Vibrio parahaemolyticus and *Vibrio alginolyticus* in *Crassostrea virginica* from the lagoon system of Mandinga, Veracruz, Mexico

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Introduction

Oyster consumption is associated with the transmission of pathogenic bacteria such as those in the genus *Vibrio*. This genus is a group of gram-negative, halophilic bacteria occurring naturally in estuarine environments. The species is distributed worldwide in sea water and is 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 *Crassostrea 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

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. The sampling sites were located in permanent oyster harvesting areas of the Mandinga lagoon system in Veracruz, Mexico. 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) (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. 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 100 g of visceral mass and intervalvar water was homogenized 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 to the selective Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar (Merck®, Alemania) and incubated at 35 ºC 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. The results were expressed as the Most Probable Number per gram of sample (MPN/g) and were interpreted using a biochemical profile.

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 transformed to $\log_{10}$MPN, and this value was correlated with water temperature and salinity.

Results and discussion

Vibrio parahaemolyticus

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

Table 1. Average concentrations (MPN/g x 10) of *V. parahaemolyticus* and *V. alginolyticus* in the oyster, *C. virginica*, from the Mandinga lagoon system, Veracruz, Mexico.

BACTERIA		Sampling site				
		1	2	3	4	Average
V. parahaemolyticus	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
V. alginolyticus	Dry	6.46±0.63x	3.40±0.3z	3.40±0.3z	2.00±1.73z	3.81
	Rainy	9.70±1.1y	n.d.	n.d.	n.d.	2.42

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

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).Thus, we confirmed that temperature is the factor that primarily determines the distribution and abundance of *V. parahaemolyticus* in lagoon systems of the Gulf of Mexico where the oyster *C. virginica* is abundant, and the minimum water temperature is 11.6ºC (Cabrera-García et al., 2004). Also, we have further confirmed that increased salinity results in a reduced concentration of *V. parahaemolyticus* in *C. virginica* (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 ppm, with a concentration of V. alginolyticus of 6.46 MPN/g. During the rainy season, the salinity decreased to 20 ppm and the bacterial concentration increased to 9.70 MPN/g (Table 1). 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 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). 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 (Albertini et al., 2006).

Conclusion

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. 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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