

**Title** The ecology of *Vibrio vulnificus* and *Vibrio parahaemolyticus* from oyster harvest sites in the Gulf of Mexico

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**Citation** Thesis, Doctor of Philosophy (Food Science), North Carolina State University. 246 pages. 2008.

**Keywords** Oyster harvest; Gulf of Mexico; *Vibrio vulnificus*; *Vibrio parahaemolyticus*; Shellfish

### Abstract

The *Vibrionaceae* are environmentally ubiquitous to estuarine waters. Two species in particular, *V. vulnificus* and *V. parahaemolyticus*, are important human pathogens that are transmitted by the consumption of contaminated molluscan shellfish. There is limited information available for the recent risk assessments; accordingly, the purpose of this study was to address some of these data gaps in the *V. vulnificus* and *V. parahaemolyticus* risk assessments. The objectives of this study were to (i) quantify the levels of total estuarine bacteria, total *Vibrio* spp., and specific levels of non-pathogenic and pathogenic *V. vulnificus* and *V. parahaemolyticus* over the harvest period; and (ii) determine if length of harvest time affects the levels of *V. vulnificus*.

Oyster and water samples were harvested seasonally from 3 U.S. Gulf Coast sites over 2 years. Environmental parameters were monitored during harvesting. Both surface and bottom water samples (1 L) were taken at the beginning of harvesting and at the end of harvesting. Oyster samples (15 specimens for each time point) were taken at 0, 2.5, 5.0, 7.5, and 10 hrs intervals after being held at ambient temperature during harvesting. Samples were processed for many different bacteria. For enumeration of total *V. parahaemolyticus*, pathogenic *V. parahaemolyticus*, and *V. vulnificus* was done using colony lift hybridization (*tlh*, *tdh* + and/or *trh* +, and *vhA* gene targets, respectively).

MPN methods were also used to obtain estimates of pathogenic *V. parahaemolyticus* (*tdh* + and/or *trh* +) counts. Representative *V. parahaemolyticus* and *V. vulnificus* isolates were subjected to phenotyping; *V. vulnificus* isolates were also subjected to genotyping. Different statistical analysis were used to establish relationships where appropriate.

The first manuscript describes the field study which examined the distribution and variation in the levels of *V. parahaemolyticus* and *V. vulnificus* (both total and pathogenic strains) in shellfish and their overlay waters, and established the effect of seasonal and environmental/ecological factors on these distributions. The best estimate of growth for *V. parahaemolyticus* (*tlh*) in oysters based on water temperature was 0.054 log<sub>10</sub> per °C, while the best estimate for growth for *V. vulnificus* (*vhA*) in oysters

based on water temperature was 0.068 log<sub>10</sub> per °C. Estimated relationships between *tlh* and *vvhA* growth rates and air temperature were consistent with the risk assessments. No statistical relationship could be established between pathogenic *V. parahaemolyticus* ( *tdh* + and/or *trh* +) growth rates and air temperatures.

In the second study, we specifically looked at the effect of extended boat deck storage of commercially harvested oysters held at ambient air temperature on the levels of *V. vulnificus* . During summer, increases in *V. vulnificus* were as high as 1.4 log<sub>10</sub> (CFU/g) after 10 h storage at ambient air temperatures; for spring/fall oysters, a 1.0 log<sub>10</sub> increase was observed; and in winter, increases were <0.5 log<sub>10</sub>. Statistically significant (p<0.05) correlations between ambient air temperature and increases in *V. vulnificus* counts were noted.

Taken together, this research provides information which can be used to fill key data gaps in the current risk assessments for *V. vulnificus* and *V. parahaemolyticus* . These data can be used in future iterations of the risk assessments to help better predict risk and inform policy as risk managers seek to reduce the disease burden associated with these important foodborne pathogens.