

Pathogenic vibrios in the oyster *Crassostrea virginica* in the lagoon system of Mandinga, Veracruz, Mexico

Vibrios patogénicos en el ostión *Crassostrea virginica* en el sistema lagunar de Mandinga, Veracruz, México

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ABSTRACT

Oyster consumption has been associated to the transmission of pathogenic bacteria, including those from the genus *Vibrio*. The objective of this investigation was to determine the concentrations of *Vibrio parahaemolyticus* and *V. alginolyticus* in *Crassostrea virginica* from the lagoon system of Mandinga, Veracruz, and their relationships with salinity and water temperature. Only the periods of greater production and consumption of oysters were examined: the dry and rainy seasons during 2008. Four sampling sites were selected, from which three samples were collected per site per season, resulting in a total of 24 samples. Each sample consisted of 30 oysters of commercial size that were subsequently analyzed using serial dilution. Biochemical analysis of the resulting bacterial colonies revealed concentrations of *V. parahaemolyticus* and *V. alginolyticus* ranged from < 3 to 150 MPN/g. A positive correlation was observed between temperature and the concentration of *V. parahaemolyticus* ($r = 0.69$, $p < 0.05$), while the correlation with salinity was negative ($r = -0.68$, $p < 0.05$). No significant correlations between the concentration of *V. alginolyticus* and water temperature and salinity were observed for the rainy season. The main contribution of this paper is to establish safe areas and periods for oyster extraction.

Key words: *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Crassostrea virginica*, Mandinga Lagoon, Mexico.

RESUMEN

El consumo de ostión ha sido asociado con la transmisión de bacterias patógenas, entre las que se encuentran las bacterias del género *Vibrio*. El objetivo de esta investigación fue determinar las concentraciones de *Vibrio parahaemolyticus* y *V. alginolyticus* en *Crassostrea virginica* del sistema lagunar de Mandinga, Veracruz, y su relación con la salinidad y temperatura del agua. Se consideraron las épocas de mayor producción y consumo de ostión, esto es, secas

y lluvias de 2008. Se seleccionaron cuatro sitios de muestreo y se tomaron 3 muestras por sitio por época, resultando un total de 24 muestras. Cada muestra consistió de 30 organismos de talla comercial que se analizaron mediante la técnica de diluciones seriadas. Se hizo una caracterización bioquímica de las colonias encontradas para determinar las concentraciones de *V. parahaemolyticus* y *V. alginolyticus* en NMP/g, cuyos valores fueron de < 3 a 150 NMP/g. Se observó una correlación positiva entre la temperatura y la concentración de *V. parahaemolyticus* ($r = 0.69$, $p < 0.05$), mientras que la correlación de ésta última y la salinidad fue negativa ($r = -0.68$, $p < 0.05$). No se pudo encontrar una correlación entre la concentración de *V. alginolyticus* y la temperatura y salinidad del agua en la época de lluvias. El principal aporte de este trabajo fue el establecer zonas y períodos seguros de extracción de ostión.

Palabras clave: *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Crassostrea virginica*, Laguna Mandinga, México.

INTRODUCTION

The Gulf of Mexico is considered one of the most important coastal zones in the world, and encompasses major fishing activities including that for the American oyster, *Crassostrea virginica* (Gmelin, 1791) (Baquero-Cárdenas *et al.*, 2007). Given that the soft body of the oyster is consumed whole, and either raw or lightly cooked, it is generally classified as a high-risk food (Daniels *et al.*, 1998; Fukushima & Seki, 2004). Oyster consumption is associated with the transmission of pathogenic bacteria such as those in the genus *Vibrio*. This genus contains more than 70 species, of which 11 are known to be human pathogens such as *V. parahaemolyticus* Fujino and *V. alginolyticus* Miyamoto (Chakraborty *et al.*, 1997; Thompson *et al.*, 2004). *V. alginolyticus* (Fujino *et al.* 1951), like other species of *Vibrio*, can produce infections in the ears and open wounds through contact with water containing this species (Parveen *et al.*, 2008). *V. parahaemolyticus* (Miyamoto *et al.* 1961) is one of the more pathogenic species and can be found in a proportion of up to 100 times more in cupped oysters than in the aquatic environment, and is associated with gastroenteritis, wound infection, and septicemia (Crocini *et al.*, 2002; Cabrera-García *et al.*, 2004).

The genus *Vibrio* is a group of Gram-negative, halophilic bacteria occurring naturally in estuarine environments. The species are distributed worldwide in sea water and are associated with the resident aquatic organisms. Their presence is independent of anthropogenic pollution, but is dependent on salinity, temperature and organic matter (Hervio-Heath *et al.*, 2002). Their concentration in the aquatic environment and in foods of marine origin is a function of the geographic and hydrographic conditions in the area, and varies according to the time of year and location within the lagoon systems (Sousa *et al.*, 2004). *Vibrio* are the principal bacteria causing sickness and death as a consequence of consuming contaminated shellfish.

Based on this information and considering that Mexico is the sixth largest producer of oysters globally (FAO, 2006), the objective of the present study was to determine the concentrations of *V. parahaemolyticus* and *V. alginolyticus* in *C. virginica* from the lagoon system of Mandinga, Veracruz, and their relationships

with water temperature and salinity during times of greater oyster production and consumption.

MATERIALS AND METHODS

Study Area. The Mandinga lagoon system is located on the coast of the Gulf of Mexico in the central part of the state of Veracruz (19° 00' to 19° 06' N, and 96° 02' to 96° 06' W), and covers an area of 3,250 ha (Fig. 1).

Sample collection and recording of water temperature and salinity. Samples and recorded data were collected during May (the dry season), and July (the rainy season) of 2008. These

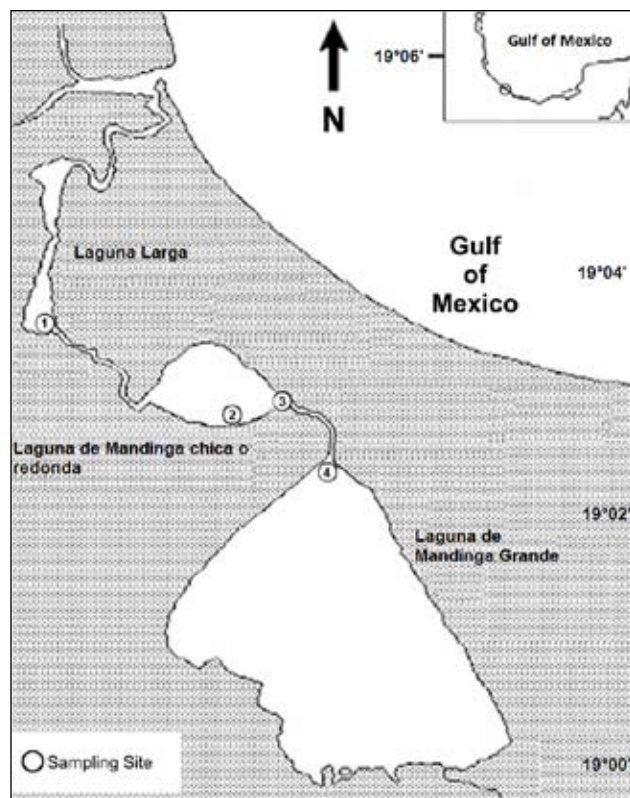


Figure 1. The Mandinga lagoon system, Veracruz, Mexico.

seasons are considered to be the highest for the production and consumption of oysters in the region. The sampling sites were located in permanent oyster harvesting areas of the Mandinga lagoon system in Veracruz, Mexico (SEFIPLAN, 1999). Four sampling sites were selected and three samples were taken per season, for a total of 24 samples. Each sample consisted of 30 oysters of commercial size (7 ± 3 cm) that were cleaned, and packaged for transportation according to specifications provided in the Norma Oficial Mexicana NOM-109-SSA1-1994 (DOF, 1994). In each sampling site, water temperature and salinity were measured using a combined probe (Model YSI-6600 V2, YSI Inc., Yellow Springs, Ohio, USA) (DOF, 1994; Castañeda *et al.*, 2005).

Determination of *Vibrio parahaemolyticus* and *V. alginolyticus*. In a sterile laboratory area, oysters were opened to obtain the entire visceral mass and the intervalvar liquid which were blended and stored in sterile glass measuring cups (Norma Oficial Mexicana NOM-031-SSA1-1993; DOF, (1993)) and the mixtures were processed in a time no longer than 1 hour, at laboratory conditions, as established by Dileep *et al.*, 2003.

The technique used for the determination of *Vibrio* spp. was based on the methodology developed by the USDA Food and Drug Administration (FDA-BAM, 2004). A 25 g subsample was removed from each mixture of visceral mass and intervalvar water, and the subsample homogenized for 2 minutes in a sterile food processor (Waring, Hartford, Connecticut, USA) with the addition of 225 ml of alkaline peptone water (APW) enriched with 3 % sodium chloride. Subsequently, a series of three hexadecimal dilutions was performed on each one of the homogenized mixtures (1:100, 1:1000 y 1:10000), which were then incubated at 35 °C for 6-8 hours. An aliquot for inoculation was extracted from each dilution and was applied, using cross-streaking, to the selective Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar (Merck®, Alemania) in Petri dishes and incubated at 35 °C for 24-48 hours (Olafsen *et al.*, 1993).

Yellow (sucrose positive) (typical of *V. alginolyticus*) and green (sucrose negative) (typical of *V. parahaemolyticus*) colonies were selected from the TCBS plates. Petri dishes containing a nonselective medium (trytone casein and salt, T1N3, Merck®, Germany) were then inoculated with samples of these colonies in order to promote growth of the selected bacteria. These plates were incubated for a period of 18 to 24 hours at 35 °C. Samples were taken from the colonies produced and placed on indicator strips of Bactident oxidase (Merck®, Germany) to test for oxidase. If a violet color appeared, the reaction to the oxidase was considered positive, or when there was no observed color change, the reaction was considered negative.

A small sample was taken from the colonies considered positive for oxidase, and inoculated into media for the biochemi-

Table 1. Biochemical characteristics of responses by *Vibrio* sp.¹

	<i>V. alginolyticus</i>	<i>V. parahaemolyticus</i>
TCBS Agar	Y	G
Oxidase	+	+
Arginine decarboxylase	-	-
Ornithine decarboxylase	+	+
Lysine decarboxylase	+	+
% salinity		
0%	-	-
3%	+	+
6%	+	+
8%	+	+
10%	+	-
Growth at 42 °C	+	+
Acid from:		
Sucrose	+	-
Lactose	-	-
Voges-Proskauer	+	-
Urease Sensitivity	-	v

¹ Adapted from FDA-BAM (2004).

Abbreviations: (TCBS) = thiosulfate-citrate-bile salts-sucrose; (Y) = yellow colonies; (G) = green colonies; (v) = response variable; (+) = growth or positive response; (-) = no growth or negative response.

cal testing of mobility, decarboxylation of ornithine, lysine and arginine, acidity or fermentation of lactose and sucrose, salinity tolerance, the Voges-Proskauer reaction, urea metabolism, and growth at 42 °C. The results were obtained using the biochemical profile described in Table 1, and were expressed as the Most Probable Number per gram of sample (MPN/g). The latter was determined from dilutions.

Statistical analysis. Data were analyzed using the software Statistica v7.0 (Statsoft, Inc., Tulsa, Oklahoma, USA). A nonparametric Kruskal-Wallis test was used to look for significant differences ($P < 0.05$) between seasons and sampling sites. Concentrations of *Vibrio* sp. were correlated with water temperature and salinity.

RESULTS

Average concentrations of *V. parahaemolyticus* and *V. alginolyticus* in *C. virginica* by season of production and consumption and by sampling site in the Mandinga lagoon system are presented in Table 2. Results from the Kruskal-Wallis test between seasons and sampling sites are shown in Figures 2 and 3. A possible trend of a correlation between average concentrations of *V. parahaemolyticus* and water temperature and salinity is shown in Figures 4 and 5.

Table 2. Average concentrations (MPN/g × 10) of *V. parahaemolyticus* and *V. alginolyticus* in the oyster, *C. virginica*, from the Mandinga lagoon system, Veracruz, Mexico.

Bacteria		Sampling site				Average
		1	2	3	4	
<i>V. parahaemolyticus</i>	Dry	3.00 ± 0.0 ^a	3.40 ± 0.3 ^a	4.43 ± 1.4 ^a	9.20 ± 1.9 ^b	5.00
	Rainy	12.06 ± 3.5 ^{bc}	2.20 ± 1.9 ^a	15.00 ± 1.0 ^c	3.40 ± 0.3 ^a	8.16
<i>V. alginolyticus</i>	Dry	6.46 ± 0.63 ^x	3.40 ± 0.3 ^z	3.40 ± 0.3 ^z	2.00 ± 1.73 ^z	3.81
	Rainy	9.70 ± 1.1 ^y	n.d.	n.d.	n.d.	2.42

Values with different superscripts letters are significantly different ($p < 0.05$).
n.d. = Not detectable.

DISCUSSION

Various studies exist on the capacity for oysters to filter and accumulate bacteria directly from the water, especially marine bacteria such as *V. parahaemolyticus* and *V. alginolyticus* which are pathogenic to humans and the latter which also is known to affect larval fish and crustaceans (Barbieri *et al.*, 1999; Parveen *et al.*, 2008). The results from the present study in the Mandinga lagoon system indicate that the American oyster, *C. virginica*, is a natural reservoir of bacteria such as *V. parahaemolyticus* and *V. alginolyticus* (Table 2), which is also confirmed by Di pinto *et al.* (2006).

Vibrio parahaemolyticus. This bacteria species has been little studied in bivalve molluscs from Mexico and in the world in general. However, when this bacterium is detected and determined to be the causative agent of gastrointestinal illness in humans by the consumption of raw fish and shellfish, the studies of this organism begin to escalate. The presence and concentration of this bacterial species are not regulated by the mexican regulations. The presence of *V. parahaemolyticus* has been reported in *C. virginica* and other shellfish (Cabrera-García *et al.*, 2004; Cabanillas-Beltrán *et al.*, 2006), but no studies have been reported in Mexico that evaluate the concentration by unit weight in bivalve

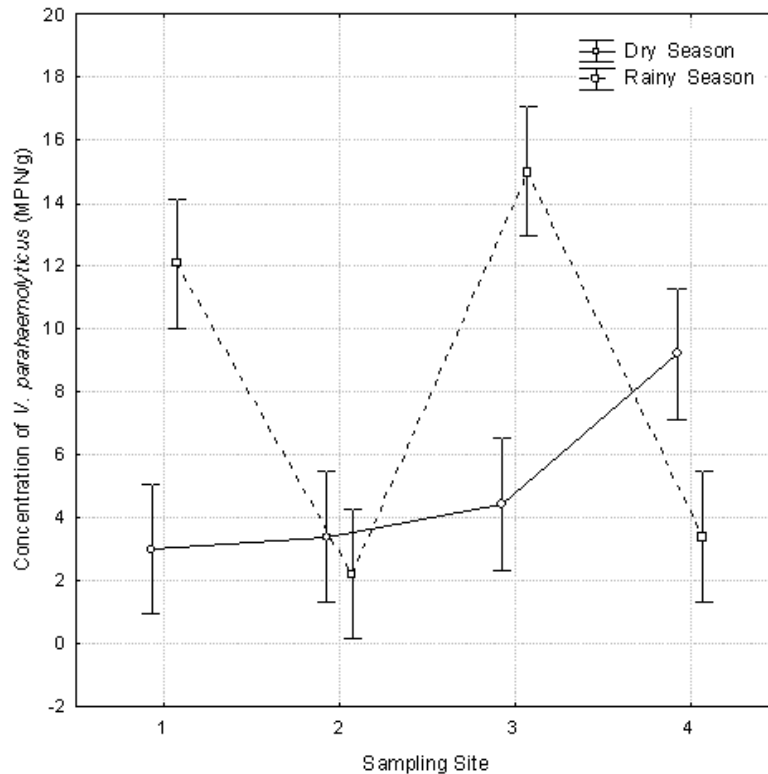


Figure 2. Average concentrations of *V. parahaemolyticus* obtained from samples of *C. virginica* collected during the dry and rainy seasons from the Mandinga lagoon system, Veracruz, Mexico.

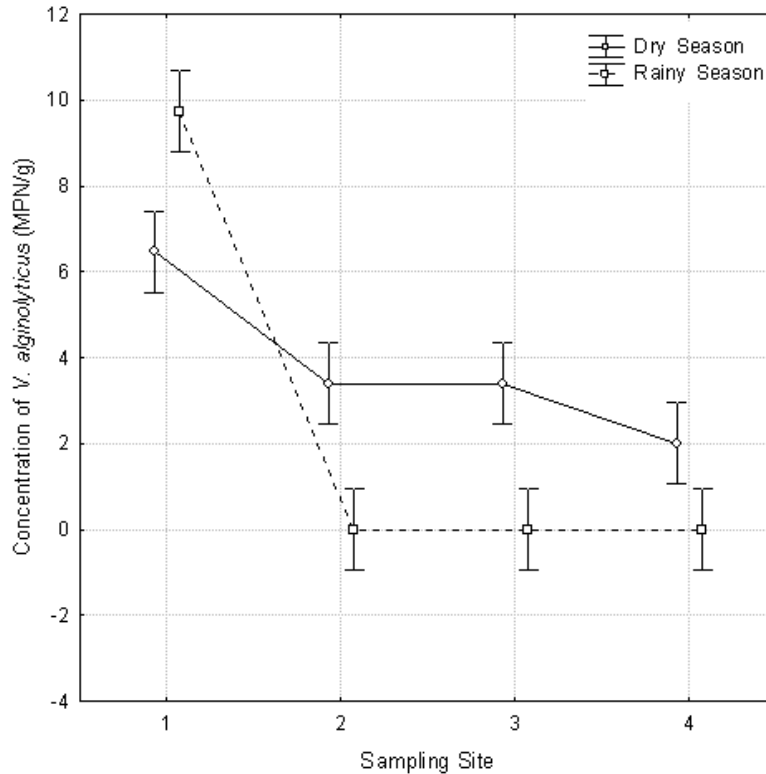


Figure 3. Average concentrations of *V. alginolyticus* obtained from samples of *C. virginica* collected during the dry and rainy seasons from the Mandinga lagoon system, Veracruz, Mexico.

molluscs. Countries such as China, Japan, Taiwan, Malaysia, Italy, and the United States of America have reported concentrations of *V. parahaemolyticus* in bivalve molluscs greater than 1100 MPN/g (Alam *et al.*, 2003; Bilung *et al.*, 2005; Di Pinto *et al.*, 2006; Yi-Chen & Chengchu, 2007; Parveen *et al.*, 2008).

In this study, *V. parahaemolyticus* was detected among seasons and sampling sites with concentrations that varied between 3 and 150 MPN/g (Table 2). Significant differences were detected between sampling seasons ($p < 0.05$), with the highest concentration of *V. parahaemolyticus* during the rainy season (Figure 2). The highest concentrations were found in sites 1 and 3, which coincided with the high temperatures and lower salinities (Table 3). The observed concentrations may also be produced by seasonal

Table 3. Water temperature and salinity in the Mandinga lagoon system, Veracruz, Mexico.

Parameter		Sampling Site			
		1	2	3	4
Temperature (°C)	Dry	29	29	29	30
	Rainy	31	30.5	31	30
Salinity (o/oo)	Dry	35	35	38	37
	Rainy	20	21	23	25

variation of the *V. parahaemolyticus* population by “hibernating” in the sediment or in partnership with the marine fauna, followed by population growth from runoff into the lagoon having high concentrations of organic matter and nutrients. This latter process not only promotes the growth of these bacteria, it eutrophies the Mandinga lagoon system (Aguilar-Ibarra *et al.*, 2006).

The distribution, dynamics and occurrence of populations of *V. parahaemolyticus* also are influenced by gradients of environmental factors such as temperature, salinity, nutrients, and by biological factors such as the abundance and predation by dinoflagellates and other hosts (Alam *et al.*, 2003; Thompson & Polz, 2006).

In spite of the few available data, the correlation between water temperature and concentrations of *V. parahaemolyticus* was significant and positive ($r = 0.69$, $P < 0.05$), while the correlation between bacterial concentration and salinity was significant and negative ($r = -0.68$, $P < 0.05$) (Figures 4, 5). Similar results have been reported by DePaola *et al.* (1990) and Parveen *et al.* (2008) who studied the incidence of *V. parahaemolyticus* in *C. virginica* along the Atlantic coast of the United States of America.

Thus, we confirmed that temperature is the factor that primarily determines the distribution and abundance of *V. parahaemolyticus*.

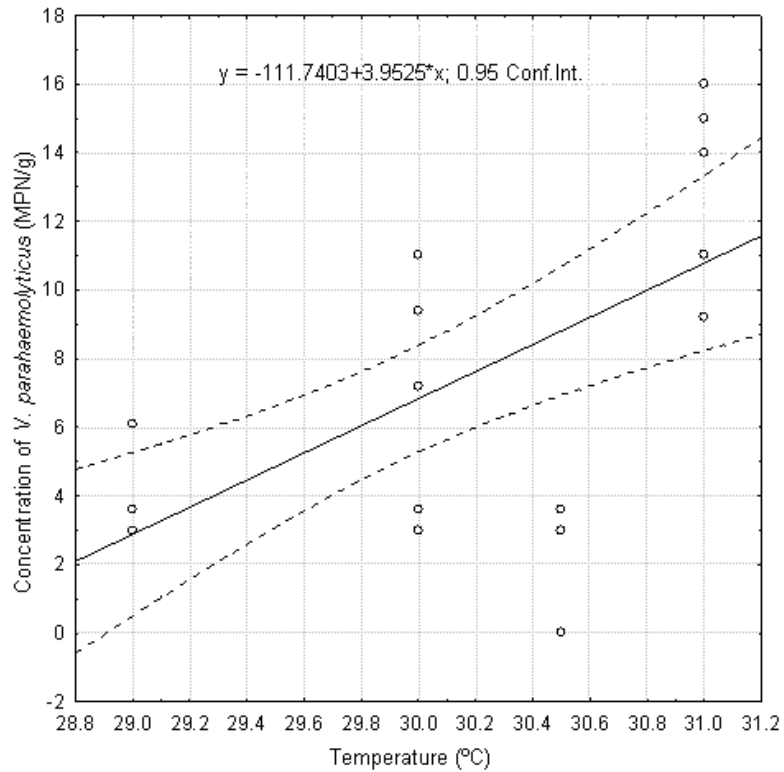


Figure 4. Correlation between the concentration of *V. parahaemolyticus* and water temperature in both seasons in the Mandinga lagoon system, Veracruz, Mexico.

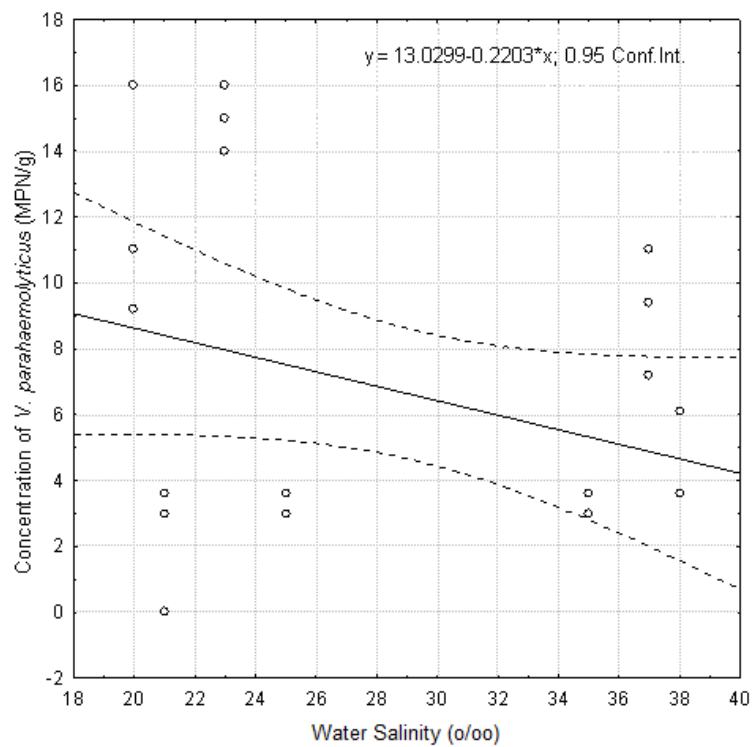


Figure 5. Correlation between the concentration of *V. parahaemolyticus* and water salinity in both seasons in the Mandinga lagoon system, Veracruz, Mexico.

molyticus in lagoon systems of the Gulf of Mexico where the oyster *C. virginica* is abundant, and the minimum water temperature is 11.6 °C (Cabrera-García *et al.*, 2004). Also, we have further confirmed that increased salinity results in a reduced concentration of *V. parahaemolyticus* in *C. virginica* (Table 3) (Cook *et al.*, 2002; DePaola *et al.*, 2003).

Vibrio alginolyticus. *V. alginolyticus* was present during the dry season in all four sampling sites, but was only present in site 1 during the rainy season. During the dry season, the salinity in site 1 was 35 o/oo, with a concentration of *V. alginolyticus* of 6.46 MPN/g. During the rainy season, the salinity decreased to 20 o/oo and the bacterial concentration increased to 9.70 MPN/g (Table 2). This trend in salinity and bacterial concentration is similar to that for *V. parahaemolyticus* where the bacterial concentration declined with increasing salinity.

The lack of detection of *V. alginolyticus* in sampling sites 2, 3, and 4 (Figure 3) can be explained by the low primary productivity in Redonda Lagoon in the Mandinga lagoon system during the rainy season where sampling sites 2 and 3 were located. It is likely that a similar condition may have occurred in the mouth of Mandinga Grande Lagoon where site 4 was located in this lagoon system (Arreguin, 1978). The low productivity may be the result of a short time of residence of nutrients in the lagoon systems during the rainy season (Gutiérrez-Mendieta *et al.*, 2006).

Similar conditions can cause *V. alginolyticus* to emerge from stasis, but it cannot be cultivated at this time of year. This is due to unfavorable conditions such as competition between bacteria for nutrients, space, and light (Avenidaño-Herrera *et al.*, 2005; Albertini *et al.*, 2006).

This species was not correlated with temperature, and due to the seasonal fluctuations its occurrence, does not appear to be dependent on temperature in tropical areas (Molitoris *et al.*, 1985). However, to corroborate this assumption, it would be necessary to have field data that span a minimum period of three years. Such efforts would better define trends in bacterial concentrations relative to the environmental conditions and co-occurring environmental management actions.

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