolated more frequently in waters with a salinity greater than 17.0‰ and in a higher proportion of samples greater than 23.0‰. Interestingly, *V. vulnificus* was found more often in waters with a fecal coliform MPN of less than 3 per 100 ml of seawater.

The numbers of *V. vulnificus* detected at

TABLE 1. Levels of *V. vulnificus* and environmental data[a] at selected seasonal intervals at Apalachicola Bay

Date and station no.	Temp (°C)	Salinity (°/oo)	Fecal coliform MPN	*V. vulnificus* MPN	
				Oyster	Seawater
April 1980					
29	20.5	0.0	15	<0.3	<0.3
36	18.0	12.0	<0.3	<0.3	<0.3
37CB	23.4	12.1	<0.3	<0.3	<0.3
8C	22.4	1.2	23	<0.3	<0.3
34	23.3	5.4	<0.3	<0.3	<0.3
19	21.2	0.0	7	<0.3	<0.3
16	22.5	0.9	11	<0.3	<0.3
15B	21.9	0.9	15	<0.3	<0.3
July 1980					
29	29.4	28.0	<0.3	<0.3	430
36	29.0	10.9	<0.3	2,300	2,300
37CB	29.0	14.5	<0.3	43	930
8C	29.5	31.0	<0.3	93	<0.3
34	28.9	8.2	<0.3	43	43
19	29.9	30.3	<0.3	4.3	930
16	29.4	31.2	<0.3	<0.3	430
15B	29.4	30.4	<0.3	93	430
November 1980					
29	18.6	21.9	93	9.3	23
36	18.9	23.8	4	46	240
37CB	18.9	23.5	23	110	280
8C	19.3	25.3	9	24	150
34	19.2	22.5	<0.3	110	93
19	19.2	20.7	43	15	93
16	19.2	32.2	<0.3	15	43
15B	19.1	25.3	93	9.3	43
February 1981					
29	10.7	0.8	240	<0.3	<0.3
36	7.7	22.3	4	<0.3	<0.3
37CB	8.5	19.5	<0.3	<0.3	<0.3
8C	9.2	12.7	9	<0.3	<0.3
34	3.1	18.5	23	<0.3	<0.3
19	9.4	10.3	9	<0.3	<0.3
16	9.5	21.0	9	<0.3	<0.3
15B	9.5	13.3	15	<0.3	<0.3
May 1981					
29	24.3	17.3	13	<0.3	<0.3
36	24.0	20.8	7	<0.3	46,000
37CB	24.3	17.3	<0.3	<0.3	<0.3
8C	24.5	25.8	7	1.1	<0.3
34	24.3	17.1	13	<0.3	24,000
19	25.1	15.3	13	<0.3	<0.3
16	23.8	25.2	<0.3	110	46,000
15B	24.4	24.7	<0.3	<0.3	<0.3
August 1981					
29	30.4	20.2	43	<0.3	150
36	29.3	19.8	<0.3	<0.3	240
37CB	29.6	19.8	<0.3	3,000	<0.3
8C	29.7	25.1	<0.3	<0.3	<0.3
34	29.5	19.7	<0.3	<0.3	93
19	30.3	16.4	<0.3	<0.3	<0.3
16	29.4	25.7	<0.3	<0.3	43
15B	29.5	22.7	7	<0.3	<0.3

[a] Values for fecal coliform MPN are per 100 ml of seawater. Oyster-associated *V. vulnificus* MPN calculated per gram of oyster (wet weight); seawater-associated *V. vulnificus* MPN calculated per 100 ml of seawater.

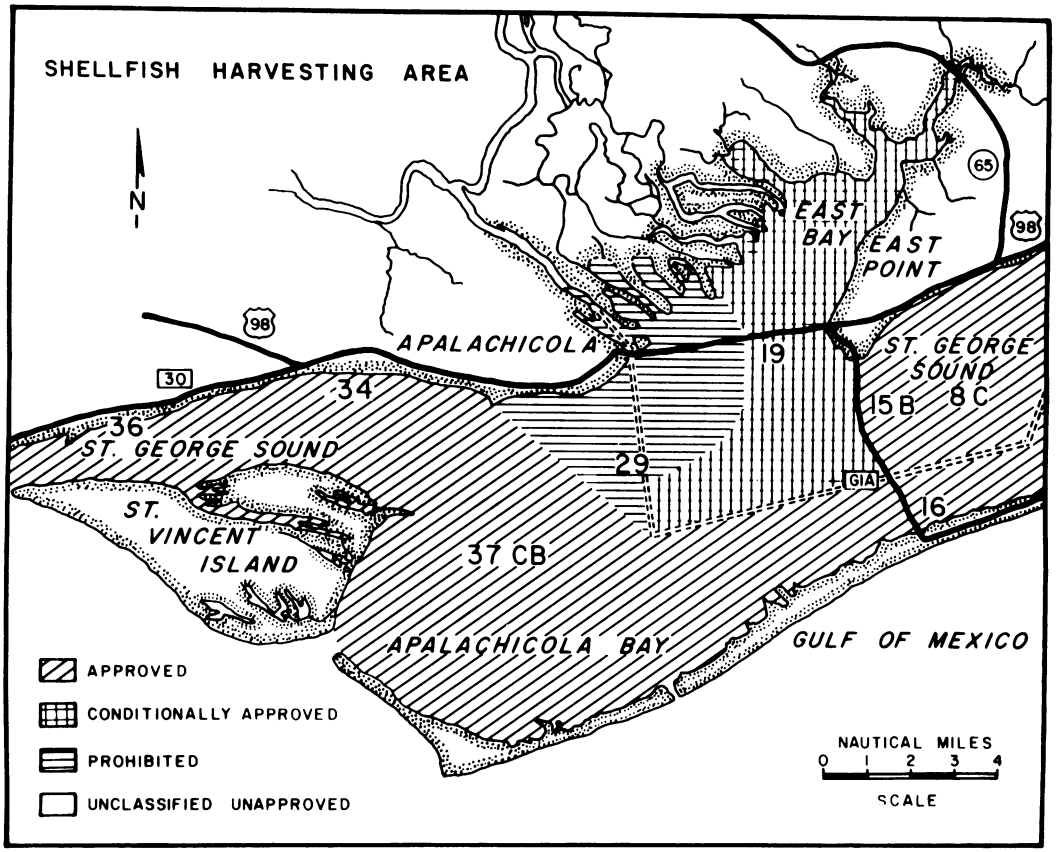

FIG. 1. Eight collection stations in Apalachicola Bay, Fla. (stations no. 29, 36, 37CB, 8C, 34, 19, 16, and 15B).

TABLE 2. Levels of *V. vulnificus* and environmental data[a] at selected seasonal intervals at Tampa Bay

Date and station no.	Temp (°C)	Salinity (‰)	Fecal coliform MPN	*V. vulnificus* MPN		
				Oyster	Clam	Seawater
May 1980						
1	28.0	25.6	240	<0.3	<0.3	<0.3
2	28.0	31.5	43	—	<0.3	<0.3
3	28.2	32.4	9	<0.3	<0.3	<0.3
October 1980						
1	30.8	20.7	4,600	930	—	15,000
2	31.6	27.1	23	—	9.3	12,000
3	36.2	31.4	<0.3	<0.3	<0.3	110
January 1981						
1	8.5	18.2	110,000	<0.3	—	<0.3
2	9.6	28.2	460	—	<0.3	<0.3
3	13.2	28.1	<0.3	<0.3	<0.3	<0.3
April 1981						
1	24.7	29.0	39	<0.3	—	<0.3
2	25.1	32.7	4	—	<0.3	<0.3
3	27.6	33.0	11	<0.3	<0.3	<0.3
July 1981						
1	31.0	30.5	39,000	<0.3	—	700,000
2	31.0	34.0	<0.3	—	<0.3	<0.3
3	31.0	35.0	23	<0.3	<0.3	<0.3

[a] Values for fecal coliform MPN are per 100 ml of seawater. MPNs for oyster- and clam-associated *V. vulnificus* are per gram of shellfish (wet weight); seawater-associated organism counts are per 100 ml of seawater. —, Not available for testing at the site.

Tampa Bay are shown in Table 2. *V. vulnificus* was not detected in the January or April samplings. However, a single seawater sample taken in July contained relatively high numbers of *V. vulnificus*. In addition, high numbers and frequent isolations were observed during the October sampling in both shellfish and seawater samples from unapproved sites. A comparison of environmental data and *V. vulnificus* numbers at Tampa Bay differed slightly from that of Apalachicola Bay. Specifically, *V. vulnificus* was isolated only in waters with a temperature greater than 30°C. In addition, *V. vulnificus* was found only in waters with a salinity greater than 17.0°/₀₀. *V. vulnificus* was also isolated from waters with either a high or low fecal coliform MPN.

The two estuaries which we examined contained significant numbers of *V. vulnificus* that fluctuated greatly between samplings. In both estuaries, there were times when seawater, oysters, and clams, all showed no detectable levels of *V. vulnificus*. Preliminary data suggest that seawater temperature and salinity may play important roles in this variance in numbers from sampling to sampling. *V. vulnificus* was detected frequently when the water temperature was greater than 17°C, but never below 17°C. In addition, salinities greater than 17.0°/₀₀ appear to be favorable for isolation of *V. vulnificus*. There appeared to be higher numbers and more frequent isolation of *V. vulnificus* from samples taken during the summer and fall months. Waste comtamination and land runoff may contribute to the presence of this bacterium, either directly by supplying needed nutrients or indirectly by enriching for some other physical or biological factor. The ecology of *V. vulnificus* at this time is unclear. Obviously, more ecological investigations are needed to understand the relationship of this organism within the marine environment. It must be emphasized that our studies have dealt with one-point-in-time analyses at different stations over seasonal intervals. Fluctuations in biological or physical factors may occur over shorter time intervals, necessitating intensive samplings for a truly statistical analysis of the presence and numbers of *V. vulnificus* in the marine environment.

It is interesting to note that reported human vibrio infections in Florida occurred primarily

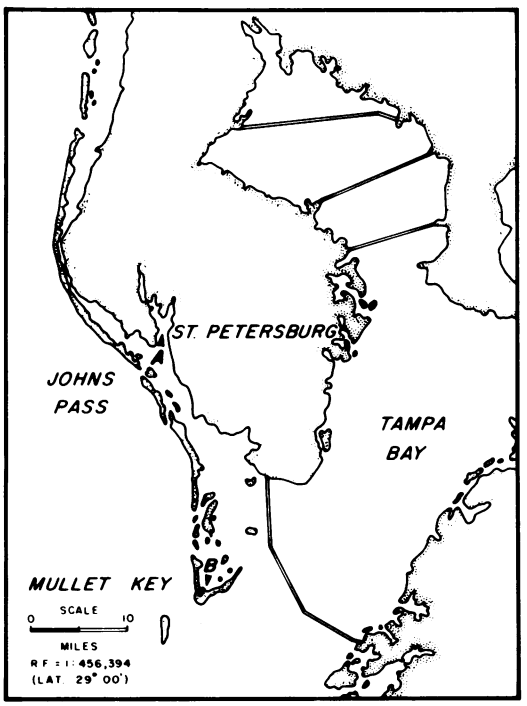

FIG. 2. Collection sites (A and B) in Tampa Bay, Fla.

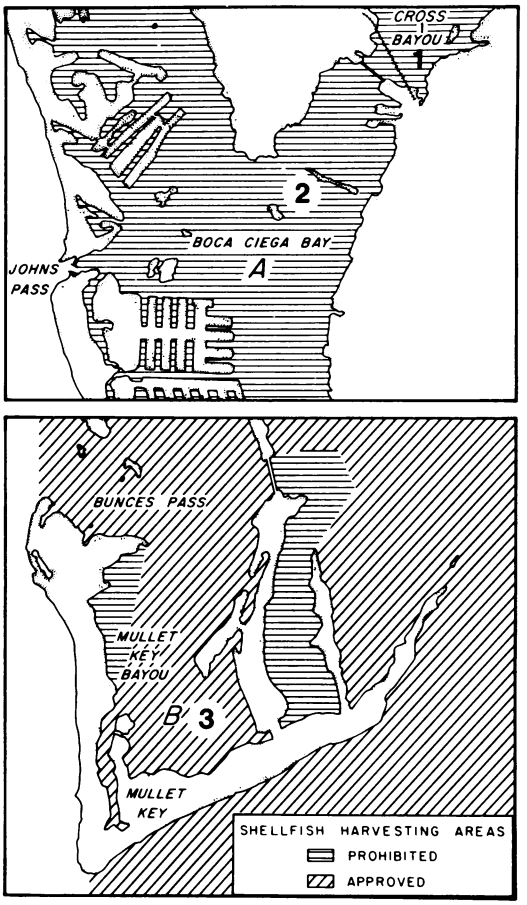

FIG. 3. Shellfish harvesting stations (A, 1 and 2 and B, 3) at the Tampa Bay collection sites.

during the fall months, correlating with our isolation of vibrios in shellfish and seawater for that period.

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