

Raw oysters can be a risk for infections

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ABSTRACT

The aims of this study were to count and identify sucrose positive and negative vibrios isolated from cultivated *Crassostrea rhizophorae* oysters during their growing cycle. Every month for 12 months, 10 to 18 oysters were collected for study. Collections occurred at the Center for Studies of Coastal Aquaculture (CSCA), which is associated with the Institute of Marine Science, Labomar, located in Euzébio, Ceará, Brazil. Approximately 150 oysters and their intervalvular liquor were studied. *Vibrio* Standard Plates Counts (SPC) from oyster meat and their intervalvular liquor varied from 25 to 59,000,000 CFU/g. For most of the 12 months of the oysters' life, it was possible to identify *Vibrio parahaemolyticus*. *Vibrio carchariae* was identified in four collections. Among other isolated species, the most important, considering public health risks, was *V. vulnificus*, although only one strain was confirmed. We concluded that retail purchased oysters should never be eaten raw or undercooked because many species of the genus *Vibrio* are known to be pathogenic to humans and live naturally on and in shellfish throughout their life cycle.

Keywords: *Vibrio* spp, oyster, public health, *Vibrio parahaemolyticus*, *Vibrio carchariae*.

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INTRODUCTION

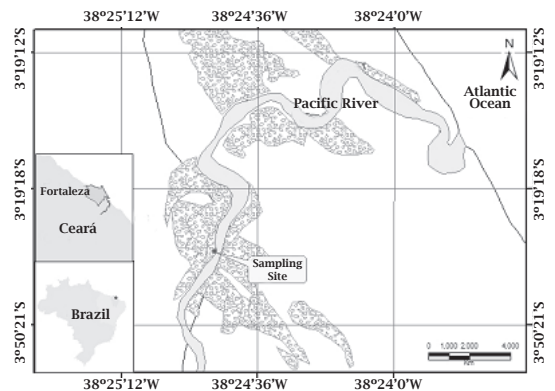
Over the last two decades, the intensification of penaeid shrimp culture industry and transportation of aquatic organisms worldwide have been accompanied by increased incidence of infectious pathogens¹ and illnesses. In this context, *Vibrio* spp., a bacterium indigenous to coastal and marine environments, occasionally causes opportunistic human infections.^{2,3} Being an aquatic bacteria, species of the genus *Vibrio* infect not only humans but also all aquatic animals, resulting in problems in shrimp and shellfish farming. Aquatic animals once infected with any *Vibrio* species should not be consumed raw to avoid the risk of illness. *Vibrios* are prevalent in warm estuarine areas throughout the world. In the United States virtually all human infections caused by *V. vulnificus* result from the consumption of oysters harvested from the Gulf of Mexico.^{4,5} *Vibrio parahaemolyticus* and *Vibrio cholerae* are species that can also cause gastroenteritis and may result in more severe infections than those caused by sewage-borne viral and bacterial pathogens.⁵ The aims of this study were to count and identify sucrose positive and negative vibrios, isolated from cultivated oysters, from juvenile to commercial marketing size.

MATERIAL AND METHODS

Field sampling sites

Crassostrea rhizophorae oysters were collected from the Center for Studies of Coastal Aquaculture (CSCA) which is associated with the Institute of Marine Science, Labomar, located in Euzébio, Ceará, Brazil (Figure 1). Oysters were selected for study based on their size and growth cycle stage.

Figure 1: Map of the sampling site for mangrove oyster, *Crassostrea rhizophorae*, located at Center for Studies of Coastal Aquaculture (CSCA) in Euzébio, Ceará state, Brazil.



Sample collection

Every month for 12 months, 10 to 18 oysters were collected for study. After 12 months, the oysters reached commercial size and were sold at market places. Approximately 150 oysters and their intervalvular liquor were studied. The pH of oyster meat and intervalvular liquor was measured immediately after opening.

Bacteriological count and isolation

Monthly, oysters were collected from their oyster bed at CSCA in Euzébio. They were put in a box with ice and transported under aseptic conditions to the Environmental Microbiology Laboratory at Labomar where the samples were processed. One gram of oyster meat was mixed with 10 mL of a sterile saline solution (1%). From these, serial dilutions were prepared (10^{-2} to 10^{-4}) using sterile saline solution (1%) in test tubes. From each dilution 0.2 mL was spread onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar media in duplicate and the plates were incubated at 36°C for 18 to 24 hours. The colonies that grew on the plates were counted and the sucrose positive and negative colonies were recorded. Sucrose positive and negative *Vibrio* isolates were streaked for purity onto Tryptone Soy Agar (TSA) supplemented with 1% NaCl. The pure strains were sent to the Oswaldo Cruz Foundation in Rio de Janeiro where they were identified.

Vibrio identification

From growth on TCBS agar, 3 to 4 colonies were transferred to Trypticase Soy Agar (TSA) slants and incubated at 35°C/24h. Isolates were identified using the phenotypic tests described by Alsina and Blanch⁶ and Elliot *et al.*⁷ Tests included bacterioscopy by Gram staining; motility (SIM), production of cytochrome oxidase test; TSI reaction; arginine hydrolysis, and lysine and ornithine decarboxylation; halophilic differ-

entiation test (at 0, 3, 6, 8 and 10% NaCl); acetoin production (Voges-Proskauer test); growth at 42°C; hydrolysis of o-nitrophenyl- β -D-galactopyranoside (ONPG); carbohydrate fermentation (glucose, mannitol, lactose, sucrose, arabinose, and mannose), and the indole production test.

RESULTS AND DISCUSSION

The *Vibrio* Standard Plate Counts (SPC) from the oysters and their intervalvular liquor varied from 25 to 59,000,000 CFU/g (Table 1). *Vibrio* counts varied enormously and followed no logic. Even smaller individuals (less than 2.5 cm in diameter, collection 1) showed higher *Vibrio* counts than those with 4,67 cm in diameter of collection 5 (Figure 2). Sometimes, even on the lowest dilutions it was not possible to find counting between 25 and 250 colonies, and that's why, in results, the estimated colony forming unit per gram (CFU/g est) was used (collections 2, 5 and 6 – Table 1).

After 12 months, oysters reach a 6.41 cm diameter or larger and may be commercialized. From previous studies and according to literature, it is known that there are two main environmental conditions influencing numbers of cultivable *Vibrio* spp. from coastal environments. First, temperature has a marked influence on the occurrence of vibrios and secondly, seawater salinity exerts a strong influence on the survival of *Vibrio* spp.^{8,9} Northeastern Brazilian coastal waters temperatures are warm and this might explain the high *Vibrio* counts recorded in this study (Table 1). Barros *et al.*¹⁰ found lower values for *Vibrio* counts from raw oyster meat. Their counts ranged from 93 to 9,300 *Vibrio* spp./g and these differences may be a result of using the Maximum Probable Number count method and not SPC. In their study, oyster meat was stored frozen before being investigated and we have already shown that freezing negatively influences growth of *Vibrio* species.¹¹ Brazilian regulations neither define safe *Vibrio* numbers limits for fish

Figure 2: Monthly variation of the estimates of log *Vibrio* CFU/g and individual size for farmed mangrove oyster, *Crassostrea rizophorae*, collected in Euzébio, Ceará state, Brazil.

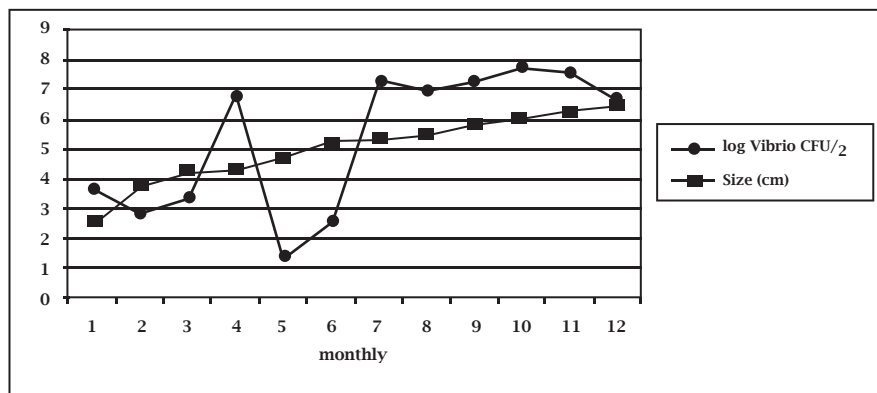


Table 1. *Vibrio sucrose* positive and negative standard plate count (spc), individual size and pH on Mangrove Oyster, *Crassostrea rizophorae*, collected in Euzébio, Ceará state, Brazil

Month	Total count of <i>Vibrio</i> CFU/g	Log	Sucrose +ive	Log	Sucrose -ive	Log	Size (cm)	pH
1	5,200	3.71	3,500	3.54	1,700	3.23	2.50	-
2	750 est.	2.87	750	2.87	<10	-	3.77	6.43
3	2,500	3.39	2,500	3.39	<10	-	4.25	6.47
4	7,025,000	6.84	2,775,000	6.44	4,250,000	6.62	4.34	6.64
5	25 est.	1.39	25 est.	1.39	<10	-	4.67	6.41
6	375 est.	2.57	100 est.	2.0	275 est.	2.43	5.23	7.78
7	23,850,000	7.37	19,775,000	7.29	4,075,000	6.61	5.35	6.54
8	9,575,000	6.98	5,450,000	6.73	4,125,000	6.61	5.48	6.65
9	20,750,000	7.31	9,750,000	6.98	11,000,000	7.04	5.85	6.69
10	59,000,000	7.77	41,500,000	7.61	17,500,000	7.24	6.05	6.45
11	37,500,000	7.57	7,250,000	6.86	30,250,000	7.48	6.3	6.69
12	4,975,000	6.69	1,500,000	6.17	3,475,000	6.54	6.41	6.56

products nor restrict consumption based on numbers.¹² One of the consequences of this lack of regulations is that it has been difficult to monitor and develop indicators for *Vibrio* spp. due to their ubiquitous occurrence in coastal waters and the lack of physiological characteristics shared with human enteric bacteria.¹³⁻¹⁵

The pH of oysters' meat plus the intervalvular liquor varied from 6.41 to 7.78 (Table 1). Usually, these are not the best values for *Vibrio* growth. These bacteria grow better under alkaline conditions and Donovan & Netten¹⁶ suggest that the optimum pH range for selective growth of vibrios is 8.4-8.6. The outcome of this experiment confirms this fact since the lowest counts of vibrios (25 CFU/g est.) were found in the samples with the lowest pH (Table 1, Figure 3).

During most of the oyster's 12 month lifespan it was possible to identify *Vibrio parahaemolyticus* (Table 2). The prevalence of this species in seafood corroborates reports from other countries.¹⁷⁻¹⁹ Similar results were obtained by Lowry *et al.*²⁰ who found 100% of the raw oysters examined to contain *V. parahaemolyticus*. Studies have also shown that a population of *V. parahaemolyticus* in unrefrigerated oysters can increase rapidly from 50 to 790 times their original numbers within 24 hours of harvest if left at 26°C.²¹ A survey of 370 lots of oysters sampled from restaurants, oyster bars, retail, and wholesale seafood markets throughout the US between June 1998 and July 1999 found a seasonal distribution of *V. parahaemolyticus* in market oysters with the highest densities detected during summer months.²² The greatest challenge for

Figure 3: Monthly variation of the estimates of log *Vibrio* CFU/g and pH for farmed mangrove oyster, *Crassostrea rizophorae*, in Ceará state, Brazil.

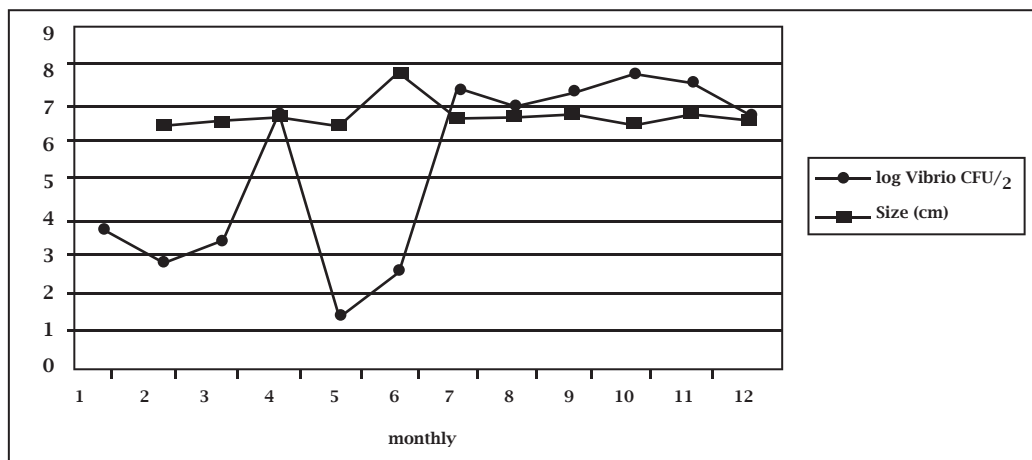


Table 2. *Vibrio* Strains Identified on Mangrove Oyster, *Crassostrea rhizophorae*, in Euzébio, Ceará State, Brazil

Month	*n	Nº of strains	<i>Vibrio</i> species
1	14	3	<i>V. fluvialis</i> (1), <i>V. parahaemolyticus</i> (1), <i>V. vulnificus</i> (1)
2	10	3	<i>Vibrio</i> sp. (1), <i>V. pelagius</i> Biogroup 2 (2)
3	15	1	<i>Vibrio</i> sp.
4	14	2	<i>V. parahaemolyticus</i> (2)
5	12	1	<i>V. carchariae</i> (1)
6	13	5	<i>V. parahaemolyticus</i> (1), <i>V. alginolyticus</i> (4)
7	18	3	<i>V. damsela</i> (1), <i>V. parahaemolyticus</i> (2)
8	12	-	-
9	11	3	<i>V. parahaemolyticus</i> (1), <i>V. carchariae</i> (1), <i>V. cholerae</i> non O1, non O139 (1)
10	12	2	<i>V. parahaemolyticus</i> (1), <i>Vibrio</i> sp. (1)
11	14	10	<i>V. furnissii</i> (1), <i>V. parahaemolyticus</i> (1), <i>V. alginolyticus</i> (2), <i>V. carchariae</i> (2), <i>V. harveyi</i> (2), <i>Vibrio</i> sp. (2).
12	9	13	<i>V. alginolyticus</i> (2), <i>V. parahaemolyticus</i> (4), <i>V. harveyi</i> (2), <i>V. carchariae</i> (1), <i>V. damsela</i> (1), <i>Vibrio</i> sp. (3)

* n= number of oyster.

the safe consumption of oysters is to get them refrigerated or frozen immediately after harvest and to market them as soon as possible. Consumption of raw or undercooked seafood, particularly shellfish, contaminated with *V. parahaemolyticus* may lead to the development of acute gastroenteritis characterized by diarrhea, headache, vomiting, nausea, abdominal cramps, and slight fever. This bacterium is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States and an important seafood-borne pathogen throughout the world.²³ Although the gastroenteritis caused by *V. parahaemolyticus* infection is often self-limited, the infection may cause septicemia that is life-threatening to people having underlying medical conditions, such as liver disease or immune disorders. Two deaths were reported among three cases of wound infections caused by *V. parahaemolyticus* in Louisiana and Mississippi after Hurricane Katrina in 2005.²⁴ *Vibrio carchariae* was identified in four of the monthly oyster collections and it has also been found on a chronic skin ulcer on a shark, *Carcharhinus plumbeus* (Nardo), kept in the Genoa Aquarium, Italy. Identification through fatty acid composition analysis and biochemical tests showed that *V. carchariae* were the predominant bacteria isolated from the ulcer.²⁵ Pedersen *et al.*²⁶ described *V. carchariae* as a synonym of *V. harveyi* based on ribotyping. *Vibrio carchariae*, a species isolated from sharks, belongs to its normal skin flora, but may become pathological when the animal is under stress. *Vibrio carchariae* has been described as an etiologic agent for some fish in cultivations.²⁷ Pereira *et al.*²⁸ reported that forty samples of mangrove oysters (*Crassostrea rhizophorae*) served raw in 15 restaurants in Rio de Janeiro were evaluated in order to investigate the presence of *Vibrio* spp. They found that *Vibrio parahaemolyticus*,

Vibrio carchariae, *Vibrio alginolyticus* and *Vibrio vulnificus* were the main species (> 60 %) isolated from raw oysters. Other species were identified as well, and despite being found in smaller numbers they were of no less importance (Table 2). Among those of most importance regarding public health was *V. vulnificus*, but only one strain was confirmed. Because oysters, in most places, are consumed without being cooked they can easily turn into a potentially health-hazard in food consumption.

In this study we counted and identified large numbers of potentially pathogenic *Vibrio* spp. from juvenile to commercial sized oysters. They are ubiquitous and can multiply to dangerous numbers very quickly. We concluded that market purchased shellfish should never be eaten raw or undercooked because many species of the genus *Vibrio*, which naturally live on and in shellfish throughout their life cycle, are known to be pathogenic to humans.

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