

## QUANTIFICATION AND DISTRIBUTION OF VIBRIO SPECIES IN WATER FROM AN ESTUARY IN CEARÁ-BRAZIL IMPACTED BY SHRIMP FARMING

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### ABSTRACT

Vibrios were quantified and their distribution determined for the estuary of the Coreaú river, in Northeastern Brazil, based on 24 water samples collected between June and October 2005. The most probable number of vibrios per 100 mL ranged from  $230 \times 10^3$  to  $240 \times 10^{11}$ . The pH value was the environmental factor most strongly associated with the abundance of vibrios. Sixty-two vibrio strains were isolated belonging to 15 species (6 of which observed in June-September and 8 in October). The most frequently isolated species were *V. parahaemolyticus* and *V. cholerae*.

### RESUMO

Foram realizadas análises concernentes à quantificação e distribuição de *Vibrio* em 24 amostras de água do estuário do Rio Coreaú (CE) no período de junho a novembro de 2005. O Número Mais Provável (NMP/100 mL) de *Vibrio* oscilou de  $230 \times 10^3$  a  $240 \times 10^{11}$ . O pH foi o fator ambiental que mais favoreceu ao aumento da microbiota de *Vibrio*. Foram isoladas 62 cepas de *Vibrio*, sendo observada uma distribuição de sete espécies nos meses de junho a setembro, e oito espécies no mês de outubro. As espécies mais frequentes nos isolamentos foram *V. parahaemolyticus* e *V. cholerae*.

Descriptors: *Vibrio*, Estuary, Shrimp farming.  
Descritores: *Vibrio*, Estuário, Carcinicultura.

### INTRODUCTION

Bacteria of the genus *Vibrio* are ubiquitous in estuaries (HEIDELBERG et al., 2002; THOMPSON et al., 2004; BLACKWELL; OLIVER, 2008) and have been known to infect cultivated marine shrimp (AGUIRRE-GUZMÁN et al., 2001; ALAVANDI et al., 2006; VASEEHARAN et al., 2008). According to Alvarez et al. (2003), the shrimp farming industry is permanently on guard against infectious diseases and other setbacks that might harm production.

Vibrios are not only a major economic threat to marine shrimp farming, but represent a public health concern as they are easily transmitted to humans through the consumption of seafood (BLACKSTONE et al. 2003; FUENZALIDA et al., 2007; LEE et al., 2008).

On the other hand, shrimp farming in estuaries is generally associated with the outlet of nutrients and organic matter into the environment, causing a negative impact on the native microbiota. Research on the impact of shrimp farming has shown that discharges from farms significantly raise the levels of nutrients in coastal environments (PÁEZ-OSUNA et al., 1998; TROTT; ALONGI, 2000; PÁEZ-OSUNA, 2001). Sousa et al. (2006) observed increasing vibrio counts in water samples from an estuary in Ceará exposed to the discharge of nutrients from shrimp farms.

Thus, the objective of the present study was to quantify and determine the distribution of vibrio species in an estuary in Ceará-Brazil impacted by shrimp farming.

## MATERIALS AND METHODS

### Sampling

Twenty-four water samples were collected from two locations, twelve at each location (Location 1 – 3°5'39.3"S, 40° 49'8.05"W and Location 2 – 3°5'46.42"S, 40°49'7.78"W, Fig. 1) in the Coreaú river, 250 km west of Fortaleza, Ceará, bimonthly between June and October 2005. The period covered the end of the rainy season and 4 months of the dry season. The samples were collected in sterilized amber-colored 1-liter vials and stored in a refrigerator until the moment of analysis, no longer than 2 hours after sampling.

### Sample Preparation

The samples were diluted at 1:9 in 0.85% saline solution in order to prepare serial decimal dilutions from  $10^{-1}$  to  $10^{-8}$ .

### Quantification of *Vibrio*

The multiple-tube technique was used to determine the most probable number (MPN) of vibrios in the samples (KAYSNER; DEPAOLA, 2004). Presumptive tests were performed with 1mL of each dilution ( $10^{-1}$  to  $10^{-8}$ ) inoculated in alkaline peptone water containing 1% NaCl (pH 8.5), followed by incubation for 24 hours at 35°C. The confirmatory

tests were made by spread-plating 0.1-mL inoculates from tubes with positive growth onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar, followed by incubation for 18 hours at 35°C. MPN was calculated by multiplying the critical series by the average dilution, and results were expressed as MPN/100mL (GARTHRIGHT, 2001).

### Isolation and Identification of *Vibrio*

Approximately three colonies (saccharose-positive and negative) were selected from the TCBS plate and isolated in tryptic soy agar (TSA) containing 1% NaCl, followed by incubation for 24 hours at 35°C. Pure strains were submitted to phenotypical identification as described in the literature (ALSINA; BLANCH, 1994a; ALSINA; BLANCH, 1994b). Biochemical testing with arginine dihydrolase, lysine and ornithine decarboxylase was used as screening to select lineages for Alsina's identification key. The tests used to identify the species were as follows: oxidase, growth at 0, 3, 6, 8 and 10% NaCl, sucrose acid, arabinose acid, mannitol acid, glucose gas, lactose acid, mannose acid, indole, citrate, Voges Proskauer test, ONPG (alfa-nitrophenyl- $\beta$ -D-galactopyronoside), growth at 4, 35 and 40°C, gelatinase, resistance to O/129 10  $\mu$ g and resistance to ampicillin 10  $\mu$ g.

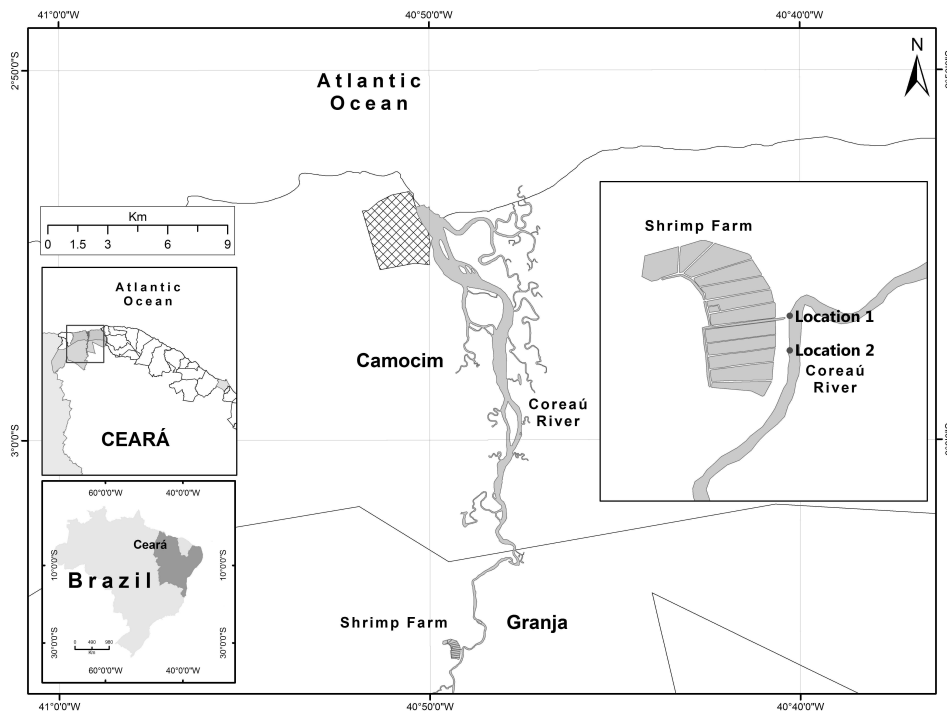


Fig. 1. Sampling points in the Coreaú river.

Environmental Variables

Temperature, salinity and pH were measured with a pH meter (Hanna instruments pH 211) and a handheld digital refractometer (Digit, model 211).

RESULTS

The most probable number of vibrios ( $\log_{10}$  NMP) per 100mL in water from sampling points 1 and 2 ranged from 6.36 to 10.04 and from 5.36 to 13.38, respectively (Table 1). The corresponding pH values were 6.53–8.22 and 7.66–8.19. Small variations were

observed for temperature (28.7–31.6°C and 29.0–31.4°C), but variations for salinity were considerable (0–36‰ and 1–43‰), respectively (Table 1).

Pearson’s correlation coefficients (r) between environmental variables (pH, temperature and salinity) and MPN of vibrios are shown in Table 2.

Altogether, 62 vibrio strains belonging to 15 species were isolated from the 24 water samples (Table 3). The predominant species were *V. parahaemolyticus* (16.12%), *V. cholerae* (14.51%) and *V. alginolyticus* (12.9%).

Table 1. Most probable number ( $\log_{10}$  MPN) of vibrios per 100 mL water and corresponding environmental variables in 24 samples from the estuary of the Coreaú river, Ceará.

Samples	Point 1				Point 2			
	MPN	T.	pH	S.	MPN	T.	pH	S.
1	7,63	31,3	6,53	0	8,17	29,5	7,85	1
2	6,36	28,7	7,58	0	9,66	30,2	7,83	1
3	7,36	29,5	7,70	1	8,96	29,3	8,10	5
4	10,04	29,4	7,57	1	7,36	31,4	7,28	14
5	7,36	30,03	8,18	9	6,36	31,0	7,78	25
6	7,20	31,0	7,45	13	5,36	30,5	7,66	24
7	6,36	31,0	7,77	25	7,36	28,4	7,71	32
8	8,63	29,9	8,22	24	6,63	30,4	7,72	30
9	7,55	28,8	7,73	34	6,96	29,8	7,83	36
10	6,96	30,3	7,79	30	7,47	29,3	7,76	30
11	6,55	29,2	7,83	36	9,38	29,0	7,90	43
12	8,46	29,7	7,74	33	13,38	29,6	8,19	34

\*T: Temperature; S: Salinity.

Table 2. Pearson’s correlation coefficients (r) between environmental variables (pH, temperature [°C] and salinity [‰]) and the most probable number (MPN) of vibrios per 100mL water in 24 samples from the estuary of the Coreaú river, Ceará.

Point	Environmental Variables	MPN/100mL de <i>Vibrio</i>
1	pH	0,5283
	Temperature	-0,2959
	Salinity	0,1699
2	pH	0,6670
	Temperature	-0,3332
	Salinity	-0,0103

Table 3. Distribution of 15 vibrio species in 24 water samples from the estuary of the Coreaú river, Ceará, collected between June and October 2005.

Month	Species
June (n=12)	<i>Vibrio</i> spp (3), <i>V. cholerae</i> (2), <i>V. damsela</i> (2), <i>V. fluvialis</i> (2), <i>V. mimicus</i> (1), <i>V. hollisae</i> (1), <i>V. anguillarum</i> (1)
July (n=11)	<i>V. cholerae</i> (4), <i>V. damsela</i> (2), <i>V. parahaemolyticus</i> (1), <i>Vibrio</i> spp (1), <i>V. carchariae</i> (1), <i>V. harveyi</i> (1), <i>V. vulnificus</i> (1)
August (n=11)	<i>V. parahaemolyticus</i> (2), <i>V. cholerae</i> (2), <i>V. splendidus</i> (2), <i>V. harveyi</i> (2), <i>Vibrio</i> spp (1), <i>V. hollisae</i> (1), <i>V. fluvialis</i> (1)
September (n=12)	<i>V. parahaemolyticus</i> (4), <i>Vibrio</i> spp (3), <i>V. alginolyticus</i> (1), <i>splendidus</i> (1), <i>V. cincinnatiensis</i> (1), <i>V. metschnikovii</i> (1), <i>V. carchariae</i> (1).
October (n=16)	<i>V. alginolyticus</i> (7), <i>V. parahaemolyticus</i> (3), <i>V. harveyi</i> (1), <i>V. cholerae</i> (1), <i>V. damsela</i> (1), <i>carchariae</i> (1), <i>V. fluvialis</i> (1), <i>V. furnissi</i> (1)

n= number of isolates

## DISCUSSION

The great abundance of vibrios in the estuary of the Coreáú river may be related to several anthropogenic effects, perhaps primarily to the increasing amount of nutrients and organic matter discharged by shrimp farms. Booming socioeconomic activities, such as shrimp farming and tourism, have exposed the estuary and surrounding mangrove lands to unprecedented impacts (MEIRELES; SILVA, 2002). However, according to Lacerda (2006), the amount of nitrogen and phosphate discharged into the Coreáú river by organized shrimp farming is much smaller than the amounts contributed by agriculture and animal husbandry. On the other hand, Das et al. (2004) reported the discharge of nutrients from shrimp farms along Indian estuaries to have caused severe impacts on the environment.

The highest MPN values were found in samples with high pH (8.22 at Point 1 and 8.19 at Point 2). In fact, Pearson's correlation coefficient between environmental variables and MPN indicates that high pH values were the environmental factor which most contributed to the increase in the abundance of vibrios in the Coreáú river. pH values in the range 8.4–8.6 are considered ideal for vibrio growth (DONOVAN; NETTEN, 1996). Gonçalves et al. (2004) observed a significant correlation between pH values (6.2–9.8) of water samples from the estuary of the Baía de São Marcos (São Luís, Maranhão, Brazil) and the amount of *V. cholerae* isolated.

The low salinity observed in samples collected in June may be explained by the influence of seasonal rainfall. However, in the dry season between August and October salinity rose as evaporation increased. As shown by Pearson's correlation coefficient, salinity and MPN values were not significantly correlated in our samples. In fact, according to Murakami et al. (1998), vibrios are indeed very flexible with regard to salinity.

The absence of a correlation between temperature and MPN values may be due to the modest variation (2.9°C) observed. Serra et al. (2003) reported similar results in a study on the relation between vibrios and physicochemical parameters in the estuary of the Anil river (São Luís, Maranhão, Brazil) and attributed the absence of significant variation to the geographical location near the Equator. Vibrios are mesophilic and are most common in tropical coastal waters (HERVIO-HEATH et al., 2002). Cervino et al. (2004) also found that vibrios grow best in temperatures between 17 and 35°C.

The distribution of vibrio species in our samples presented insignificant variations, probably because of the relatively stable temperature and pH values. Molitoris et al. (1985) studied the distribution of *V. parahaemolyticus* in the bay of Jakarta (Indonesia) and

reported the species to be more abundant during the dry season. The present findings lend support to this observation since *V. parahaemolyticus* was most frequently isolated in samples collected in September and October. The presence of *V. cholerae* strains in our water samples also corroborates the findings of Thompson et al. (2003), who reported *V. cholerae* to be ubiquitous and abundant in aquatic environments, especially in coastal areas, estuaries and rivers. The authors also reported that *V. cholerae* has caused many deaths in Brazil over the past decade and suggest that *V. cholerae* strains may be successfully adapting to changes in environmental conditions.

The frequency with which vibrios were isolated from our samples may be compared to the results of Barbieri et al. (1999) who isolated a large number of *V. alginolyticus*, *V. parahaemolyticus*, *V. cholerae* non-O1 and *V. vulnificus* in water samples from two estuaries on the Italian Adriatic coast. The authors report that some of the most toxigenic strains have been associated with a range of infections, from gastroenteritis to severe septicemia.

The occurrence of *V. harveyi* in the Coreáú river may represent a concern to local shrimp farmers. The species has been a major etiologic agent in vibrio infections in shrimp populations and responsible for serious economic losses in Southeast Asia (CONEJERO; HEDREYDA, 2003).

Macián et al. (2000) found a great abundance of vibrios in seawater samples from Valencia, Spain, especially *V. harveyi* (24% of strains), *V. splendidus* (19%) and *V. alginolyticus* (8%). This is comparable to our findings for the Coreáú river, with the exception of *V. splendidus* with only 3 strains isolated from water samples at 28.4–31.4°C.

The occurrence of *V. vulnificus* in our samples is in accordance with Yano et al. (2004), who say the species is a typical component of the estuarine microbiota which has been implicated in food contamination and septicemia. In their study on the ecology of *V. vulnificus* in estuaries, Pfeffer et al. (2003) only isolated the species in samples at 15–27°C, and reported the abundance of vibrios to be dependent mainly on temperature, turbidity and dissolved oxygen levels. Their results do not match our own since the only *V. vulnificus* strain isolated in our study came from a sample at more than 28°C.

Vibrios were abundant during the entire study period. Although the study design did not include a control, the authors suspect the consistently high indices of vibrios may be explained by the discharge of nutrients from local shrimp farms. The frequent isolation and abundance of certain vibrio species in samples from the Coreáú river indicate a potential risk to intensive aquaculture and to public health, considering the amount of seafood extracted from the region for human consumption.

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