

Quantitative Risk Assessment on the Public Health Impact of Pathogenic *Vibrio parahaemolyticus* In Raw Oysters

Center for Food Safety and Applied Nutrition
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RESPONSE TO PUBLIC COMMENTS

A notice of availability of the Food and Drug Administration (FDA) draft risk assessment on the relationship between *Vibrio parahaemolyticus* in raw molluscan shellfish and public health was published in the Federal Register of January 19, 2001 (66 FR 5517). A comment period was established during which FDA actively sought comments, suggestions, and additional data sources. The results of the draft risk assessment were presented for clarification during a public meeting on March 20, 2001 (66 FR 13544). Comments were submitted to the FDA Docket (No. 99N-1075) from nine institutions or individuals. The data and information acquired during the comment period were reviewed and used, as appropriate, to further enhance the risk assessment.

We appreciate the time and effort expended to submit these comments, and have addressed these in this revised risk assessment to the best of our ability. A summary of the modifications made to the draft risk assessment in response to the comments, new data and modeling techniques is provided below. A more detailed discussion of our response to the public comments can be found in Appendix 2.

Modifications Made to the 2001 Draft *Vibrio parahaemolyticus* Risk Assessment

Topic	Modifications
Assumptions	<p>Additional information was obtained that further the following assumptions:</p> <ul style="list-style-type: none"> • Growth rates of pathogenic and non-pathogenic <i>V. parahaemolyticus</i> are similar; • Time required for refrigerated oysters to cool down to temperatures that do not support the growth of <i>V. parahaemolyticus</i> is variable and may range from 1 to 10 hours.
Additional Data/ Information	<ul style="list-style-type: none"> • Prevalence of total and pathogenic <i>V. parahaemolyticus</i> at harvest for Pacific Northwest region (PNW) and Gulf Coast regions; • Relationship between water temperature and <i>V. parahaemolyticus</i> levels in oysters; • Time-to-refrigeration after harvest for the PNW region.
Modeling techniques	<ul style="list-style-type: none"> • Included intertidal harvesting in the PNW as an additional harvest region; • Evaluated mitigation effect of specific reduction levels of <i>V. parahaemolyticus</i> in addition to types of interventions; • Included regression-based sensitivity analysis; • Added two additional uncertainty parameters (total <i>V. parahaemolyticus</i> in oysters based on water temperature and dose-response relationship) to the examination of factors that influence risk predictions; • Oyster meat weights at retail were used rather than those at harvest; • Comparison of the model-predicted number of illnesses using both retail survey and epidemiological data

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EXECUTIVE SUMMARY

Background

The Food and Drug Administration (FDA) conducted a quantitative risk assessment to characterize the factors influencing the public health impact associated with the consumption of raw oysters containing pathogenic *Vibrio parahaemolyticus*. This effort was initiated in January 1999 and a draft risk assessment was made available for public comment in 2001. The risk assessment was conducted in response to four outbreaks in 1997 and 1998 in the United States involving over 700 cases of illness. These outbreaks renewed concern for this pathogen as a serious foodborne threat to public health and raised new concerns about the effectiveness of risk management guidance available at that time. These outbreaks also raised questions about the criteria used to close and reopen shellfish waters to harvesting and the FDA guidance for the maximum number of *V. parahaemolyticus* per gram in shellfish. FDA decided to conduct a quantitative risk assessment to provide new insights into how to better manage the presence of this pathogenic microorganism in shellfish.

This risk assessment focused on raw oysters, because that is the food in the United States predominately linked to illness from this pathogen. The risk assessment gathers available knowledge of *V. parahaemolyticus* in a systematic manner, and includes sophisticated, mathematical models. The levels of the pathogen in oysters were estimated beginning with harvest of the oysters through post-harvest handling, processing, and storage to predict human exposure from consumption of raw oysters and subsequent illnesses. The number of illnesses (on a per serving and a per year basis) were predicted for six regions in the United States and each season for a total of 24 region/season combinations. Total cases of illness include both gastroenteritis and septicemia. In addition, the probability of gastroenteritis progressing to septicemia in individuals with underlying medical conditions (such as diabetes, alcoholic liver disease, hepatitis, and those receiving immunosuppressive treatments for cancer or AIDS) was compared to that of healthy individuals. Once developed, the baseline model was used to develop “what-if” scenarios to evaluate the likely impact of potential intervention strategies on the exposure to pathogenic *V. parahaemolyticus* from consumption of raw oysters.

Vibrio parahaemolyticus is a gram-negative, salt tolerant bacterium that occurs naturally in estuaries. It has been long recognized as an important bacterial seafood-borne pathogen throughout the world. It was first isolated and implicated in an outbreak of food poisoning in Japan in 1950. *Vibrio parahaemolyticus* has been associated with outbreaks and individual cases of illness in the United States since 1969. These bacteria are normally present in many types of raw seafood, including fish, crustaceans, and molluscan shellfish. The microorganism concentrates, colonizes, and multiplies in the gut of filter-feeding molluscan shellfish such as oysters, clams, and mussels. Not all strains of *V. parahaemolyticus* cause illness; on the contrary, pathogenic strains represent a small percentage of the total *V. parahaemolyticus* present in the environment or seafood.

Scope and General Approach

This risk assessment is a quantitative product pathway analysis in which the key steps from harvest through post-harvest handling and processing to consumption were modeled. The likelihood of illness following exposure to pathogenic *V. parahaemolyticus* from consumption of raw oysters was calculated. The levels of *V. parahaemolyticus* in oysters at the time of consumption are influenced by the harvest methods and conditions, as well as the handling of oysters after harvest. These practices and conditions vary considerably among different geographic areas and at different times of year. The baseline risk assessment model was also used to estimate the likely impact of intervention strategies (referred to as “what-if” scenarios) on the predicted number of illnesses.

The risk assessment considered six oyster harvest regions and four seasons for a total of 24 region/season combinations. The oyster harvest regions included: Gulf Coast (Louisiana), Gulf Coast (non-Louisiana), Mid-Atlantic, Northeast Atlantic, Pacific Northwest (Dredged) and Pacific Northwest (Intertidal). In the Gulf Coast, the harvest duration (i.e., the time between removal of the oyster from the water to unloading them at the dock) for Louisiana is typically much longer than for other states in that region (Florida, Mississippi, Texas, and Alabama). Since harvest duration can affect the levels of *V. parahaemolyticus* in raw oysters, the Gulf Coast was divided into two distinct regions. Likewise, the Pacific Northwest was divided into two distinct regions, but in this case it was based on harvest methods, dredging and intertidal. Oysters harvested in intertidal areas are typically exposed to higher temperatures before refrigeration than those harvested using dredging. For the intertidal harvest method, oysters are hand-picked when oyster reefs are exposed during the tide cycle and left in baskets until the tide rises to a sufficient depth to allow a boat to retrieve the basket.

The risk assessment had two main objectives:

- determine the factors that contribute to the risk of becoming ill from the consumption of pathogenic *V. parahaemolyticus* in raw oysters; and
- evaluate the likely public health impact of different control measures, including the effectiveness of current and alternative microbiological standards.

Data for this risk assessment were obtained from many sources, including both published and unpublished scientific literature and reports produced by various organizations such as State shellfish control authorities, the Centers for Disease Control and Prevention (CDC), the shellfish industry, the Interstate Shellfish Sanitation Conference (ISSC), and State Health Departments. In some instances the conduct of the risk assessment required that assumptions be made when data were incomplete. To the extent possible, research was specifically undertaken during the period between issuing the original draft and the current version to address data gaps previously identified. These new data have been incorporated into the risk assessment.

Results

The model predicts illnesses (gastroenteritis alone and gastroenteritis followed by septicemia) associated with the consumption of *V. parahaemolyticus* in raw oysters for the 24 region/season combinations. Summary Table 1 provides the risk on a “per serving basis” (i.e., the risk of becoming ill per serving of raw oysters) and Summary Table 2 provides the risk on a “per annum basis” (i.e., the predicted number of illnesses per year).

Summary Table 1. Predicted Mean Risk per Serving Associated with the Consumption of *Vibrio parahaemolyticus* in Raw Oysters

Region	Mean Risk Per Serving ^a				
	Summer	Fall	Winter	Spring	Total
Gulf Coast (Louisiana)	4.4×10^{-4}	4.3×10^{-5}	2.1×10^{-6}	1.7×10^{-4}	6.6×10^{-4}
Gulf Coast (Non-Louisiana) ^b	3.1×10^{-4}	1.9×10^{-5}	1.1×10^{-6}	1.2×10^{-4}	4.5×10^{-4}
Mid-Atlantic	9.2×10^{-5}	2.2×10^{-6}	1.1×10^{-8}	3.1×10^{-5}	1.3×10^{-4}
Northeast Atlantic	1.8×10^{-5}	4.0×10^{-7}	1.1×10^{-8}	3.6×10^{-6}	2.2×10^{-5}
Pacific Northwest (Dredged)	1.0×10^{-5}	2.6×10^{-8}	8.1×10^{-10}	8.7×10^{-7}	1.1×10^{-5}
Pacific Northwest (Intertidal) ^c	1.4×10^{-4}	3.9×10^{-7}	1.7×10^{-9}	1.3×10^{-5}	1.5×10^{-4}

^a Risk per serving refers to the predicted risk of an individual becoming ill (gastroenteritis alone or gastroenteritis followed by septicemia) when they consume a single serving of raw oysters. Values rounded to 2 significant digits.

^b Includes oysters harvested from Florida, Mississippi, Texas, and Alabama. The time from harvest to refrigeration in these states is typically shorter than for Louisiana.

^c Oysters harvested using intertidal methods are typically exposed to higher temperature for longer times before refrigeration compared with dredged methods.

Summary Table 2. Predicted Mean Annual Number of Illnesses Associated with the Consumption of *Vibrio parahaemolyticus* in Raw Oysters

Region	Mean Annual Illnesses ^a				
	Summer	Fall	Winter	Spring	Total
Gulf Coast (Louisiana)	1,406	132	7	505	2,050
Gulf Coast (Non-Louisiana) ^b	299	51	3	193	546
Mid-Atlantic	7	4	<1	4	15
Northeast Atlantic	14	2	<1	3	19
Pacific Northwest (Dredged)	4	<1	<1	<1	4
Pacific Northwest (Intertidal) ^c	173	1	<1	18	192
TOTAL	1,903	190	10	723	2,826

^a Mean annual illnesses refers to the predicted number of illnesses (gastroenteritis alone or gastroenteritis followed by septicemia) in the United States each year. Note: Actual values for the illness predictions are provided in Appendix 7.

^b Includes oysters harvested from Florida, Mississippi, Texas, and Alabama. The typical time from harvest to refrigeration of oysters for these states is shorter than for Louisiana.

^c Oysters harvested using intertidal methods are typically exposed to higher temperature for longer times before refrigeration compared with dredged methods.

Below are the responses to the questions that the risk assessment team was charged with answering.

What is known about the dose-response relationship between consumption of *Vibrio parahaemolyticus* and illnesses?

- Although an individual may become ill from consumption of low levels of *V. parahaemolyticus*, it is much more likely that he or she will become ill if the level is high. The probability of illness is relatively low (<0.001%) for consumption of 10,000 *V. parahaemolyticus* cells/serving (equivalent to about 50 cells/gram oysters). Consumption of about 100 million *V. parahaemolyticus* cells/serving (500 thousand cells/gram oysters) increases the probability of illness to about 50%.
- Anyone exposed to *V. parahaemolyticus* can become infected and develop gastroenteritis. However there is a greater probability of gastroenteritis developing into septicemia (and possibly death) among the subpopulation with concurrent underlying chronic medical conditions.
- The model predicts about 2,800 *V. parahaemolyticus* illnesses from oyster consumption each year. Of infected individuals, approximately 7 cases of gastroenteritis will progress to septicemia each year for the total population, of which 2 individuals would be from the healthy subpopulation and 5 would be from the immunocompromised subpopulation.

What is the frequency and extent of pathogenic strains of *Vibrio parahaemolyticus* in shellfish waters and in oysters?

- Levels of pathogenic *V. parahaemolyticus* usually occur at low levels in shellfish waters.
- Levels of pathogenic *V. parahaemolyticus* in oysters at the time of harvest are only a small fraction of the total *V. parahaemolyticus* levels.

What environmental parameters (e.g., water temperature, salinity) can be used to predict the presence of *Vibrio parahaemolyticus* in oysters?

- The primary driving factor to predict the presence of *V. parahaemolyticus* in oysters is water temperature. Salinity was a factor evaluated but not incorporated into the model. Salinity is not a strong determinant of *V. parahaemolyticus* levels in the regions that account for essentially all the commercial harvest. Other factors such as oyster physiology and disease status may also be important but no quantifiable data were available to include these factors in the model.
- There are large differences in the predicted levels of *V. parahaemolyticus* in oysters at harvest among regions and seasons. For all regions, the highest levels of *V. parahaemolyticus* were predicted in the warmer months of summer and spring and the lowest levels in the fall and winter.
- Overall, the highest levels of total and pathogenic *V. parahaemolyticus* were predicted for the Gulf Coast (Louisiana) and the lowest levels in the Pacific Northwest (Dredged) harvested oysters.
- After harvest, air temperature is also an important determinant of the levels of *V. parahaemolyticus* in oysters. *Vibrio parahaemolyticus* can continue to grow and multiply in oysters until they are adequately chilled.

- Levels of *V. parahaemolyticus* are lower in oysters after harvest in the cooler vs. warmer months. This means that reducing the time between harvest and cooling will be more important in the summer and spring than in the fall and winter.

How do levels of *Vibrio parahaemolyticus* in oysters at harvest compare to levels at consumption?

- With no mitigation treatments, levels of *V. parahaemolyticus* are higher in oysters at consumption than at harvest. The difference between *V. parahaemolyticus* densities at-harvest versus at-consumption is largely attributable to the extent of growth that occurs before the oysters are cooled to no-growth temperatures.
- Levels of *V. parahaemolyticus* in oysters vary by region and season and are highest during the summer.
- During intertidal harvest, oysters are exposed to ambient air temperatures for longer times, allowing additional growth of *V. parahaemolyticus* in oysters and leading to higher predicted risk of illness.
- Preventing growth of *V. parahaemolyticus* in oysters after harvest (particularly in the summer) will lower the levels of *V. parahaemolyticus* in oysters and, as a consequence, lower the number of illnesses associated with the consumption of raw oysters.

What is the role of post-harvest handling on the level of *Vibrio parahaemolyticus* in oysters?

- Post-harvest measures aimed at reducing the *V. parahaemolyticus* levels in oysters reduced the model-predicted risk of illness associated with this pathogen.
- Reducing the time between harvest and chilling has a large impact on reducing levels of *V. parahaemolyticus* in oysters and the number of illnesses. Predicted reductions were greater for shorter times to refrigeration using ice (oysters reach no-growth temperature in 1 hour) compared to cooling under conventional refrigeration (which may take up to 10 hours until oysters reach a no-growth temperature).

What reductions in risk can be anticipated with different potential intervention strategies?

- Overall. The most influential factor affecting predicted risk of illness is the level of total *V. parahaemolyticus* in oysters at the time of harvest. Intervention strategies should be aimed at reducing levels of *V. parahaemolyticus* and/or preventing its growth in oysters after harvest. These strategies, either at-harvest or post-harvest, may need to consider regional/seasonal differences.
- Regional/seasonal Differences. The risk of *V. parahaemolyticus* illness is increased during the warmer months of the year, with the magnitude of this increase a function of the extent to which the growing waters (and ambient air temperatures) are at temperatures that support the growth of the pathogen (e.g., temperatures above 10 °C). For each region, the predicted numbers of illnesses are much higher for the summer compared to the winter months. Intervention measures that depend on cooling oysters to no-growth temperatures for *V. parahaemolyticus* may be more important in warmer seasons and regions.

The risk of *V. parahaemolyticus* illness is substantial in the Gulf Coast region where water temperatures are warm over a large part of the year as compared to the Northeast Atlantic region where water temperatures support the growth of *Vibrio parahaemolyticus* only during a relatively small portion of the year. A difference is seen among the regions due to different harvesting methods. Within the Gulf Coast, the predicted number of illnesses is much higher in Louisiana compared to other states in this region because the harvest boats in Louisiana are typically on the water longer, i.e., leading to a longer time from harvest to refrigeration. Harvest volume is also a determining factor; in the summer, Louisiana accounts for approximately 77% of the Gulf Coast harvest. This is also seen in the Pacific Northwest by comparing intertidal versus dredged harvesting. Intertidal harvesting accounts for 75% of the Pacific Northwest harvest and exposes oysters to higher temperatures longer, allowing greater growth of *V. parahaemolyticus*. Overnight submersion for a single tidal cycle, reduces levels of *V. parahaemolyticus* in oysters and the risk of illness.

- Post-Harvest Treatments. Post-harvest treatments that reduce levels of *V. parahaemolyticus* by 2 to 4.5-logs were found to be effective for all seasons and regions, with the most pronounced effects seen for regions and seasons with higher baseline risk. The model shows that any treatment that causes at least a 4.5-log decrease in the number of *V. parahaemolyticus* bacteria reduces the probability of illness to such an extent that few illnesses would be identified by epidemiological surveillance. However, some outbreak strains (e.g., O3:K6) are more resistant to mitigations than endemic pathogenic *V. parahaemolyticus* strains, and the duration or extent of treatment may need to be more stringent to achieve an equivalent degree of reduction. Studies have shown that both *V. parahaemolyticus* and *V. vulnificus* respond similarly to control measures such as ultra high pressure, mild heat treatment, and freezing. Therefore, mitigations aimed at decreasing levels of *V. parahaemolyticus* will also likely decrease levels of *V. vulnificus*.

The model also demonstrated that if oysters are not refrigerated soon after harvest, *Vibrio parahaemolyticus* rapidly multiply resulting in higher levels. For example, the model indicates that for the Gulf Coast there is a significant reduction (~10-fold) in the probability of illness when the oysters are placed in a refrigerator immediately after harvest. Less pronounced reductions are predicted for the other regions. Predicted reduction in illness is less in colder seasons because oysters harvested in cooler weather are already at or below the temperature threshold for *V. parahaemolyticus* growth and as such refrigeration has little additional impact on levels of *V. parahaemolyticus*.

- At-Harvest and At-Retail Controls. Controlling the levels of *V. parahaemolyticus* in oysters at-harvest or at-retail (after refrigeration and storage) drastically reduces the number of predicted illnesses but would require diversion of oysters from the raw market or modification of handling practices to reduce post-harvest *V. parahaemolyticus* growth. For the Gulf Coast (Louisiana) region in the summer, excluding all oysters with at least 10,000 *V. parahaemolyticus*/g at-harvest would reduce illness by approximately 16% with an impact of approximately 3% of the total

harvest; and this same control level at-retail would reduce illness by about 99% with a 43% loss from the raw consumption market. The effectiveness of the control level either at-harvest or at-retail to reduce illnesses depends on the extent of compliance with that control level.

In a sample-based control strategy, a reasonable surrogate for pathogenic *V. parahaemolyticus* may be total levels of this microorganism. Criteria for rejection of oysters based on the levels of this surrogate might have to vary by region. For example, an at-harvest control criterion based on total *V. parahaemolyticus* levels in the Pacific Northwest might need to be more stringent than in the Gulf Coast because the incidence of pathogenic strains appears to be higher in the Pacific Northwest. However, in an outbreak, the ratio of pathogenic to total *V. parahaemolyticus* may not be the same or consistent, and the model does not evaluate how well total *Vibrio parahaemolyticus* would serve as a surrogate for pathogenic *V. parahaemolyticus* in an outbreak situation.

Conclusions

Although the risk assessment modeled sporadic *V. parahaemolyticus* illnesses, steps taken to reduce sporadic cases from TDH⁺ strains could also proportionally reduce the size of outbreaks. However, some outbreak strains (e.g., O3:K6) may be more resistant to mitigations than endemic *V. parahaemolyticus* strains and may also require fewer cells to cause illness. The risk assessment illustrates that the levels of *V. parahaemolyticus* at-harvest play an important role in causing human illness. However, other factors that either reduce or allow growth of *V. parahaemolyticus* in oysters are also important in determining the number of illnesses. For example, shortening the time-to-refrigeration of oysters in the summer controls growth of *V. parahaemolyticus* in oysters and subsequently reduces illnesses associated with this microorganism.

The results of this risk assessment are influenced by the assumptions and data sets that were used to develop the Exposure Assessment and Dose-Response models. The predicted risk for illness among consumers of raw oysters and the most significant factors which influence the incidence of illness could change as a result of future data obtained from continuing surveillance studies. It is anticipated that periodic updates to the model when new data and knowledge become available will continue to reduce the degree of uncertainty associated with the factors that influence the risk, and that this will assist in making the best possible decisions, policies, and measures for reducing the risk posed by *V. parahaemolyticus* in raw oysters. This risk assessment provides an understanding of the relative importance and interactions among the factors influencing risk. It will hopefully provide a useful tool to facilitate the formulation of effective guidance and requirements and the evaluation of risk mitigation strategies.

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GLOSSARY

Term	Definition
Case series	Study of cases of similar illness occurring over a period of time.
Compliance	Voluntarily choosing to follow the guidelines
Depuration	The process of reducing pathogenic organisms that may be present in shellfish using a controlled aquatic environment, such as land-based tanks, as the treatment process.
Dose	The number of pathogenic <i>V. parahaemolyticus</i> consumed in oysters at one sitting.
Dose-response	The relationship of the levels of <i>V. parahaemolyticus</i> ingested with the frequency and magnitude of illness.
Gastroenteritis	Inflammation of the gastrointestinal tract; symptoms typically include diarrhea, vomiting, and/or abdominal cramps, caused by an infecting organism which is present in feces.
Gyrase B	A prokaryotic gene which codes for the enzyme gyrase that unwinds DNA so it can be replicated.
Imputation (impute)	The statistical practice of substituting missing data with plausible values. For example, in regard to samples with densities less than the sensitivity of an enumeration method (e.g., <0.3 cfu/g) plausible values in the range between zero and 0.3 may be imputed using statistical methods.
Isolate	A single colony identified from a mixed bacterial culture on an agar plate
Iteration	A single calculation of model output(s) based on a set of sampled variability and/or uncertainty model inputs (factors).
Kanagawa phenomenon	Hemolysis induced by the thermostable direct haemolysin on a special blood agar, Wagatsma medium.
Maximum likelihood estimate (MLE)	An estimate (e.g., of a model parameter) such that the observed outcome is the most likely of all possible outcomes.
Midday temperature	Temperature taken at noon.
Mode	A statistical term; most likely value.
Monte-Carlo Simulation	Computer experiments of modeled relationships that simulate probabilistic variation using random numbers generated by specified distribution functions.
Outbreak	The occurrence of similar illness involving 2 or more persons resulting from the ingestion of a common food.
Pathogenic <i>V. parahaemolyticus</i>	For the purpose of this risk assessment, pathogenic <i>V. parahaemolyticus</i> strains are those that produce thermostable direct hemolysin (TDH) and/or hemolyse red blood cells on a blood agar plate, which is referred to as the Kanagawa Phenomenon -positive (KP-+ve).
Relaying	The process of reducing pathogenic organisms or deleterious substances that may be present in shellfish by transferring shellfish from a contaminated growing area to one that is not.

Term	Definition
Sensitive subpopulation	Group of people with greater vulnerability to more severe <i>V. parahaemolyticus</i> disease (i.e., septicemia) as a result of some underlying state of compromised health, such as liver disease, blood disorder, or immunodeficiency.
Septicemia	A systemic disease caused by the multiplication of pathogenic microorganisms and/or the presence and persistence of their toxins in the circulating blood.
Skow	A flat bottomed, flat decked "barge" towed by another boat; some may be motorized, have a cabin, and a boom hoist.
Species	Bacterial collections of similar strains.
Sporadic case	When a single individual becomes ill; an isolated event not documented as occurring in the context of an outbreak.
Strain	A group of organisms of the same species, having distinctive characteristics but not usually considered a separate breed or variety.
Thermocouple	A device for measuring temperature. A pair of wires of dissimilar metals are joined and the free ends of the wires are connected to an instrument (as a voltmeter) that measures the difference in potential created at the junction of the two metals.
Thermostable direct hemolysin	A toxin produced by <i>V. parahaemolyticus</i> that lyses red blood cells in Wagatsuma agar.
Thermostable-related hemolysin	A toxin very similar in action and characteristics to, but genetically distinct from the thermostable direct hemolysin.
Tobit regression	A type of regression model, applicable to limit-of-detection truncated or censored data, whereby unbiased parameter estimates are obtained without the need for imputation in place of missing values
Total <i>V. parahaemolyticus</i>	The summation of pathogenic (<i>tdh</i> +) and non-pathogenic (<i>tdh</i> -) <i>V. parahaemolyticus</i> cells in a specified unit of volume or mass.
Uncertainty	An expression of the lack of knowledge, usually expressed as a probability distribution; pertaining to the lack of knowledge concerning a fixed but unknown quantity.
Uncertainty Distribution	A description of the range of plausible values for a prediction.
Variability	A description of differences of an attribute among the individual members of a series or population.
Virulence	The capacity of a microbial pathogen to invade and/or produce illness in the host. Mediated by the presence of specific genes and their protein products that interact with the host.
Water activity	The ratio of the water vapor pressure in any kind of food system to the water vapor pressure of pure water; $aw = P_{\text{product}} / P_{\text{water}}$.

ACRONYMS AND ABBREVIATIONS

Acronym/ Abbreviation	Definition
CDC	Centers for Disease Control and Prevention
CFSAN	Center for Food Safety and Applied Nutrition
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
GCSL	FDA Gulf Coast Seafood Laboratory, Dauphin Island
GCVSS	Gulf Coast <i>Vibrio</i> Surveillance System
IAFP	International Association for Food Protection
ICP	ISSC Interim Control Plan for monitoring levels of pathogenic <i>V. parahaemolyticus</i> in oysters at time of harvest
ISSC	Interstate Shellfish Sanitation Conference
MSI	Molluscan Shellfish Industry
NACMCF	National Advisory Committee on Microbiological Criteria for Foods
NCTR	National Center for Toxicological Research
NERR	National Estuarine Research Reserve System
NBDC	National Buoy Data Center
NOAA	National Oceanic and Atmospheric Administration
NOS	National Ocean Services
NSSP	National Shellfish Sanitation Program
NWS	National Weather Service
PCSGA	Pacific Coast Shellfish Growers Association
RAC	Interagency Risk Assessment Consortium
SGE	Special Government Employee
STORET	EPA Storage and Retrieval of U.S. Waterways Parametric Data database
WHO	World Health Organization

Acronym/ Abbreviation	Definition
Bp	base pairs
C	Celsius
CFU	Colony Forming Units
DIG	digoxigenin
F	Fahrenheit
/g	per gram
g	grams
<i>gyrB</i>	gyrase B
HGMF	Hydrophobic Grid Membrane Filtration procedure
h	hours
ID ₅₀	Infective Dose at which 50% of infected subjects become ill
KP+	Kanagawa-positive
LD ₅₀	Lethal Dose at which 50% of infected subjects die
LOD	Limit Of Detection
Mb	mega base pairs
min	minute
ml	milliliters
MLE	Maximum likelihood estimates
MPa	Mega Pascals
MPN	Most Probable Number
PBS	phosphate buffered saline
ppt	parts per thousand
RITARD	removable intestinal tie adult rabbit diarrhea
TDH	thermostable direct hemolysin
TRH	thermostable-related hemolysin
TTSS	Type III Secretion System
VBNC	viable but not culturable
Vp	<i>Vibrio parahaemolyticus</i>
Vp _{path}	pathogenic strains of <i>V. parahaemolyticus</i>

I. INTRODUCTION

The Food and Drug Administration (FDA) conducted this risk assessment on the public health impact of *Vibrio parahaemolyticus* transmitted by raw oysters. This is a “product pathway” risk assessment and provides a systematic evaluation of the factors affecting *V. parahaemolyticus* in oysters and the sequence of events leading to consumer illnesses.

Background

Vibrio parahaemolyticus is a marine bacterium that occurs naturally in the estuarine environment and can accumulate in filter-feeding molluscan shellfish. This microorganism was first identified as a foodborne pathogen in Japan in the 1950s. It has been associated with outbreaks and individual cases of illness in the United States since 1969. In 1997 and 1998, over 700 cases of illness from four outbreaks were associated with consumption of raw oysters in three regions of the country, the Gulf Coast, Pacific Northwest, and Northeast. These outbreaks renewed concern for this pathogen as a serious foodborne threat to public health and raised new concerns about the effectiveness of current risk management guidance.

The Centers for Disease Control and Prevention (CDC) estimates that each year there are approximately 2,800 cases of *V. parahaemolyticus* illness associated with the consumption of raw oysters. The most common clinical manifestation of *V. parahaemolyticus* infection is gastroenteritis. In at-risk populations (individuals with underlying chronic medical conditions), infection can lead to more serious outcomes (septicemia and death).

FDA announced the initiation of this risk assessment in 1999 in the Federal Register (FDA, 1999). The public was invited to comment on the planned assessment and submit scientific data and information for use in the assessment. The advice and recommendations of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) were sought on the assumptions and the model structure to be used. During the conduct of this risk assessment, FDA solicited the technical advice and opinions of scientific experts both within and outside of the Federal government. The availability of the draft risk assessment was announced in the Federal Register (Federal Register Docket No. 99N 1075) in January 2001 (FDA, 2001). A comment period was established during which FDA actively sought comments, suggestions, and additional data sources. The draft risk assessment was presented to stakeholders and other interested parties during a public meeting on March 20, 2001. The risk assessment report and model were modified based on the public comments received and availability of new data. The revised document and model were subjected to extensive review. A chronology of the technical and scientific review involved in the development of this risk assessment is provided in Appendix 1. A summary of the modifications made to the 2001 model is provided in Appendix 2.

Scope

This risk assessment is a quantitative product pathway analysis in which the key steps from harvest through post-harvest handling and processing to consumption were modeled. The likelihood of illness following exposure to pathogenic *V. parahaemolyticus* from consumption of raw oysters was calculated. The levels of *V. parahaemolyticus* in oysters at the time of consumption can be influenced by the harvest methods and handling of oysters after harvest and these practices may vary considerably in different geographic areas and at different times of year. The impact of regional and seasonal conditions on the predicted risk was evaluated.

The risk assessment had two main objectives: (1) to determine the factors that contribute to the risk of becoming ill from the consumption of pathogenic *V. parahaemolyticus* in raw oysters and (2) to evaluate the likely public health impact of different control measures, including the effectiveness of current and alternative microbiological standards.

The risk assessment addresses the following questions:

- What is known about the dose-response relationship between consumption of *V. parahaemolyticus* and illnesses?
- What is the frequency and extent of pathogenic strains of *V. parahaemolyticus* in shellfish waters and in oysters?
- What environmental parameters (e.g., water temperature, salinity) can be used to predict the presence of *V. parahaemolyticus* in oysters?
- How do levels of *V. parahaemolyticus* in oysters at-harvest compare to levels at consumption?
- What is the role of post-harvest handling on the level of *V. parahaemolyticus* in oysters?
- What reductions in risk can be anticipated with different potential intervention strategies?

Risk Assessment Overview

The *Vibrio parahaemolyticus* Risk Assessment follows the risk assessment structure of the Joint Food and Agriculture Organization/World Health Organization Expert Consultation on the Application of Risk Analysis to Food Standards Issues (FAO/WHO, 1998). The structure consists of four components: (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization. Figure I-1 shows the organization and components of the risk assessment including the types of data and modeling techniques used.

Hazard Identification

The Hazard Identification component of a microbial risk assessment is the identification of the pathogenic organism that may be present in a particular food or group of foods that are capable of causing adverse health effects. The hazard on which this risk assessment is focused is pathogenic *V. parahaemolyticus* in raw oysters. The adverse health effect considered is the number of illnesses characterized by gastroenteritis and septicemia. See Chapter II: Hazard Identification for details.

Hazard Characterization/Dose Response/Severity Assessment

The Hazard Characterization component of a microbial risk assessment is often referred to as Dose-Response because it characterizes the relationship between the level of exposure to a pathogen (the dose) and the likelihood of an adverse health effect for individuals and populations (the response). For this risk assessment, a quantitative relationship was developed to predict the number and severity of illnesses resulting from ingesting different amounts of pathogenic *V. parahaemolyticus*. The Dose-Response model was developed using human clinical volunteer feeding studies and epidemiological surveillance data. See Chapter III: Hazard Characterization for details.

Exposure Assessment

The Exposure Assessment component of a microbial risk assessment defines the frequency and likely level of exposure to a pathogenic microorganism. In this risk assessment, the likelihood of exposure to pathogenic *V. parahaemolyticus* from consumption of raw oysters was evaluated. The Exposure Assessment was divided into three modules: Harvest, Post-Harvest, and Consumption. The levels of *V. parahaemolyticus* in oysters at the time of consumption can be influenced by the harvest methods and handling of oysters after harvest and these practices may vary considerably in different geographic areas and at different times of year.

Oysters are harvested in the United States from the Gulf Coast, Mid-Atlantic, Northeast Atlantic, and Pacific Northwest. In the Gulf Coast, the harvest duration for Louisiana is typically much longer than for other states in that region (Florida, Mississippi, Texas, and Alabama), therefore it was divided into two distinct regions: Gulf Coast (Louisiana) and Gulf Coast (Non-Louisiana). Likewise, the Pacific Northwest was divided into two distinct regions: Pacific Northwest (Intertidal) and Pacific Northwest (Dredged). In the Pacific Northwest, oysters are harvested by two methods: dredging and intertidal. For the intertidal harvest method, oysters are hand-picked when oyster reefs are exposed during

the tide cycle and left in baskets until the tide rises to a sufficient depth to allow a boat to retrieve the basket. The risk assessment considered six oyster harvest regions and four seasons, for a total of 24 region/season combinations. See Chapter IV: Exposure Assessment for details.

Risk Characterization

Risk Characterization is the integration of the Dose-Response relationship with the Exposure Assessment to predict the probability of potential adverse outcomes for individuals or populations. For this risk assessment, the likelihood and severity of illness (gastroenteritis alone or gastroenteritis followed by septicemia) from the consumption of raw oysters containing pathogenic *V. parahaemolyticus* was predicted on both a per serving and a per annum basis. The uncertainties associated with the predicted risk estimates were also determined. See Chapter V: Risk Characterization for details.

Using the Model as a Tool: “What-If” Scenarios

The baseline risk assessment model can be used to estimate the likely impact of intervention strategies on the predicted number of illnesses. “What-if” scenarios were conducted by changing one or more model inputs and measuring the resulting change to the model outputs. Various control measures and mitigation strategies were evaluated. See Chapter VI: What-If Scenarios for details.

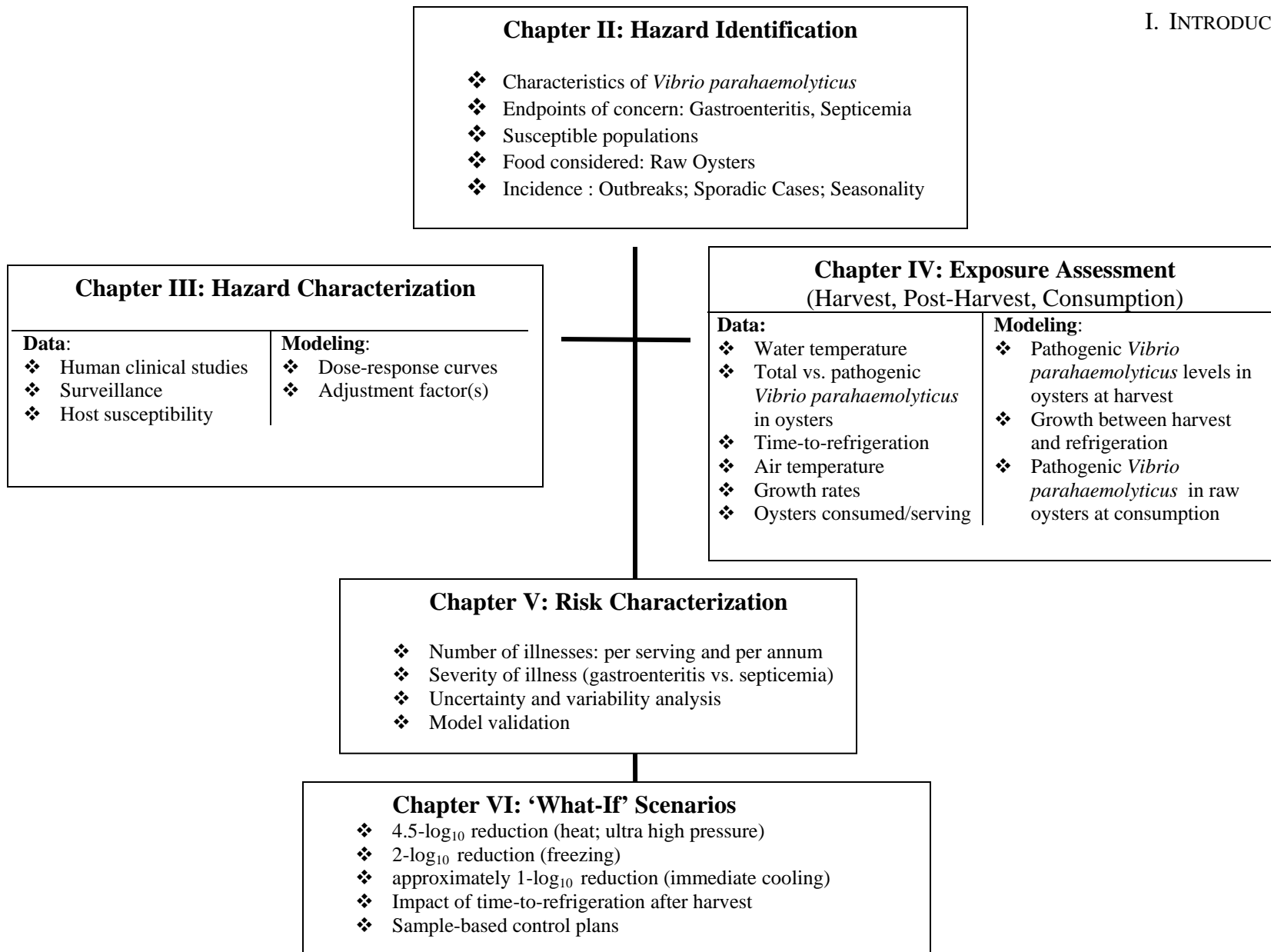


Figure I-1. Overview of *Vibrio parahaemolyticus* Risk Assessment Document

II. HAZARD IDENTIFICATION

The Hazard Identification component of a microbial risk assessment is the identification of the pathogenic microorganism that is capable of causing adverse health effects and is present in a particular food or group of foods. The hazard on which this risk assessment is focused is pathogenic *V. parahaemolyticus* in raw oysters and the adverse health effects include gastroenteritis and septicemia.

Vibrio parahaemolyticus

Vibrio parahaemolyticus is a Gram-negative, halophilic bacterium that occurs naturally in estuaries and is recognized as an important bacterial seafood-borne pathogen throughout the world (Fujino *et al.*, 1953; Sakazaki, 1973). *Vibrio* spp. are found in the estuarine environment in the tropical and temperate zones (Joseph *et al.*, 1983). These bacteria are normally present in many seafoods, including fish, crustaceans, and molluscan shellfish. They concentrate in the gut of filter-feeding molluscan shellfish such as oysters, clams, and mussels where they multiply and cohere.

The genome of *V. parahaemolyticus* was sequenced (Makino *et al.*, 2003) and was found to consist of two circular chromosomes of 3,288,558 bp and 1,877,212 bp, and contains 4,832 genes. Although *V. parahaemolyticus* is phylogenetically close to *V. cholerae*, comparison of the *V. parahaemolyticus* genome with that of *V. cholerae* showed there are many rearrangements within and between the two chromosomes. Chromosome 1 does not differ much in size between the two genomes (3.3 vs. 3.0 Mb), but chromosome 2 is much larger in *V. parahaemolyticus* than in *V. cholerae*. Genes for the type III secretion system (TTSS) identified in the genome of *V. parahaemolyticus* are not found in *V. cholerae*. The TTSS is a central virulence factor of diarrhea-causing bacteria such as *Shigella* spp., *Salmonella* spp., and enteropathogenic *Escherichia coli*, which cause gastroenteritis by invading or intimately interacting with intestinal epithelial cells. These results suggest that *V. parahaemolyticus* and *V. cholerae* use different mechanisms to establish infection.

Serotypes

Isolates of *V. parahaemolyticus* can be differentiated by serotyping. The system for identifying *V. parahaemolyticus* serotypes is based on the different antigenic structures of the lipopolysaccharides groups (referred to as O groups) and capsular types (referred to as K types) (Joseph *et al.*, 1983). Thirteen O groups and 71 K types have been identified by commercial antisera (Iguchi *et al.*, 1995). Of these, 11 O groups and 38 K types have been isolated from *V. parahaemolyticus* strains collected in the United States (Fishbein *et al.*, 1974). In a recent study, 27 different O:K serotypes were found among 178 strains isolated from various sources including seafood, sediment and clinical samples (DePaola *et al.*, 2003a).

Historically, *V. parahaemolyticus* infections have been characterized by sporadic cases caused by multiple, diverse serotypes. However, three serotypes (O4:K12, O1:K56, and O3:K6) predominated in outbreaks associated with the consumption of raw molluscan

shellfish in 1997 and 1998. The serotypes isolated from patients in the 1997 outbreak in the Pacific Northwest included O4:K12 and O1:K56 (Daniels *et al.*, 2000a). In outbreaks in 1998 in Texas and New York, the serotype O3:K6 was the predominant isolate and principal cause of illness. Prior to the 1998 outbreak, the O3:K6 serotype had only been reported in Asia; this was the first time it was reported in the United States. This serotype may have a lower infectious dose than other pathogenic *V. parahaemolyticus* strains (Daniels *et al.*, 2000b).

Strains

Strains of *V. parahaemolyticus* are isolates of the same serotype that have been characterized or distinguished from each other. Not all strains of *V. parahaemolyticus* cause illness in humans; in fact, the majority of strains isolated from the environment or seafood are not pathogenic. For the purpose of this risk assessment, pathogenic strains of *V. parahaemolyticus* are those that produce thermostable direct hemolysin (TDH). TDH is an enzyme that lyses (breaks down) red blood cells on Wagatsuma blood agar plates, which is referred to as the Kanagawa phenomenon. The role of the toxin in illness is not known.

Illnesses Caused by *Vibrio parahaemolyticus*

The most common clinical manifestation of *V. parahaemolyticus* infection is gastroenteritis, an inflammation of the gastrointestinal tract. Gastroenteritis is usually a self-limited illness with moderate severity and short duration (Barker, 1974; Barker and Gangarosa, 1974; Hlady, 1997; Levine *et al.*, 1993). A summary of clinical symptoms associated with *V. parahaemolyticus* gastroenteritis infection is presented in Table II-1. Symptoms of illness include explosive watery diarrhea, nausea, vomiting, abdominal cramps, and less frequently headache, fever and chills. Diarrhea may also be characterized by full-blown dysentery with blood and pus and superficial ulceration on proctoscopic examination (Carpenter, 1995).

Table II-1. Clinical Symptoms Associated with Gastroenteritis Caused by *Vibrio parahaemolyticus*

Symptoms	Incidence of Symptoms	
	Median	Range
Diarrhea	98%	80 to 100%
Abdominal cramps	82%	68 to 100%
Nausea	71%	40 to 100%
Vomiting	52%	17 to 79%
Headache	42%	13 to 56%
Fever	27%	21 to 33%
Chills	24%	4 to 56%

Source of data: Barker and Gangarosa, 1974; Levine *et al.*, 1993

On rare occasion, infection can lead to septicemia. Septicemia is a severe, life-threatening, systemic disease caused by the multiplication of pathogenic microorganisms and/or the presence and persistence of their toxins in the circulating blood. It is characterized by fever or hypotension and the ability to isolate the microorganism from the blood. In cases of septicemia, subsequent symptoms can include swollen, painful extremities with hemorrhagic bullae (Hlady, 1997; Klontz, 1990). Death may also occur subsequent to the occurrence of septicemia.

Duration of illness can range from 2 hours to 10 days (Barker and Gangarosa, 1974; Barker *et al.*, 1974). Information from several United States outbreaks revealed that the incubation period ranges from 12 to 96 hours with a median of approximately 15 to 24 hours (CDC, 1998; CDC, 1999a; Lowry *et al.*, 1989; Nolan *et al.*, 1984).

At-Risk Populations

Any exposed individual can become infected with *V. parahaemolyticus* and develop illnesses (such as gastroenteritis). However, infected individuals with underlying chronic medical conditions often develop septicemia. Therefore, although all raw shellfish consumers are “at risk” for infection, there is a subpopulation of individuals with increased risk of severe disease.

Individuals with Chronic Medical Conditions. Chronic medical conditions include liver disease, immunodeficiency, peptic ulcer disease, diabetes, alcoholism, hematological disease, gastric surgery, heart disease, renal disease, cancer or malignancy, treatment with corticosteroids, and transplant recipients (Klontz, 1990; Klontz, 1997; Angulo and Evans, 1999).

The percentage of the population that is at increased risk for development of septicemia from *V. parahaemolyticus* infection is not known precisely. The Center for Science in the Public Interest reported that approximately 20% of the United States population (60 million) have immunocompromised conditions and are at increased risk for *V. vulnificus* septicemia (CSPI, 1997). However, it is not known how many of these individuals consume raw oysters. Based on studies showing that certain persons are at greatest risk for illness from raw-oyster associated *V. vulnificus* infection (Desenclos *et al.*, 1991 and Klontz, 1990), it was estimated that approximately 7% of the population have immunocompromising health conditions associated with increased risk of infection (Klontz, 1997). Analysis of epidemiological surveillance data (Angulo and Evans, 1999) indicates that approximately 30% of 107 cases of gastroenteritis were identified in individuals with underlying chronic illnesses. However, immunocompromised individuals may be over represented in case series data because of a “reporting phenomenon” driven by the severity of illness. An immunocompromised individual may be more likely to seek medical care for the symptoms of *V. parahaemolyticus* illness than an otherwise healthy individual with the same symptoms.

Raw Shellfish Consumers. Surveys conducted by FDA in 1993 and 1998 indicate that consumption of raw shellfish is not uniformly distributed in the United States population (Levy and Fein, 1999). For example, a higher percentage of men consume raw oysters than women (16% vs. 7%), and raw shellfish consumption is higher for those living along the coastline of the United States than for those living inland (22% vs. 13%). The trend in raw shellfish consumption, as evidenced in the 1998 FDA survey, is toward lowered consumption of raw shellfish. This may be the result of education efforts by the Agency concerning the risks associated with the consumption of raw or undercooked protein foods, such as beef, chicken, eggs, and shellfish.

Annual Incidence

In 1999, CDC conducted a comprehensive evaluation of the national burden of infectious food-related illnesses in the United States. The total annual incidence of *Vibrio* illness was estimated as 7,880 illnesses and of that 65% were estimated to be food related (Mead *et al.*, 1999). This estimate was based on the frequency of reported cases obtained by passive surveillance from 1988 through 1996 and the cases reported through FoodNet. The estimate also considers that this illness is under reported and under diagnosed and for every reported illness there are assumed to be 20 cases that are not reported (Kennedy, 2000; Mead *et al.*, 1999).

Based on FoodNet data, the yearly estimates of food-related illness attributed to *V. parahaemolyticus* for 1996, 1997 and 1998 were approximately 2,700, 9,800, and 5,600, respectively (Tauxe, 2000). The 1997 estimate reflects the increased reporting of cases from a large outbreak in the Pacific Northwest. Some variation in estimated cases from year to year is expected, even in the absence of any inter-annual variation attributable to differing environmental conditions.

Specifically for this risk assessment (see Chapter III Hazard Characterization), CDC conducted an in-depth analysis of the available data on the incidence of illness from consumption of raw oysters reported over a 5-year period (1998-2002). CDC estimated there are approximately 2,790 cases of *V. parahaemolyticus* illness in the United States as result of oyster consumption (Painter, 2003). To obtain this estimate, CDC compared the reported cases from the National Notifiable Diseases Surveillance System (NNDSS) and the Cholera and Other *Vibrio* Illness Surveillance System (COVISS) because these systems collect reports from all states. Some cases are reported in both systems. A comparison of case information (using “capture-recapture” method for surveillance evaluation) indicated the number of reported cases was 1,125 for the 5-year period (or 225 cases per year). This compares well with FoodNet surveillance data (which represents 13% of the United States population) which indicate there are 300 cases per year in the United States. As noted above, CDC estimates that the number of cases is underestimated by a factor of 1:20 due to underreporting. So the estimated number of cases is 4,500 (225 x 20). Using information relating to *V. parahaemolyticus* exposure from COVISS, CDC estimates that 62% of all *V. parahaemolyticus* illness cases are caused by consumption of raw oysters. Therefore, the estimated number of cases of illness from *V. parahaemolyticus* in raw oysters used in the dose-response modeling was 2,790 (0.62 x 4,500). See Chapter III Hazard Characterization for details.

CDC's Active Surveillance Systems

- **FoodNet**. The Foodborne Diseases Active Surveillance Network (FoodNet) is the principal foodborne disease component of CDC's Emerging Infections Program (EIP). FoodNet is a collaborative project of the CDC, 10 EIP sites (California, Colorado, Connecticut, Georgia, New York, Maryland, Minnesota, Oregon, Tennessee and New Mexico), the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA).
- **CDC Gulf Coast *Vibrio* Surveillance System (GCVSS)**. The CDC Gulf Coast *Vibrio* Surveillance System (GCVSS) is a unique regional system that began in 1988 (Levine *et al.*, 1993). Four states initially participated in this program (Alabama, Florida, Texas, and Louisiana). Mississippi was added soon after, and the system has grown to include any and all states that are willing to participate; indeed, in the last few years, the West Coast states have become very active in reporting cases (Crowe, 2002). Investigators in state and county health departments complete standardized *Vibrio* illness investigation forms on all patients from whom *Vibrio* isolates are reported. *Vibrio* reporting comes from individual physicians, hospitals, or laboratories. Illness investigation forms contain clinical data concerning signs and symptoms, underlying illnesses, use of medications, as well as epidemiological information concerning seafood consumption in the week prior to illness. Data from this surveillance system has also been used for case series analysis (see discussion below).

Outbreaks and Sporadic Cases

An outbreak is defined as the occurrence of two or more cases of a similar illness resulting from the ingestion of a common food. The term “sporadic cases” refers to an irregular pattern of occurrence, with occasional cases occurring at irregular intervals. Sporadic cases can be reported as either “case reports” which present pertinent information on individual cases, or as a “case series” which is a study of sporadic cases over a specified period of time.

Outbreaks

The first confirmed case of foodborne illness-associated *V. parahaemolyticus* infection in the United States occurred in Maryland in 1971 with an outbreak associated with consumption of steamed crabs (Dadisman *et al.*, 1972). Between 1973 and 1998, forty outbreaks were reported to the CDC from 15 states and the Guam Territories (Daniels *et al.*, 2000a). These outbreaks were associated with raw seafood or cooked seafood cross-contaminated with raw or undercooked seafood. Since 1998, there have been three outbreaks caused by *V. parahaemolyticus*, and all were associated with consumption of oysters (Agasan, 2002; New Jersey Dept of Environmental Protection, 2002; Potempa, 2004).

Table II-2 summarizes the major outbreaks of *V. parahaemolyticus* gastroenteritis in the United States from 1997 to 2002. In 1997, an outbreak involving 251 cases occurred in

the Pacific Northwest (202 in the United States and 49 in British Columbia) (Sample and Swanson, 1997). Of these cases, *V. parahaemolyticus* infection was confirmed in 209 persons who consumed raw oysters harvested from California, Oregon and Washington and from Canada (CDC, 1998). The most common *V. parahaemolyticus* serotypes isolated from patients involved in this outbreak were O4:K12 and O1:K56 (Daniels *et al.*, 2000a). In the United States, oyster-associated *V. parahaemolyticus* outbreaks are more common than other shellfish-associated *V. parahaemolyticus* outbreaks (Daniels *et al.*, 2000; Agasan, 2002; New Jersey Dept of Environmental Protection, 2002; Potempa, 2004).

Three separate outbreaks occurred in the United States in 1998. In the Pacific Northwest, 48 cases were reported (Therien, 1999). In Texas, a total of 416 *V. parahaemolyticus* infections were associated with consuming raw oysters harvested from Galveston Bay (Daniels *et al.*, 2000a). Also in 1998, New York reported the first outbreak associated with raw molluscan shellfish harvested from that state and this outbreak included 23 cases, 10 of which were associated with raw oysters (CDC, 1999a).

In the summer of 2002, a cluster of seven cases with *V. parahaemolyticus* infection appeared to be linked to the consumption of shellfish that was harvested and purchased locally in the Long Island and New York City area (Agasan, 2002). In another outbreak that same year, a total of 11 cases with two fatalities were reported in New Jersey (Mulnick, 2002). These cases were attributed to the above average water temperatures that year and resulted in closing 110 square miles of oyster beds (New Jersey Dept. of Environmental Protection, 2002).

Table II-2. Outbreaks of Illnesses from *Vibrio parahaemolyticus* Associated with Consumption of Raw Oysters in the United States

Year	Location	Number of Cases
1997	Pacific Northwest ^a	209 ^b
1998	Pacific Northwest ^a	48
1998	Texas	416 ^c
1998	Northeast Atlantic	10 ^b
2002	New York	7
2002	New Jersey	11
2004	Alaska	46 (8 ^b)

^aThe Pacific Northwest includes California, Oregon, Washington State, and British Columbia.

^bNumber of cases that were culture-confirmed.

^cIncludes 296 cases in Texas and 120 cases in other states traced back to oysters harvested from Texas.

Case Reports

Several case reports have been published that outline clinical presentations and outcomes of patients with *V. parahaemolyticus*. One such case report describes a 35-year-old woman who sought medical attention for abdominal pain after she had consumed raw fish (Tamura *et al.*, 1993). She presented with gastrointestinal symptoms, redness on lower extremities, fever, polyarthritis and weakness. *Vibrio parahaemolyticus* was isolated in the stool culture. She was diagnosed as having reactive arthritis induced by *V. parahaemolyticus* infection. Another clinical case report describes a 31 year-old female with a history of alcohol abuse, hepatitis C virus infection, and cirrhosis (Hally *et al.*, 1995). She presented with diarrhea, weakness, leg pain, and urine retention. The patient had ingested raw oysters and steamed shrimp 72 hours prior to being admitted to the hospital. *Vibrio parahaemolyticus* was isolated from blood samples. The patient developed cardiac arrest and died six days after presentation.

A suspected case of a laboratory-associated infection was reported in 1973 (Sanyal *et al.*, 1973). One day prior to the development of diarrheal disease the laboratory worker had been handling *V. parahaemolyticus* strains for the first time. The illness was associated with severe upper abdominal pain, bloody stools, nausea and fever. Weakness and abdominal discomfort continued for two days beyond the onset of illness. No other source of *V. parahaemolyticus* could be identified, and it was believed that the infection was caused by a relatively small inoculum.

Case Series

Case series data (Angulo and Evans, 1999) was used to analyze the relationship between illness outcomes and pre-existing health conditions. The data were from oyster-related culture-confirmed cases reported to the CDC GCVSS from 1997 to 1998. There were a total of 107 *V. parahaemolyticus* cases, of which 102 were gastroenteritis only, 5 that progressed to septicemia and 1 death. The overall incidence of septicemia among culture-confirmed *V. parahaemolyticus* infections was approximately 5% (5 out of 107). Of the cases with information on health conditions, 29% (23 out of 79) of the gastroenteritis illnesses and 75% (3 out of 4) of the septicemia illnesses occurred in individuals with an identified underlying (immunocompromising) health condition. The underlying medical conditions included liver disease, alcoholism, diabetes, malignancy, renal disease, immunodeficiency, hematological disease, and gastric surgery. The data from this case series was used in “Chapter III Hazard Characterization,” to estimate the annual number of septicemia cases in susceptible and healthy populations.

Case series have also been reported by others including Bonner *et al.* (1983), Noland *et al.* (1984), Kelly and Stroh (1988b), and Levine and Griffin (1993). These studies have also illustrated the association of septicemia with underlying medical conditions. Three case series for illnesses and deaths associated with *V. parahaemolyticus* infections from consumption of shellfish in Florida from 1981 to 1991 are described below.

- A case series of 4 patients who died in Florida due to *V. parahaemolyticus* infection from 1981 to 1988 was reported by Klontz (1990). All patients were male and all were over the age of 60 years. All died of septicemia. Two of the patients reported eating raw oysters during the week before onset of illness. The

- median duration of illness was 24 hours. All patients had underlying medical conditions, including cirrhosis, heart disease, prostate cancer and lung cancer.
- A case series of 690 *Vibrio* infections related to raw oyster consumption in Florida during 1981 to 1993 was reported by Hlady and Klontz (1996). There were 355 cases of gastroenteritis, of which 68% were associated with the consumption of raw oysters and 120 (34%) were due to *V. parahaemolyticus*. Of the 118 cases of septicemia, 83% were associated with raw oyster consumption and 16 (14%) were due to *V. parahaemolyticus*. Of 467 patients with infections presenting as either gastroenteritis or septicemia, 35% had a preexisting medical condition, such as liver disease, alcoholism, peptic ulcer disease, gastrointestinal surgery, diabetes, antacid medication or immune disorders. While the prevalence of underlying illness was high in the septicemia patients, the majority of patients with raw-oyster associated *Vibrio* gastroenteritis had no underlying conditions. The reported cases of gastroenteritis caused by *V. parahaemolyticus* infection were more common during warm weather months.
 - A case series of 339 *Vibrio* infections reported in Florida between 1981 and 1994 was reported by Hlady (1997). Culture-confirmed case reports of *Vibrio* infections, reported to the Florida Department of Health and Rehabilitation Services were investigated. Oyster-associated *Vibrio* infection was defined as a history of raw oyster consumption in the week prior to onset of gastroenteritis or septicemia. *Vibrio parahaemolyticus* accounted for 77 of the 339 reported *Vibrio* infections. Of the 237 raw oyster-associated cases of gastritis, 68 (30%) of the infections were due to *V. parahaemolyticus*. Of the 193 patients who were hospitalized, 37 (19%) had infection with *V. parahaemolyticus*. *Vibrio parahaemolyticus* accounted for 4 (8%) of reported deaths. Patients with septicemia had underlying illness including, but not limited to, cancer, liver disease, alcoholism and diabetes *mellitus*.

Implicated Foods

Raw oysters are the most common food associated with *Vibrio* infection in the United States (Hlady, 1997). While thorough cooking destroys *Vibrio*, oysters are often eaten raw. However, there have been reports of *V. parahaemolyticus* illnesses associated with other seafood, including crayfish, lobster, shrimp, and crab. In a study from Levine *et al.* (1993), of 15 patients who ate seafood, the most commonly ingested foods were crabs, shrimp and raw clams. In addition, studies demonstrated the presence of *V. parahaemolyticus* in fresh fish, mussels and clams (Baffone *et al.*, 2000). In an outbreak of *V. parahaemolyticus* in the Northeast in 1998, 16 of 23 ill persons ate either raw oysters or raw clams (CDC, 1999a).

Cooked seafood has also caused illnesses. Seafood cooked using seawater from the ships' fire systems caused outbreaks of *V. parahaemolyticus* gastroenteritis aboard two Caribbean cruise ships in 1974 and 1975 (Lawrence *et al.*, 1979). Half of the 1,200 persons who ate boiled shrimp at a feast in Louisiana became ill with *V. parahaemolyticus* gastroenteritis in 1972 (Barker *et al.*, 1974). Samples of the uncooked

shrimp tested positive, indicating that the shrimp were colonized prior to arrival at the shrimp feast and were not cooked at an adequate temperature to kill *V. parahaemolyticus* or were re-contaminated after cooking.

Steamed crabs were implicated in two outbreaks in the United States from a cross-contamination with live crabs (Dadisman *et al.*, 1972). In another United States outbreak, crab salad was prepared from packaged processed crabmeat, opened the day the meal was served. The crabmeat likely became contaminated prior to final packaging (Dadisman *et al.*, 1972). A case-control study of sporadic *Vibrio* illnesses in two coastal areas of Louisiana and Texas was conducted from 1992-1993. Cooked crayfish consumption was reported by 5 of 10 persons affected with *V. parahaemolyticus* infection (Bean *et al.*, 1998). In a study by Lowry *et al.*, (1989), the presence of *V. parahaemolyticus* was surveyed from raw and cooked seafood from New Orleans restaurants. *Vibrio parahaemolyticus* was isolated from all of the raw oysters sampled; the microorganism was isolated in 50% of cooked oyster samples, 67% of boiled shrimp samples, 33% of crab salad samples and in none of the boiled crabs.

Seasonality

The majority of outbreaks of foodborne illnesses associated with *V. parahaemolyticus* in the United States occur in the warmer months, with 94% occurring between April and October (Daniels *et al.*, 2000a). CDC data (Smith, 2003b) indicates that of the oyster-related, culture-confirmed illnesses due to *V. parahaemolyticus* from 1988 to 2001, 60% occurred in the summer and only 4% occurred in the winter months. The breakdown of the number of reported cases of illnesses by season is provided in Table II-3. The same associations have been reported in other countries. In India, the monthly isolation of *V. parahaemolyticus* was more predominant in warmer months (Okuda *et al.*, 1997) and in Japan the monthly outbreaks of food-related *V. parahaemolyticus* are more prevalent in summer with a peak in August (International Disease Surveillance Center, 1999; IASR, 1998).

Table II-3. Culture-confirmed *Vibrio parahaemolyticus* Illnesses Associated with Consumption of Oysters

Season	2000 ^a	2001 ^a	1988 to 2001 ^a
Winter	1	2	22
Spring	14	17	146
Summer	39	49	354
Fall	8	7	71
TOTAL	62	75	593

^a Analysis based on oyster-related culture-confirmed *V. parahaemolyticus* infections reported to the Centers for Disease Control and Prevention (CDC) for which either a date of oyster consumption or a date of illness onset was reported (Smith, 2003b).

Geographic Distribution of Illness

Oysters are harvested in the United States from the Gulf Coast, Mid-Atlantic, Northeast Atlantic, and Pacific Northwest. The climate in these regions is different and there are different harvesting methods and handling practices within the regions that can have an impact on levels of *Vibrio* in oysters. For example, in the Pacific Northwest, oysters harvested in intertidal areas are typically exposed to higher temperatures longer before refrigeration than those harvested using dredging.

Of the four major oyster-harvest regions in the United States, the majority of oysters (approximately 50%) are harvested from the Gulf Coast and approximately 24% are harvested from the Pacific Northwest (Chapter IV: Exposure Assessment, Table IV-15). During the 1998 outbreaks, the Pacific Northwest shellfish harvested from the Hood Canal area of Washington were responsible for 32 of 48 (67%) of cases in the state of Washington (Therien, 1999). In the Gulf Coast, 20 of 30 harvest sites in Galveston Bay were implicated in the 1998 outbreak. In the Atlantic Northeast region, Oyster Bay Harbor (Area 47) was the only area implicated in the 1998 outbreak of that region (CDC, 1999a).

International Reports of *Vibrio parahaemolyticus* Cases

Vibrio parahaemolyticus was first identified as a foodborne pathogen in Japan in the 1950s (Fujino *et al.*, 1953). By the late 1960s and early 1970s, *V. parahaemolyticus* was recognized as a cause of diarrheal disease worldwide. Below is a brief description of recent reports of *V. parahaemolyticus* illnesses in different parts of the world.

Japan. Prior to 1994, the incidence of *V. parahaemolyticus* infections in Japan had been declining; however, from 1994 to 1995 there were a total of 1,280 reports of infection due to *V. parahaemolyticus* (IDSC, 1999). During this time period, the incidents of *V. parahaemolyticus* food poisoning outnumbered those of *Salmonella* food poisoning. For both years, the majority of the cases occurred in the summer, with the largest number appearing in August.

Food poisoning due to *V. parahaemolyticus* in Japan is usually restricted to relatively small-scale outbreaks involving fewer than 10 cases. From 1996 to 1998, there were 1,710 incidents, including 496 outbreaks, with 24,373 cases of *V. parahaemolyticus* reported. The number of cases of *V. parahaemolyticus* food poisoning doubled in 1998 as compared to 1997 and again exceeded the number of *Salmonella* cases (IDSC, 1999). Similar to the 1994 to 1995 period, outbreaks were more prevalent in the summer with a peak in August and relatively few outbreaks occurred during winter months. Boiled crabs caused one large-scale outbreak, involving 691 cases. However, the majority of outbreaks were small in scale, but occurred frequently. There were 292 outbreaks and sporadic reports of *V. parahaemolyticus* involving 5,241 cases in 1996. In 1997, the incidence increased to 568 outbreaks and sporadic reports, with 6,786 cases, and in 1998, there were 850 outbreaks and sporadic reports (IDSC, 1999). The increased incidence during 1997 to 1998 has been attributed to an increased incidence of serovar O3:K6.

India. A hospital-based active surveillance of *V. parahaemolyticus* infections in Calcutta, India, conducted from 1994 to 1996, identified 146 patients (Okuda *et al.*, 1997b). The incidence suddenly increased in February of 1996 and remained elevated until August of that year when surveillance ended. The increased incidence of *V. parahaemolyticus* infections was associated with an increased prevalence of O3:K6 strains. This serovar had not been isolated in Calcutta prior to February of 1996. The incidence of diarrhea due to *V. parahaemolyticus* strain O3:K6 accounted for 63% of the strains isolated from patients in Calcutta between September 1996 and April 1997. The virulent O3:K6 strains isolated from travelers arriving in Japan from Southeast Asian countries was indistinguishable from O3:K6 strains found in Calcutta, India (Matsumoto *et al.*, 1999).

Vietnam. Five hundred forty eight cases of *V. parahaemolyticus* infection were detected between 1997 and 1999 in the Khanh Hoa province of Vietnam (Tuyet *et al.*, 2002). Of these, 90% occurred in persons over 5 years of age, 421 (77%) reported vomiting, 258 (53%) presented with watery stools, 34 (6%) reported bloody stools. None of the patients died at the time of discharge from the health care service. A risk factor for infection was high socioeconomic status, which led the authors to believe that the source of infection was fresh seafood since only the most affluent members of the community can afford this delicacy. There was no definitive information on consumption.

Chile. Between November 1997 and April 1998, several gastroenteritis cases were reported in Antofagasta, a city in northern Chile (Cordova *et al.*, 2002). The outbreak was associated with consumption of shellfish. This was the first report of *V. parahaemolyticus* causing an outbreak in Chile. Isolates were obtained from patient stool specimens and fresh shellfish. It was speculated that the exceptionally warm seawater caused by "El Nino" may have favored a bacterial bloom.

Spain. Between August and September 1999, an outbreak with 3 clusters of illness occurred in Galicia, Northwest Spain (Lozano-Leon *et al.*, 2003). Sixty four persons were ill, 9 case patients were hospitalized. The most common symptom was diarrhea; other symptoms included abdominal cramps, nausea, headache, fever and vomiting. The median duration of illness was 3 days, and onset was within 12 to 24 hours after consumption of raw oysters in a typical outdoor street market. *Vibrio parahaemolyticus* was isolated in stool of all case patients. All patients resided in one of 2 cities near the outbreak site.

Taiwan. *Vibrio parahaemolyticus* has become a leading cause of foodborne disease outbreaks in Taiwan (Chiou *et al.*, 2000). *Vibrio parahaemolyticus* accounted for 64% (542/850) of the food-associated outbreaks in Taiwan between 1995 and 1999. The O3:K6 serovar accounted for 0.6% of *V. parahaemolyticus* infections in Taiwan in 1995. This increased to 50% in 1996 and reached a peak of 84% in 1997. Comparison of outbreak data indicates that the high incidence of foodborne *V. parahaemolyticus* outbreaks from 1996 to 1999 can be attributed to the increase in O3:K6 infections.

III. HAZARD CHARACTERIZATION/DOSE-RESPONSE

The Hazard Characterization component of a risk assessment describes the adverse effects on the host of a particular substance, organism, or other hazard. In the current risk assessment, a quantitative evaluation was conducted of the dose-response relationship between the levels of *V. parahaemolyticus* ingested and the frequency and severity of illness. The dose-response relationship for *V. parahaemolyticus* was derived using human clinical feeding trial studies and epidemiological surveillance data. The probability of illnesses (gastroenteritis and septicemia) and the incidence of severe disease (septicemia) were evaluated.

Factors Influencing the Dose-Response Relationship

Dose-response relationships are influenced by three factors: the pathogen (e.g., virulence characteristics), the environment (e.g., the food matrix), and the host (e.g., susceptibility and immune status). These factors are described below.

Virulence Characteristics of *Vibrio parahaemolyticus*

Several different virulence traits have been associated with the pathogenesis of *V. parahaemolyticus* strains. These include their ability to:

- produce a thermostable direct hemolysin (TDH) (Miyamoto *et al.*, 1969);
- produce a thermostable-related hemolysin (TRH) (Okuda *et al.*, 1997a);
- produce urease (Kelly and Stroh, 1988a);
- invade the enterocytes (Akeda *et al.*, 1997);
- produce an enterotoxin (Honda *et al.*, 1976b); and
- produce pili as possible attachment/colonization factors (Nakasone and Iwanaga, 1990).

Currently, the only trait that has definitively been demonstrated to reliably distinguish pathogenic from non-pathogenic *V. parahaemolyticus* is the production of TDH. The *tdh* gene was first cloned from a Kanagawa-positive strain by Kaper *et al.* (1984). The so-called, Kanagawa Phenomenon (KP) is the exhibition of β -hemolysis induced by this haemolysin on a special blood agar (Wagatsuma) medium. This phenotype is strongly associated with clinical strains (Miyamoto *et al.*, 1969). Pathogenic strains possess a *tdh* gene and produce TDH, whereas non-pathogenic strains lack the gene and the trait. For the purpose of this risk assessment, pathogenic *V. parahaemolyticus* are defined as those strains that produce TDH.

Food Matrix Factors

Food matrix factors such as fat levels, acidity, salt content, and other characteristics can have a significant impact on the ability of a pathogen to cause disease (Foegeding, 1997). For example, gastrin, the most potent stimulant of gastric acid secretion, is released after eating a protein-rich meal, such as oysters (West, 1985). Because most enteric pathogens, including *V. parahaemolyticus*, are sensitive to acids, the increased production of gastric acid actually provides a protection against infection. On the other hand,

consumption of highly buffered foods (such as cooked rice) or antacids may decrease the number of microorganisms needed to cause illness because of their effects on gastric pH. For example, the ID₅₀ (the dose at which 50% of infected subjects become ill) observed in feeding trials with *V. cholerae* O1 was substantially lower when the microorganism was ingested with antacid vs. no antacids (Levine *et al.*, 1981).

Host Factors

Host factors such as the general health status, presence of underlying disease, nutritional status, or physical stress can play an important role in an individual's response to infections. The immune status, especially of those individuals who are immunocompromised due to disease or medical treatments can influence occurrence and/or severity of foodborne diseases. Intrinsic factors such as age, sex, and genetics further influence the immune system, and thus the susceptibility of an individual to disease. For illness associated with *V. parahaemolyticus* infection, the severity of the disease is strongly associated with the presence of underlying medical conditions. The impact of immune status on the initial colonization and infection of the gastrointestinal tract is less clear-cut.

Human Clinical Feeding Studies

Several human clinical feeding trials were conducted prior to 1974 using pathogenic *V. parahaemolyticus*. The available data from these studies are briefly summarized here. Information on non-O1 *V. cholerae* is also provided as this represents a possible surrogate microorganism with respect to future investigations.

Feeding Trials with *Vibrio parahaemolyticus*

Takikawa (1958) used a Kanagawa-positive strain in a human volunteer study and showed that *V. parahaemolyticus* caused diarrhea in 1 of 2 individuals fed a dose of approximately 10⁶ cells. Diarrhea occurred in both individuals fed approximately 10⁷ cells. The ingested doses were not directly determined, but were instead estimated assuming that *V. parahaemolyticus* cultures can reach maximum growth densities of approximately 10¹⁰ cells per milliliter. These data were selected for the dose-response model.

In a study by Aiso and Fujiwara (1963), three clinical isolates (2 Kanagawa-negative strains and 1 Kanagawa-positive strain) and one shell fish isolate (Kanagawa-negative strain) were tested. The cultures were suspended in salted milk and were fed just prior to eating a normal meal. Illness only occurred with the Kanagawa-positive strain fed at a dose of 10⁹ organisms. Symptoms developed 5 to 11 hours after challenge. Typical symptoms included violent abdominal pain, diarrhea and vomiting in each of the 4 volunteers. The data for the Kanagawa-positive strain were selected for the dose-response model.

In a third study (Sanyal and Sen, 1974), three Kanagawa-negative strains isolated from cases of gastroenteritis were fed to groups of four volunteers each. No illness was

observed in any of the volunteers at doses as high as 2×10^{10} cells. A Kanagawa-positive strain also isolated from a gastroenteritis case produced no symptoms at a low dose of 200 viable cells; however, abdominal discomfort was reported by 1 of 4 volunteers at a dose of 2×10^5 viable cells, and 2 of 4 volunteers experienced abdominal discomfort and diarrhea at 3×10^7 viable cells. All volunteers received antacid tablets prior to challenge with cultures suspended in gelatin. Only the data from the Kanagawa-positive strains were used in the dose-response model.

In another study, human exposure to 15 Kanagawa-negative strains isolated from fish produced no illnesses when doses as high as 10^9 viable cells were used (Sakazaki *et al.*, 1968). It was not reported how many volunteers were challenged in this study. These data were not used in the dose-response model.

A personal communication from Kasai (1971) reports that it took 6 to 8 hours incubation for a *V. parahaemolyticus* Kanagawa-positive strain to cause disease whereas a Kanagawa-negative strain required approximately 18 hours to cause disease after challenge. The infecting dose was reported to be approximately 10^6 organisms. No information was provided in the communication about the dose level or number of volunteers in the study. These data were not used in the dose-response model.

Feeding Trials with non-O1 *Vibrio cholerae*

Two human clinical feeding studies have been conducted with non-O1 *Vibrio cholerae*, a potential surrogate for *Vibrio parahaemolyticus*. In one study, healthy volunteers were fed 10^5 to 10^9 levels of non-O1 *V. cholerae*. One of the three strains caused no diarrhea in 2 volunteers fed 10^5 cells, 2 of 3 fed 10^6 , 1 of 2 fed 10^7 and 3 of 3 fed 10^9 . Two other strains produced no disease at doses as high as 10^9 cells (Morris *et al.*, 1990). In a second study, *Vibrio cholerae* O139 Bengal fed to volunteers caused diarrhea in 2 of 4 fed 10^4 cells and in 7 of 9 fed 10^6 cells (Morris *et al.*, 1995). The pathogenicity of this serotype more closely resembles *Vibrio cholerae* O1, and as such may be less useful as a potential surrogate.

Animal Studies

Animal studies using *V. parahaemolyticus* or a surrogate microorganism are potentially useful as a basis for extrapolating dose-response estimates for humans. Animal studies can also be useful for assessing the virulence potential of different strains and serotypes, susceptibility of sensitive subpopulations (i.e., immunocompromised), and the role of specific virulence determinants. Several *V. parahaemolyticus* animal studies have shown the virulence potential of TDH-negative strains. However, it remains to be determined whether the virulence potential of these strains also applies to humans. The effect of food matrices and other environmental factors on virulence and the dose-response relationship can be evaluated more readily in animal studies than in human studies. Potentially relevant animal dose-response data and identified factors influencing the infectivity of *V. parahaemolyticus* in animal models are described in this section. Although potentially informative, animal data were not utilized in the dose-response model for this risk

assessment because the measures of the severity of illness in relevant animal studies did not correspond with definitions of human illness on which reporting statistics are based and therefore provided little additional information with respect to quantitative risk prediction/characterization of human illness.

A limited number of animal studies have been conducted using *V. parahaemolyticus*. In one study, suckling rabbits infected orally with a Kanagawa-positive strain at doses of 10^9 to 10^{10} had positive blood cultures in 9 of 36 tested, positive spleen cultures in 11 of 21 tested and positive liver cultures in 14 of 21 tested (Calia and Johnson, 1975). Similar doses of a Kanagawa-negative crab isolate were negative for bacteremia, liver or spleen invasion in all 12 animals challenged (Calia and Johnson, 1975).

Hoashi *et al.* (1990) conducted 7 experiments in which mice were challenged intraperitoneally with 4 TDH⁺ and 3 TDH⁻ strains. In the combined results of all 7 experiments, no deaths were reported with a dose of 10^5 cells; 4% deaths with a dose of 10^6 ; 61% deaths with a dose of 10^7 , and 90% deaths with a dose of 10^8 cells. Combined results of 2 experiments in which mice were challenged orally with TDH-positive strains resulted in 38% deaths with a dose of 10^7 cells, 57% deaths with a dose of 10^8 and 80% deaths with a dose of 10^9 cells (Hoashi *et al.*, 1990). There were no significant differences in mortality between the TDH⁺ and TDH⁻ strains at any of the doses.

In rabbit ileal loop model the effective dose required to produce ileal loop dilation in 50% of rabbits for three Kanagawa-positive strains ranged from 2.6×10^5 to 7.7×10^6 cells (Twedt *et al.*, 1980). It was estimated that the initiation of positive loops occurred with doses from 10^2 to 10^5 cells (Twedt *et al.*, 1980). Seven clinical isolates were tested belonging to four different serotypes that possess one or more virulence factors: TDH, TRH, and urease, in relation to the ability to cause diarrhea (Kothary *et al.*, 2000). All strains were found to induce fluid accumulation in suckling mice and diarrhea in a ferret model after oral inoculation in a dose-dependent manner. The relationship between clinical and environmental origins of these strains was not evaluated.

Epidemiological Data

Epidemiological investigations of *V. parahaemolyticus* provide directly relevant information on the dose-response in humans. These data may be somewhat limited if there is a lack of information for the ingested dose associated with reported cases of illness. However, even when epidemiological data is not informative as to dose-response, such data often provide valuable information on the likelihood of illness (gastroenteritis) progressing to more severe outcomes (i.e., septicemia, death) in susceptible versus otherwise healthy populations. Information on the annual incidence of illness from surveillance data and outbreak investigations is provided in “Chapter II. Hazard Identification.”

CDC estimated the annual illness burden from pathogenic *V. parahaemolyticus* associated with the consumption of raw oysters as 2,790 cases of illness per year (Painter, 2003). For additional information, see Chapter II: Hazard Identification.

Data Selection and Criteria for the Dose-Response Model

The selection of data for use in the Dose-Response model considered the availability of the data and limitations of data sources. Consideration was given to using the dose-response of an appropriate surrogate bacteria and/or host (i.e., animal model), which could provide a more suitable basis for risk prediction/characterization if uncertainties such as immune status and food matrix effects were substantially reduced. If a surrogate dose-response is to be more informative than the available feeding trials data, then better information is needed with respect to response rates associated with low dose exposure (including knowledge of relevant biomarkers) and the effect of the (oyster) food matrix on the dose-response relationship. However, the potential difference between a surrogate dose-response and that of *V. parahaemolyticus* adds an additional uncertainty with respect to risk prediction/characterization. For the purpose of this risk assessment, human clinical feeding studies with pathogenic *V. parahaemolyticus* were used. A summary of the selection criteria and evaluation of each identified human clinical feeding study is provided in Table III-1.

Table III-1. Summary of Criteria and Selection of Human Clinical Feeding Studies for Dose-Response Modeling

Study	Selection Criteria			Used in Dose-Response Model?
	Dosed with <i>Vibrio parahaemolyticus</i>	Pathogenic strains? ^a	Dose Level Reported?	
Aiso and Fujiwara, 1963	Yes	Yes	Yes	Yes
Takikawa, 1958	Yes	Yes	Yes	Yes
Sanyal and Sen, 1974	Yes	Yes	Yes	Yes
Sakazaki <i>et al</i> , 1968	Yes	No	Yes	No
Kasai, 1971	Yes	Yes	No	No
Morris <i>et al.</i> , 1990	No (<i>V. cholerae</i>)	Not applicable	Yes	No
Morris <i>et al.</i> , 1995	No (<i>V. cholerae</i>)	Not applicable	Yes	No

^a For the purpose of this risk assessment, pathogenic *Vibrio parahaemolyticus* strains are those characterized as Kanagawa Phenomenon-positive.

Limitations of the Available Human Feeding Trial

The limitations of the available human feeding trial and surrogate studies for use in dose-response modeling are summarized below. Some of the studies were performed using uncharacterized strains.

- No information was available on the immune status of the volunteers. Previous exposure of the volunteer to *V. parahaemolyticus* could provide some immunity to infection.
- A dose range limited to relatively high doses of *V. parahaemolyticus* was used.
- The *V. parahaemolyticus* dose was not administered with a food matrix; except for one study, which used salted milk (Aiso and Fujiwara, 1963). This is problematic because a food matrix can either increase or decrease stomach acidity. Protein-rich meals, such as oysters, would increase stomach acidity. Because *V. parahaemolyticus* is sensitive to stomach acids, the presence of oysters may increase the infective dose.
- In most cases, antacids were administered with the *V. parahaemolyticus* dose. It is common to administer oral challenge dose either in or in conjunction with an alkaline solution or a fat emulsion (e.g., cream) in order to neutralize or minimize the impact of stomach acidity. This practice attempts to create less variability in stomach acidity among volunteers. The practice also effectively mimics achlorhydric (e.g., low stomach acid) conditions, which are common in a significant portion of the United States population, particularly in the elderly. While this helps to control the dose in the experimental context, it introduces an uncertainty with respect to inferring the dose that causes infection when *V. parahaemolyticus* is consumed with oysters. The magnitude of the difference between an infectious dose administered in an antacid, in comparison to that ingested in food, is generally unknown.
- The number of volunteer subjects is small in each study. Most studies do not provide information on the volunteers such as gender, age, and health status. In general when information was provided, the majority of the volunteer subjects were male and relatively young (aged 25 to 40).

The human feeding studies were performed prior to 1974 and it is unlikely that any future human feeding studies with *V. parahaemolyticus* will be undertaken to resolve these issues due to an apparent cardiotoxicity of TDH in animal models (Honda *et al.*, 1976a; Seyama *et al.*, 1977).

Assumptions Made for the Dose-Response Model

- All individuals are equally susceptible to probability of gastroenteritis.
- Septicemia may only occur subsequent to gastroenteritis.
- The likelihood that an infection will lead to more severe symptoms varies depending on pre-existing health conditions.
- Approximately 7% of the population has underlying medical conditions and are at higher risk of *V. parahaemolyticus* septicemia once the gastrointestinal tract is infected.
- Only 1 in 20 cases of *V. parahaemolyticus* illness is culture-confirmed.

- The Kanagawa Phenomenon-positive strains used in the human volunteer studies are representative of pathogenic *V. parahaemolyticus* with respect to estimation of the steepness of the dose-response curve.
- The slope of the dose-response curve was assumed to be the same for both the controlled feeding trials and oyster-related exposure situations.

Modeling the Dose-Response Relationship

The structure of the dose-response model is shown in Figure III-1. The *V. parahaemolyticus* dose-response model was developed by fitting a distribution to the selected human feeding trial data. The resulting estimate of the shape of the dose-response relationship was then modified by “anchoring” the mean risk predictions to be consistent with epidemiological surveillance data. The probability of cases of gastroenteritis progressing to septicemia was also calculated.

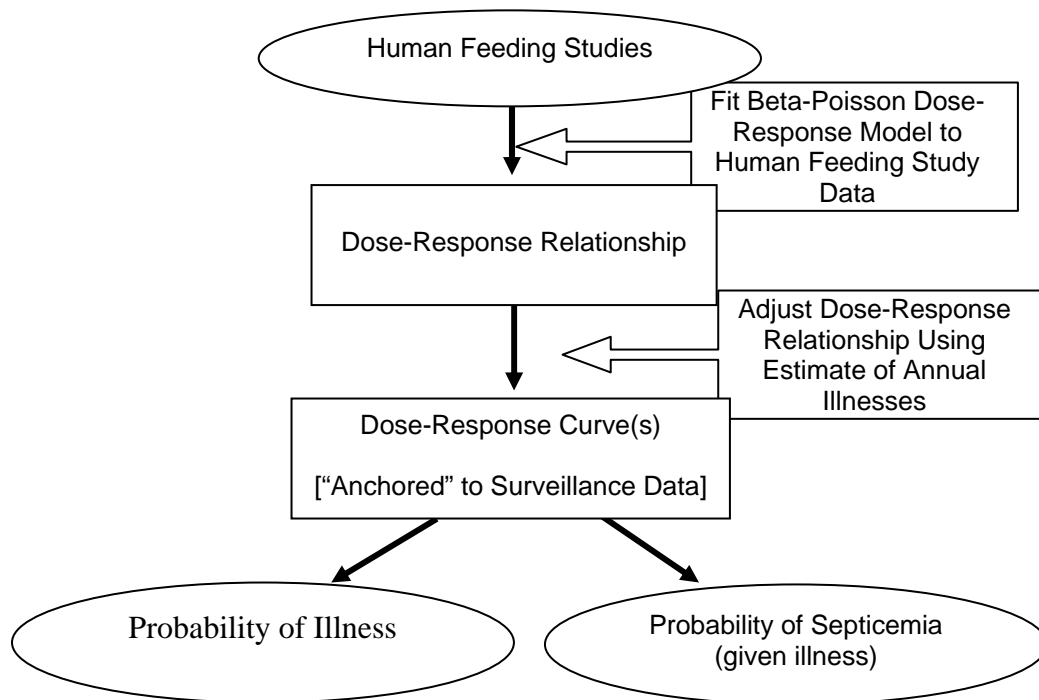


Figure III-1. Schematic Representation of the Development of the *Vibrio parahaemolyticus* Dose-Response Model

Studies and Data Sources Used for Dose-Response

- Aiso and Fujiwara, 1963. Data from human clinical trial used to fit dose-response model.
- Sanyal and Sen, 1974. Data from human clinical trial used to fit dose-response model.
- Takikawa, 1958. Data from human clinical trial used to fit dose-response model.
- Painter, 2003. Estimate of annual incidence of *V. parahaemolyticus* illness. Data used to ‘anchor’ dose-response model and adjust for limitations of the human clinical trial data.
- Angulo and Evans, 1999. Data on culture-confirmed cases with medical history used to estimate the probability of septicemia.
- Klontz, 1997. Estimate of percentage of United States population with underlying chronic medical conditions used to calculate probability of septicemia cases in this subpopulation.

Fitting Three Dose-Response Functions to Data

First, the available human feeding trial data for the incidence of gastrointestinal illness from the three selected studies [Takikawa (1958), Aiso and Fujiwara (1963), and Sanyal and Sen (1974)] were pooled. Collectively, a total of 20 healthy volunteers were administered pathogenic *V. parahaemolyticus* at doses ranging from 2.3 to 9-log_{10} cfu in a bicarbonate buffer. In these three studies, 9 of 20 subjects developed symptoms of gastroenteritis. No illnesses were reported for the lower doses of 2×10^2 and 2×10^5 cfu of *V. parahaemolyticus*. However, at higher doses ($>1 \times 10^6$ *V. parahaemolyticus* organisms) between 50% and 100% of the human subjects became ill. A summary of the dose levels, number of subjects, and number that develop illness is provided in Table III-2.

Table III-2. Summary of Data from the Human Feeding Trial Studies Used for the *Vibrio parahaemolyticus* Dose-Response Model

Dose (cfu)	Number of Subjects	Number of Illnesses	Rate of Observed Illness	Reference
2×10^2	4	0	0	Sanyal and Sen (1974)
2×10^5	4	0	0	Sanyal and Sen (1974)
1×10^6	2	1	0.5	Takikawa (1958)
1×10^7	4	2	0.5	Takikawa (1958)
3×10^7	2	2	1.0	Sanyal and Sen (1974)
1×10^9	4	4	1.0	Aiso and Fujiwara (1963)
Total Subjects = 20		Total Illnesses = 9		

Secondly, the dose-response models were selected. Dose-response models are used to define the shape of the dose-response curves, allowing the extrapolation from the observed data from the human feeding trials to other (lower) dose levels. Three dose-response models, Beta-Poisson, Gompertz, and Probit, were evaluated. These models exhibit different behaviors at low dose levels; that is they would predict different probability of illness for the same exposure levels. These models are parametric,

meaning that they can be described by a mathematical (i.e., algebraic) equation. The mathematical equations for these three models are shown in Table III-3. Additional details about the model selection are provided in Appendix 4.

Table III-3. Dose-Response Model Equations for the Probability of Illness as a Function of Ingested Dose

Dose-Response Model	Equation ^a
Beta-Poisson	$\Pr(\text{ill} d) = 1 - (1 + d/\beta)^{-\alpha}$
Probit	$\Pr(\text{ill} d) = \Phi(\alpha + \beta * \log_{10}(d))$
Gompertz	$\Pr(\text{ill} d) = 1 - \exp[-\exp[\alpha + \beta * \log_{10}(d)]]$

^aFor the Beta-Poisson, α and β are the shape (steepness) and location parameters, respectively. The approximation used for the Beta-Poisson dose-response function applies when $\alpha \ll \beta$ (and $\beta \gg 1$). For the Probit and Gompertz models, α and β are the location and shape (steepness) parameters, respectively. For all three models, d denotes the dose. For the Probit model Φ denotes the cumulative distribution function of a standard normal random variable.

Next, the dose response models were fit to the observed feeding trial data as shown in Figure III-2. The models were fit to the data by the maximum likelihood criteria; that is, the values chosen for the model equation parameters shown in Table III-3 were the values which maximized the likelihood of the model predicting data similar to the observed data. The adequacy of model fits to the data was evaluated using a likelihood ratio based goodness-of-fit measure. All of the models provided an adequate statistical fit to the data. For more information about estimated model parameters and the statistical evaluation of the model fits, see Appendix 4.

The Maximum Likelihood Estimate (MLE) is the most likely value of all possible outcomes (i.e., the best estimate of the probability of illness). The best estimates of the dose corresponding to a 50% probability of illness (i.e., the MLE of the ID₅₀) were determined to be 2.8×10^6 , 4.0×10^6 , and 3.2×10^6 organisms/serving for the Beta-Poisson, Gompertz and Probit dose-response models, respectively. Although these estimates are not substantially different at the ID₅₀, the differences are much more substantial at low dose levels as can be seen in Figure III-2. For example, the estimated risk of illness is approximately 5 cases per 10,000 servings for the Beta-Poisson model at a dose of 1,000 *V. parahaemolyticus* organisms/ serving. However, at the same dose, the estimated risk is approximately 10-fold higher based on the Gompertz and approximately 10-fold lower based on the Probit. The differences between these models are less substantial for high doses that exceed 100,000 organisms per serving.

Selection of the Beta-Poisson Dose-Response Model

An evaluation of the uncertainty distributions of the risk predications for the three dose-response models was conducted (Appendix 4). This comparison indicated that considering the residual predictions of uncertainty, the three models were comparable. Therefore, for simplicity, one model was chosen to use in the risk characterization. Of the three models evaluated, the Beta-Poisson model is the only one that meets the mechanistic criteria identified by FAO/WHO (2003). The criteria include consideration that there is no threshold level (i.e., a single cell can cause illness). The Beta-Poisson model was therefore considered the most appropriate model to use for this risk assessment.

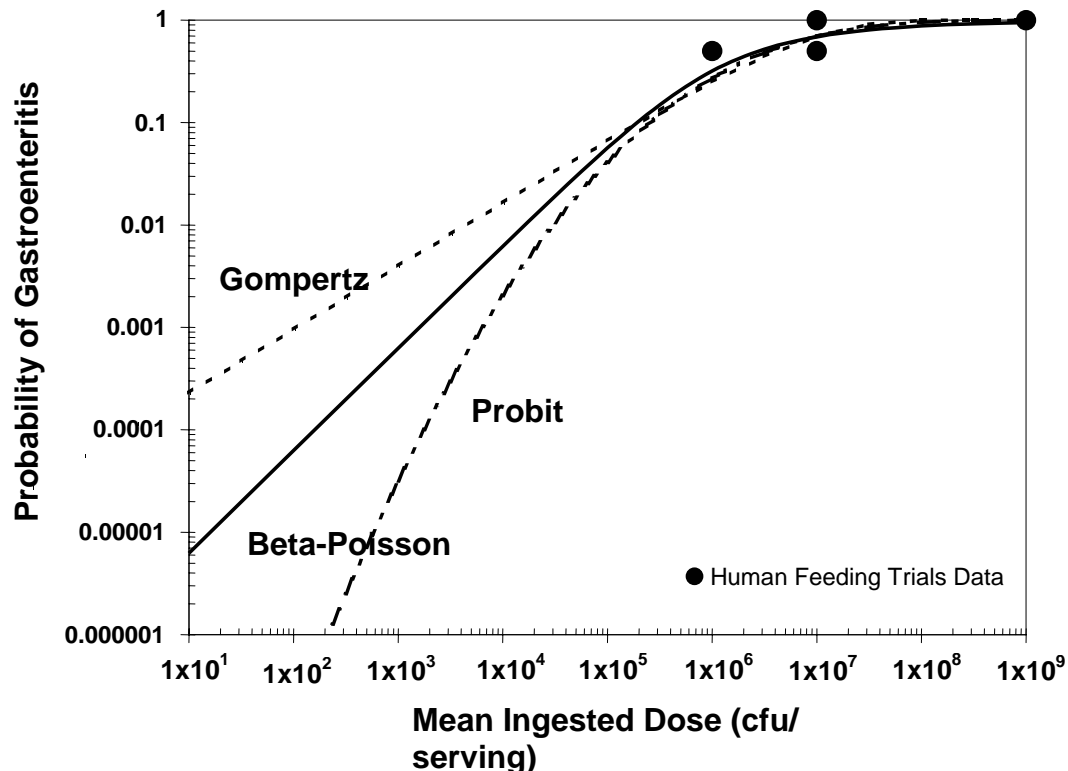


Figure III-2. Comparison of the Beta-Poisson, Gompertz, and Probit Dose-Response Models Fit to Data from Human Feeding Studies

Dose-Response Adjustment Factor

The *V. parahaemolyticus* human feeding trial data is the most complete data set available to describe the relationship between dose and the probability of illness. However, there are apparent biases in these data relative to what may be expected from exposure to *V. parahaemolyticus* by a diverse population consuming raw oysters. For example, the human feeding trials included concurrent antacid administration and no concurrent administration of oysters (food matrix) with the *V. parahaemolyticus* dose, which potentially changes the infective dose. Thus, the ID₅₀ observed in feeding trials would be expected to be lower than that of the general population based on effect of the food matrix vs. buffer on the infective dose.

Figure III-2 shows the relationship between dose and the probability of illness. Using the Beta-Poisson curve and the predicted exposure levels (see Chapter IV Exposure Assessment), the model would predict too many illnesses in comparison to epidemiological data. For example, using the Gulf Coast summer harvest, the mean exposure to pathogenic *V. parahaemolyticus* from oysters is predicted to be 20,000 organisms per serving (~100 cells per gram) (see Chapter IV: Exposure Assessment). At this level of exposure, the risk of illness would be predicted to be substantially greater than 0.001 (i.e., >1 illness in 1,000 servings). Accounting for the number of servings per year, this rate of illness would be approximately equivalent to 4,000 illnesses/year associated with the Gulf Coast summer harvest. This predicted rate is too high, considering that CDC estimates there are only 2,790 cases/year (Painter, 2003) for the entire United States population.

Based on the above considerations, the dose-response model was adjusted or “anchored” to be consistent with both the CDC’s estimate of the average annual number of cases occurring per year and the estimated number of servings consumed (Chapter IV: Exposure Assessment). This adjustment factor represents the effect of the apparent differences between the dose-response observed in human volunteers under controlled conditions versus that in the general population when exposure is associated with the oyster food matrix.

The shape of the dose-response curve (i.e., the slope or steepness) was assumed to be the same for both the controlled feeding trials and oyster-related exposure situations. However, the location of the curve was shifted, using the adjustment factor. For the Beta-Poisson model, the resulting expression used for risk prediction was taken to be:

$$\text{Pr}(\text{ill} | d) = 1 - \left(1 + \frac{d}{\gamma * \beta}\right)^{-\alpha}$$

where γ is the dose-response adjustment factor.

The magnitude of the adjustment factor was estimated by iteratively running the risk characterization model and adjusting the location of the curve to be consistent with CDC’s estimated average annual illness burden of approximately 2,800 cases (Painter, 2003). For the Beta-Poisson model, the resulting dose-response adjustment factor was estimated to be 27, which corresponds to a difference of 1.4- \log_{10} between the ID_{50} under the controlled versus oyster-related exposure scenarios. The difference between the adjusted and unadjusted curves is shown in Figure III-3.

The solid line shown in Figure III-3 is the MLE of the Beta-Poisson model fit to the pooled human feeding studies data and the dashed line shows the shift adjustment (location) made so that the model predictions agree with the epidemiological surveillance data. From Figure III-3, it can be seen that the dose corresponding to a 50% probability of illness (ID_{50}) for the unadjusted curve is approximately 3 million and that of the adjusted curve is approximately 80 million.

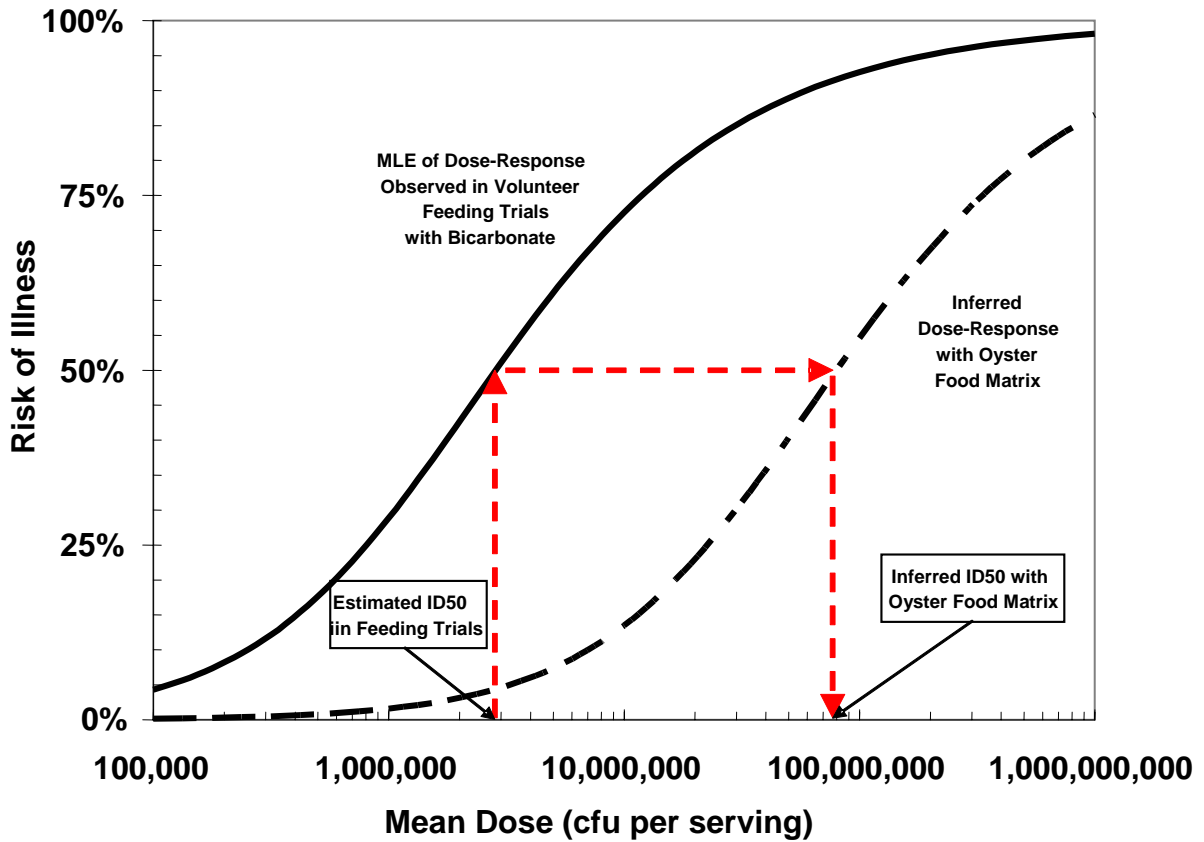


Figure III-3. The Beta-Poisson Dose-Response Model for *Vibrio parahaemolyticus* Fit to Human Feeding Trials and Adjusted Using Epidemiological Surveillance Data

[The solid line is the best estimate of the Beta-Poisson Model fit to pooled human feeding studies. The dashed line shows the shift adjustment so that the model predictions agree with epidemiological surveillance data. MLE denotes the maximum likelihood estimate. ID₅₀ is the dose corresponding to a 50% probability of illness.]

Uncertainty Characterization of the Dose-Response Relationship

Uncertainty in the dose-response relationship was characterized by performing a procedure called non-parametric bootstrapping. This procedure involves hypothetical replication of the observed human feeding study. However, given the limited number of possible outcomes (illness rates), the procedure was conducted as follows. For each possible outcome, the model was refit by the maximum likelihood criteria to obtain a set of parameter estimates, one corresponding to each possible (but unobserved) outcome. Weighting was assigned based on the probabilities of the outcomes. An uncertainty distribution was derived based on the parameter estimates and the weighting. The details of these calculations are provided in Appendix 4.

Figure III-4 shows a graphical representation of the weighted set of dose-response curves from the bootstrapping procedure. The 21 curves in this set were used in the Risk

Characterization model. For each simulation (run of the model), a single curve was randomly selected, based on the assigned weight for that curve (the uncertainty distribution). The thick black curve shown in Figure III-4 is the curve that received the most weight (i.e., had the highest probability and would be selected most frequently). The weights for each curve and other supporting information are provided in Appendix 4.

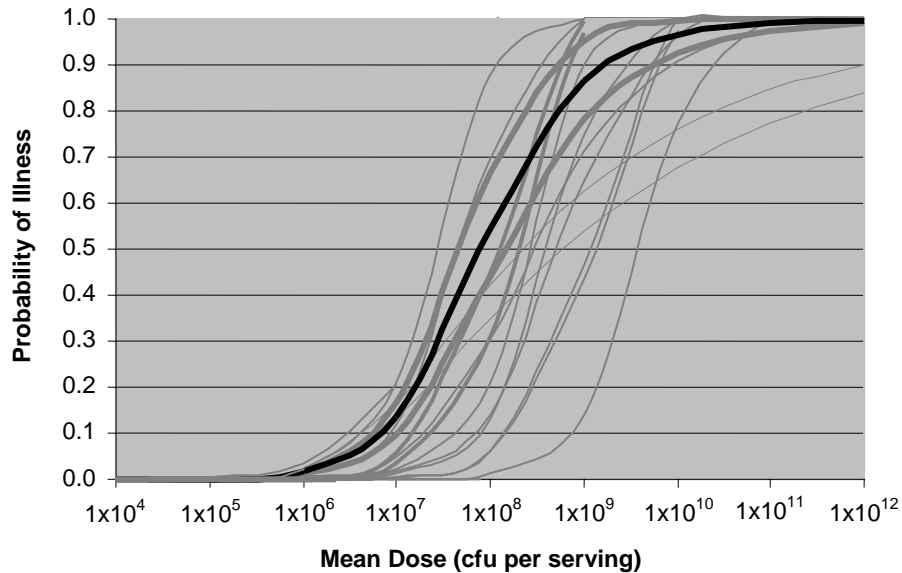


Figure III-4. *Vibrio parahaemolyticus* Dose-Response Curve and Uncertainty

[The dark line indicates the dose-response curve with the highest weighting (16.5%) and the 20 gray lines represent the dose-response curves with lower weightings (<1% to 13%).]

We did not apply uncertainty to the dose-response adjustment factor used to bring the model-predicted illnesses in alignment with the reported epidemiological illnesses (i.e., the shift shown in Figure III-3). To incorporate uncertainty in the dose-response shift an effort to assess the uncertainty in the number of illnesses occurring annually (i.e., uncertainty in the number of underreported illnesses) would need to be undertaken. See Appendix 4 for additional information regarding uncertainty in the dose-response model.

Predicted Probability of Illness

The Beta-Poisson Dose-Response model shown in Figure III-4 estimates the probability of the total *V. parahaemolyticus* risk per serving (gastroenteritis alone and gastroenteritis followed by septicemia) as a function of dose. For example, using the curve with the highest weight (the dark line in Figure III-4), the probability of illness is approximately 0.5 for a dose of approximately 100 million cfu. This means that for every 100 servings at that dose level, approximately 50 individuals will become ill. At exposure levels of approximately 1,000 cfu, the probability of illness is relatively low (<0.001). The probability of illness approaches 1.0 (i.e., 100% certainty of illness) at exposure levels around 1×10^9 cfu.

Severity of Illness

For the purpose of this risk assessment, it was assumed that there is no sensitive subpopulation with respect to the occurrence of an infection leading to gastroenteritis. However, given the occurrence of illness, it was estimated that it was more likely that the infection leads to a severe outcome (e.g., septicemia or death) among individuals with an underlying chronic medical condition.

The probability of gastroenteritis progressing to septicemia in healthy and immunocompromised individuals was estimated using an application of Bayes' Theorem (see for example, Fleiss, 1973). The equation below illustrates the relationship between the frequency of a given outcome, health status, and the probability of the outcome.

$$\begin{aligned} & \text{Pr(illness outcome | health status)} \\ &= \frac{\text{Pr(health status | illness outcome)} * \text{Pr(illness outcome)}}{\text{Pr(health status)}} \end{aligned}$$

where, Pr(illness outcome | health status) denotes the frequency or probability of an illness outcome type within a subpopulation of individuals defined by the existence of a common predisposing health condition (“health status”).

All factors on the right hand side of the equation are identifiable based on a set of CDC’s epidemiological case series data reported by Angulo and Evans (1999). The statistics of the case series were:

- 107 cases of gastroenteritis
- 5 cases of septicemia
- 1 death

Of the cases with available information:

- 23 of 79 (29%) cases occurred in individuals with underlying chronic conditions
- 3 of 4 (74%) septicemia cases had an underlying chronic condition

Substituting the observed data into the above equation provides an estimate of the probability of septicemia occurring. Thus, for the subpopulation identified as having an immunocompromised chronic health condition, the probability of septicemia (given that illness occurs) was estimated as follows:

$$\begin{aligned} & \text{Pr}(septicemia | immunocompromised) \\ &= \frac{\text{Pr}(immunocompromised | septicemia) * \text{Pr}(septicemia)}{\text{Pr}(immunocompromised)} \\ &= \frac{\frac{3}{4} * \frac{5}{107}}{\frac{23}{79}} = 0.12 \end{aligned}$$

The probability of septicemia occurring consequent to culture-confirmed illness in healthy individuals and the total United States population was estimated in a similar fashion (see Appendix 4).

It is important to recognize that the estimated probabilities based on the CDC data pertain to culture-confirmed illnesses; i.e., these are probabilities conditional on both the occurrence of illness and the identification of that illness by a confirmed culture. Analysis of the cases series data (Angula and Evans, 1999) indicates that the rate of reported illnesses that are culture confirmed is higher in individuals with an immunocompromising health condition compared to individuals with no pre-existing health condition. It was assumed that approximately 7% of the United States population has an underlying medical condition (Klontz, 1997). Therefore, the equation was modified to account for the differential reporting rates for culture-confirmed illness for immunocompromised versus healthy subpopulations. For details of this analysis, see Appendix 4.

As shown in Table III-4, the overall estimated risk of progression to septicemia occurring subsequent to *V. parahaemolyticus* illness is 0.0023, or approximately 2 cases of septicemia per 1,000 illnesses. For immunocompromised individuals, however, the probability of gastroenteritis progressing to septicemia is approximately 10-fold higher, with approximately 25 cases per 1,000 illnesses. This translates to a mean of approximately 7 cases per year of septicemia for the total population, 2 cases per year for the healthy population, and 5 cases per year for the immunocompromised population.

Table III-4. Probability of Septicemia in Patients with Gastroenteritis from *V. parahaemolyticus* Infection

Population	Probability of Septicemia	Mean Number of Cases (per 1000 Illnesses)	Mean Number of Cases (per Year) ^a
Total	0.0023	2	7
Healthy Individuals	0.00063	<1	2
Immunocompromised Individuals	0.025	25	5

^a Number of Cases per Year = (total illness/year) X (probability of septicemia) X (percentage of population). Total illness/year assumed to be 2,800 (Painter, 2003); 7% of the population assumed immune compromised (Klontz, 1997) and 93% assumed healthy.

IV. EXPOSURE ASSESSMENT

The Exposure Assessment component of a microbial risk assessment is an evaluation of the likelihood of ingesting a pathogenic microorganism via food and the likely level of exposure. In this assessment, the likelihood of exposure to pathogenic *V. parahaemolyticus* from consumption of raw oysters was evaluated. This risk assessment is a quantitative product pathway analysis in which the key steps from harvest of oysters through post-harvest handling and processing to the point of consumption were modeled. The predicted levels of pathogenic *V. parahaemolyticus* in oysters were determined at each step in the pathway.

A schematic representation of the Exposure Assessment Module is shown in Figure IV-1. The Exposure Assessment is subdivided into three modules: Harvest, Post-Harvest, and Consumption. The Harvest Module considers the factors influencing the prevalence of total *V. parahaemolyticus* in oysters up to the time of harvest. The Post-Harvest Module considers factors associated with handling and processing of oysters. The Consumption Module considers factors such as the number of oyster servings eaten per year, the quantity of oysters consumed per serving, and the levels of pathogenic *V. parahaemolyticus* in the oyster at the time of consumption.

Oysters are harvested throughout the year in the United States from four major regions: the Gulf Coast, Mid-Atlantic, Northeast Atlantic, and Pacific Northwest. Methods and conditions of harvest and handling of oysters after harvest can influence the levels of *V. parahaemolyticus* in oysters at the time of consumption. These harvest and handling practices and conditions vary considerably in different geographic areas and at different times of year. In the Gulf Coast, the harvest duration (i.e., the time between removal of the oyster from the water to unloading them at the dock) for Louisiana is typically much longer than for other states in that region (Florida, Mississippi, Texas, and Alabama). Therefore, the Gulf Coast was divided into two distinct regions: Gulf Coast (Louisiana) and Gulf Coast (Non-Louisiana). Likewise, the Pacific Northwest was divided into two distinct regions: Pacific Northwest (Intertidal) and Pacific Northwest (Dredged). In the Pacific Northwest, oysters are harvested by two methods: dredging and intertidal. For the intertidal harvest method, oysters are hand-picked when oyster reefs are exposed during the tide cycle and left in baskets until the tide rises to a sufficient depth to allow a boat to retrieve the basket.

The risk assessment modeled six oyster harvest regions [Gulf Coast (Louisiana), Gulf Coast (non-Louisiana), Mid-Atlantic, Northeast Atlantic, Pacific Northwest (Intertidal) and Pacific Northwest (Dredged)] and four seasons [Summer, Fall, Winter, Spring] for a total of 24 region/season combinations. These region/season combinations were separately modeled. Predictions of the number of pathogenic *V. parahaemolyticus* per serving of oysters at the time of consumption were determined for each of the 24 region/season combinations.

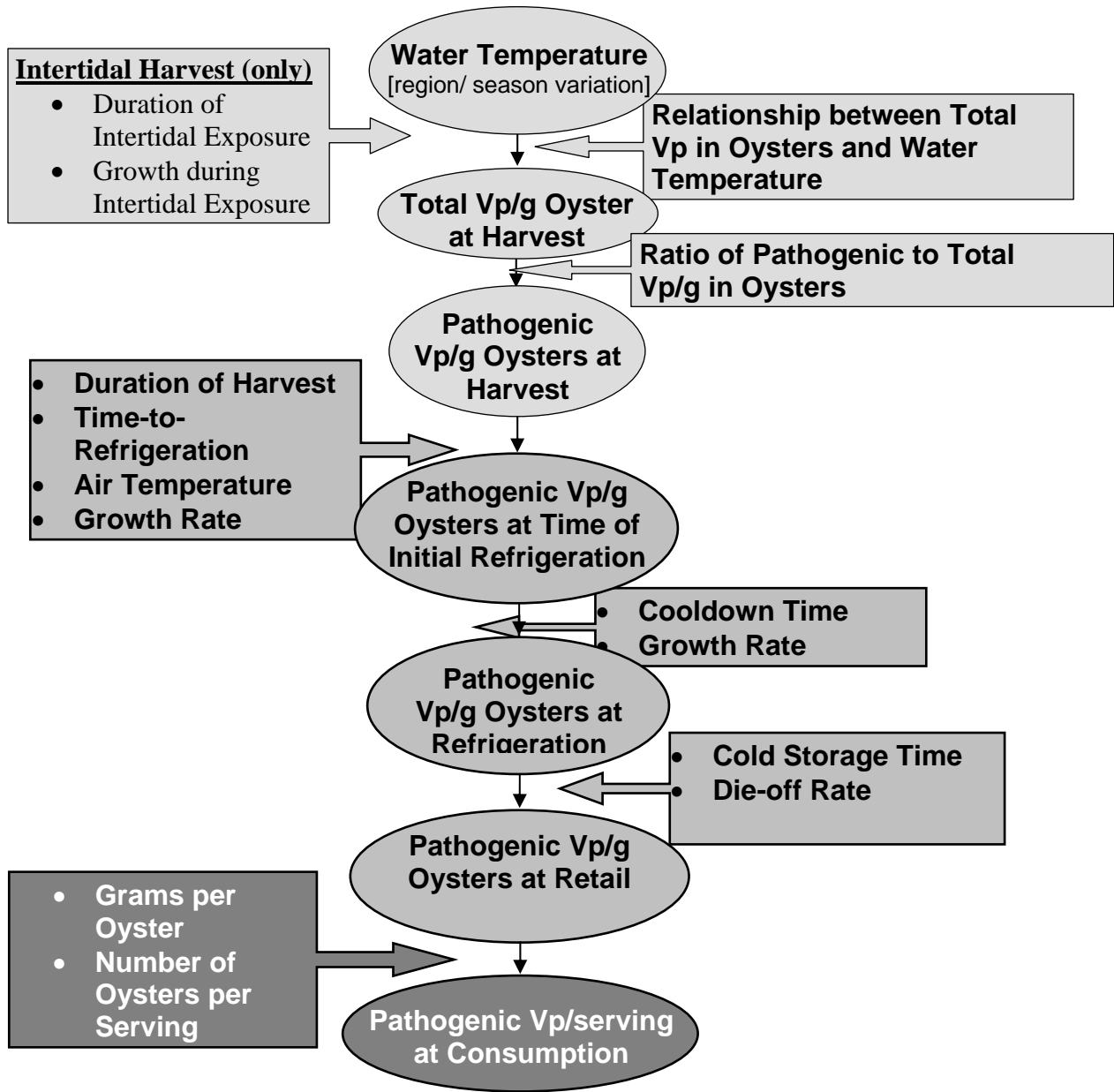


Figure IV-1. Schematic Representation of the Exposure Assessment Component of the *Vibrio parahaemolyticus* (Vp) Risk Assessment Model

[The boxes with black lettering shaded with light gray show the Harvest Module, the boxes shaded with gray show the Post-Harvest Module, and the boxes with white lettering and shaded in dark grey show the Consumption Module.]

Harvest Module

The Harvest Module considers the factors associated with the likelihood that oysters harvested from specific growing areas and at specific times of the year will contain *V. parahaemolyticus* (total and pathogenic). Factors which affect the frequency and levels of *V. parahaemolyticus* in oysters include the routes of introduction, prevalence and persistence of *V. parahaemolyticus* in the environment. These factors are discussed below.

Routes of Introduction into Oyster-Growing Areas

There are several pathways by which *V. parahaemolyticus* may occur in oyster growing areas. *Vibrio parahaemolyticus* may be indigenous to a geographical area. New strains may be introduced naturally by the activities of terrestrial and aquatic animals, or through human activities. Terrestrial and aquatic animals (including plankton, birds, fish, and reptiles) may harbor pathogenic strains of *V. parahaemolyticus* and may play a role as intermediate hosts and vehicles for its dissemination (Davis *et al.*, 1982; Sarkar *et al.*, 1985). For example, *V. parahaemolyticus* has been isolated from a number of fish species where it is associated primarily with the intestinal contents (Nair *et al.*, 1980). *Vibrio parahaemolyticus* can also be introduced into non-contaminated areas by transfer of shellfish from contaminated waters, as would occur during the process of “relaying” shellfish.

Ship ballast release is another potential mechanism of introduction of *V. parahaemolyticus* into a particular geographical area. Most cargo ships must carry substantial quantities (millions of gallons) of ballast water to operate safely when they are not carrying cargo. Cargo ships take on ballast water from the body of water in which the ship originates. Having taken water on board, it is normally retained until the ship is about to load cargo, at which point ballast water is discharged. During de-ballasting, organisms picked up from one port could be introduced into the loading port. It is possible that the non-potable water from a cargo ship could have been the source of *V. parahaemolyticus* serotype O3:K6 in the Galveston Bay in 1998. This serotype was identified during a large outbreak of culture-confirmed illnesses associated with oysters harvested from this location at this time. Prior to 1998, serotype O3:K6 had not been isolated from either environmental or clinical samples in the United States, but had established an ecological niche in Asia (Arakawa *et al.*, 1999).

Prevalence and Persistence in Oyster-Growing Areas

Prevalence and persistence of pathogenic strains of *V. parahaemolyticus* in oyster in the environment may be dependent on several parameters. Factors which may determine whether *V. parahaemolyticus* will become established in a specific area include interactions of environmental conditions, species and physiology of the shellfish, and the genetics of the microorganism. Other factors to be considered in determining the prevalence of *V. parahaemolyticus* include water temperature (including El Niño and La Niña weather patterns), salinity, zooplankton, tidal flushing (including low tide exposure of shellfish) and dissolved oxygen (Amako *et al.*, 1987; Garay *et al.*, 1985; Kaneko and Colwell, 1978; Venkateswaran *et al.*, 1990).

Environment. Favorable environmental conditions will support the establishment, survival, and growth of the microorganism. Warmer water temperatures and moderate salinities, especially those prevailing during the summer months, favor the growth and survival of *V. parahaemolyticus* (Covert and Woodburne, 1972; Jackson, 1974; Nair *et al.*, 1980; Zhu *et al.*, 1992). Most of the shellfish-borne illnesses caused by this microorganism occur in the warmer months. In an investigation of the 1998 outbreak, the CDC randomly selected 7 of the 76 existing Texas Department of Health sites for monitoring environmental conditions in Galveston Bay. At these sites, water temperature and salinity levels during May and June, 1998 were found to be significantly higher compared with data recorded over the previous five years for the same months (Daniels *et al.*, 2000b). Elevated water temperatures were also suspected to have played a role in the 1997 outbreak on the West Coast (CDC, 1998).

Vibrio parahaemolyticus often “over-winters” (survives the winter) in the sediment and is absent or below detectable levels in the water column or oysters during the winter months (Joseph *et al.*, 1983; Kaysner *et al.*, 1990a; United States Department of Health and Human Services, Food and Drug Administration, 1995). During the summer, shellfish often have levels of *V. parahaemolyticus* that are more than 100-fold greater than those in the water (DePaola *et al.*, 1990; Kaysner *et al.*, 1990a). Also, under extreme environmental conditions, *Vibrio* species, including *V. parahaemolyticus*, may enter a “viable but non-culturable (VBNC) phase” in marine waters and could be missed by traditional cultural methods (Bates *et al.*, 2000; Colwell *et al.*, 1985; Oliver, 1995; Xu *et al.*, 1982).

The potential influence of nutrients in the water on the prevalence and persistence of *V. parahaemolyticus* is unclear. Watkins and Cabelli (1985) reported that the densities of *V. parahaemolyticus* in the water column in Narragansett Bay, Rhode Island were correlated with the densities of fecal coliforms from sewage. The effect of sewage was surmised to be an indirect one, possibly mediated by stimulation of zooplankton with which the *V. parahaemolyticus* were associated, because laboratory studies showed that nutrients in the sewage did not directly increase *V. parahaemolyticus* levels. However, another study reported that organic matter does affect growth and survival of *Vibrio* species (Singleton *et al.*, 1982). In another study, the distribution of *V. parahaemolyticus* in sediment samples from the Boston Harbor were found to be independent of densities of fecal coliforms (Shiaris *et al.*, 1987).

Shellfish Physiology. *Vibrio parahaemolyticus* is frequently found on marine particulates, zooplankton and other chitin sources (Amako *et al.*, 1987). Microorganisms are internalized by shellfish through shellfish filter feeding. Factors that favor active filter feeding by shellfish increase the probability that shellfish in a given area will take up the pathogen (Murphree and Tamplin, 1991). Shellfish species and physiology (e.g., sexual maturity, immune function, and metabolic state) can affect survival and growth of disease-causing *Vibrio* spp. within shellfish. There is evidence that the immune status of the shellfish may play an important role in the prevalence and persistence of the microorganism (Fisher and DiNuzzo, 1991; Kothary *et al.*, 1997; LaPeyre and Volety, 1999; Ordás *et al.*, 1998; Volety *et al.*, 1999). There also appear to be seasonal

differences in the oyster's cellular defense system. A study by Genthner *et al.* (1999) showed that the bactericidal activity of hemocytes (oyster blood cells) was greater in summer than in winter. Other factors such as spawning or adverse environmental conditions play a role in the incorporation of *V. parahaemolyticus* in the oyster by reducing or stopping filter feeding or changing oyster physiology. For example, the presence of the oyster parasite, *Perkinsus marinus*, influences the ability of oyster hemocytes to kill the internalized microorganisms (Kothary *et al.*, 1997; LaPeyre and Volety, 1999; Tall *et al.*, 1999). The presence of chemicals in the environment (e.g., tributyltin oxide, polycyclic aromatic hydrocarbons, wood preservative leachates) may reduce filter feeding (Sujatha *et al.*, 1996; Weinstein, 1995; Wendt *et al.*, 1996).

Genetics of the Microorganism. It is not known whether the prevalence and persistence of pathogenic and non-pathogenic strains are affected in a similar fashion by environmental factors. However, the presence of a pathogenicity island (a physical grouping of virulence-related genes) in *V. parahaemolyticus* may foster rapid microevolution, promote growth and survival, and result in transmission of factors, such as those responsible for virulence, to other strains (horizontal gene transfer) (Frischer *et al.*, 1990; Ichige *et al.*, 1989; Iida *et al.*, 1998). Bacteriophages may genetically alter vibrios (Baross *et al.*, 1978; Ichige *et al.*, 1989).

Effect of Intertidal Harvest Practices.

The practice of intertidal harvest is used extensively in some of the estuaries of the Pacific Northwest region. Typically, after the tide recedes from an intertidally harvested area, the shellfish are hand picked and placed into large baskets, which are left in the harvest area until the tide rises to a sufficient depth to permit a vessel to retrieve the baskets and transport them to the processing plant. Alternatively, harvesters may transport the harvest by truck after collection, depending upon the location of the harvest area. In either case, intertidal harvest potentially exposes oysters to favorable conditions for growth of *V. parahaemolyticus*, especially on sunny summer days.

The effect of intertidal harvest practices has been shown to have a significant impact on *V. parahaemolyticus* densities in the harvested oyster. *Vibrio parahaemolyticus* levels were reported to increase (>100-fold) in oysters from the Puget Sound during intertidal exposure (Herwig and Cheney, 2001). In another study, oysters were analyzed before and after being submerged on a beach for 24 hours (DePaola *et al.*, 2002). *Vibrio parahaemolyticus* levels were found to be below or near the minimum detectable level (10 cfu/g) when they were first removed from the water and after 5 hours exposure to ambient temperature and sunlight. After 24 hours, *V. parahaemolyticus* levels were approximately 500 cfu/g in oysters harvested on a sunny day and approximately 100 cfu/g in oysters harvested on a cloudy day. With respect to oysters collected from commercial reefs, the overall mean *V. parahaemolyticus* densities were found to be as much as 8-fold higher after maximum exposure compared to samples exposed for less than 1 hour, but there was considerable variation among sites (DePaola *et al.*, 2002).

Data Selection and Criteria for the Harvest Module

A number of factors were identified that potentially affect the levels of *V. parahaemolyticus* in oysters at time of harvest. Modeling these factors required that both sufficient quantitative data were available and that the data permit consideration of regional and temporal variation. Due to the relatively low prevalence of pathogenic *V. parahaemolyticus* and limitations of current methods of detection, most quantitative studies have focused on the levels of total *V. parahaemolyticus*. Salinity can influence the prevalence and growth of *V. parahaemolyticus* in oysters, and preliminary modeling included a consideration of that parameter (see 2001 draft risk assessment at www.foodsafety.gov/~dms/fs-toc.html). However, subsequent consideration of the model indicated that water salinity is not as strong a determinant of *V. parahaemolyticus* levels in the regions that account for essentially all of the commercial harvest and was overshadowed by the impact of water temperature (Appendix 5). Accordingly, salinity was not included as a variable in the model.

There have been a number of studies conducted over a wide range of geographic locations showing the relationship of environmental factors and total *V. parahaemolyticus* levels in water and oysters. These studies were reviewed and evaluated for their utility for estimating an appropriate predictive relationship between pathogenic *V. parahaemolyticus* densities in oysters and environmental conditions. The studies are discussed in detail in this chapter and a summary of the key results of the studies is provided in Appendix 5. Most of the studies do not provide sufficient information with respect to a quantitative relationship, primarily because these studies were either limited to specific seasons with little variation of environmental parameters, measured *V. parahaemolyticus* levels in water or sediment rather than oysters or reported little quantitative data on densities *per se*.

The selection of data for use in the Harvest Module considered the availability of data and limitations of the data sources. Tables IV-1a, IV-1b, and IV-1c provide a summary of the criteria used to select the studies for the Harvest Module. Data used in this module include the following:

- water temperature distribution for each region/ season combination
- the relationship between total *V. parahaemolyticus* in oysters and water temperature
- the ratio between pathogenic and total *V. parahaemolyticus* in oysters

Water Temperature. Criteria for selecting studies used to describe the water temperature distributions for each region/season combination is summarized in Table IV-1a. The data set must include long-term historical data so that the extent of year-to-year variation can be determined. Also, because of the large number of records needed to characterize the distribution of water temperatures across regions and seasons, the data must be available electronically. See Table IV-1a for details.

In comparison to the NBDC sites, STORET and NERR are more specific to estuaries as opposed to open coastal waterways. Some NBDC sites such as Thomas Point Lighthouse

(Chesapeake) are located within estuaries but similar sites could not be identified for the Gulf Coast and Northeast Atlantic within the NBDC database. Comparison of NERR data for Weeks Bay, AL, versus that of the Dauphin Island NBDC buoy suggests that shallow water estuaries may be slightly warmer than open coastal waters but that the difference is not substantial (i.e., ~1 °C (1.8 °F) difference on average). An additional consideration is the availability of enough long-term historical data to determine extent of year-to-year variation. As already indicated, data are available from most NBDC buoys from 1988 to the present. The NERR program started data collection in 1995. Although STORET has considerable long-term historical data associated with monitoring of water quality dating back to 1964, access to STORET records is not readily available. Also, STORET records do not necessarily correspond to fixed locations, as is the case for NBDC and NERR. Additional data on water temperature measurements specific to oyster harvesting areas were made available to the FDA by State agencies in Texas, Alabama, New York, and Connecticut. The state data were not substantially different from the NBDC data selected for each region.

Table IV-1a. Summary of Criteria and Selection of Data for the Regional and Seasonal Distribution of Water Temperature.

Study	Criteria		Used in Harvest Module?
	Long-Term Historical Data Base	Electronically Available Records	
NBDC ^a	Yes (varies by buoy)	Yes	Yes (Gulf Coast, Northeast Atlantic, Mid-Atlantic)
Washington State ^b	Yes (1988 to 1999)	Yes	Yes (Pacific Northwest)
EPA STORET ^c	Yes (since 1964)	No	No
NERR ^d	No (since 1995)	Yes	No ^e
Other state Agencies ^f	Yes (varies)	No	No

^a National Buoy Data Center (NBDC) www.ndbc.noaa.gov/index.shtml. Buoys in Pacific Northwest are located in deep water and those data are not used for the risk assessment.

^b Washington State Department of Health (1999).

^c EPA Storage and Retrieval of United States Waterways Parametric Data (STORET). www.epa.gov/storet

^d National Estuarine Research Reserve Systems (NERR) www.ocrm.nos.noaa.gov/nerr/

^e When the risk assessment was initiated in 1999, there was insufficient data available from NERR to evaluate the year-to-year variation.

^f Other state agencies also provided data to FDA including Texas, Alabama, New York, and Connecticut. Not all data were in a conveniently accessible format.

Relationship of Water Temperature and Total *Vibrio parahaemolyticus* in Oysters.

Criteria for selecting studies to define the relationship between water temperature and total *V. parahaemolyticus* in oysters is summarized in Table IV-1b. A quantitative method must have been used to determine the levels of *V. parahaemolyticus* in oysters (enumerated, not presence/absence). Also, data would ideally be available over multiple years and regions. See Table IV-1b for details.

The Ratio of Pathogenic to Total *Vibrio parahaemolyticus* in Oysters. Criteria for selecting studies to define the percentage of pathogenic *V. parahaemolyticus* in oysters relative to the levels of total *V. parahaemolyticus* is summarized in Table IV-1c. Ideally, the study design should include analysis of individual oysters for the percentage of the total *V. parahaemolyticus* that are pathogenic (i.e., TDH⁺) such that the variation across individual samples can be accounted for in the model. Two different studies, DePaola *et al.* (2002) and Kaufman *et al.* (2003) were conducted in the summer of 2001. Both studies utilized a gene probe technique for enumeration of total and pathogenic *V. parahaemolyticus* in replicate aliquots from all samples collected. See Table IV-1c for details.

Table IV-1b. Summary of Criteria and Selection of Data on the Relationship between *Vibrio parahaemolyticus* (Vp) Levels in Oysters and Water Temperature

Study	Criteria				Used in Harvest Module?
	Levels Vp/g in Oyster Tissue Reported?	Measured Water Temperature	Multistate	All Seasons	
DePaola <i>et al.</i> , 1990	Yes	Yes	Yes	Yes	Yes
FDA/ISSC, 2001 ^a	Yes	Yes	Yes	Yes	Yes
Washington State Department of Health, 2000	Yes	Yes	No (Washington State only)	Yes	Yes
Washington State Department of Health, 2001	Yes	Yes	No (Washington State only)	Yes	Yes
Kelly and Stroh, 1988a	No	No	No	Yes	No
Kelly and Stroh, 1988b	No	Yes	No	Yes	No
Chan <i>et al.</i> , 1989	Yes	No	Not U.S.	No	No
Kiiyukia <i>et al.</i> , 1989	Yes	Yes	Not U.S.	No	No
Ogawa <i>et al.</i> , 1989	Yes	Yes	Not U.S.	Yes	No
Kaysner <i>et al.</i> , 1990a	Yes	No	No	No	No
Tepedino, 1982	Yes	No	No	No	No
Herwig and Cheney, 2001	Yes	Yes	No	No	No
Depaola <i>et al.</i> , 2000	Yes	No	Yes	No	No
DePaola <i>et al.</i> , 2002	Yes	No	No	No	No
Kaufman <i>et al.</i> , 2003	Yes	Yes	No	No	No

^a These data were also reported in Cook *et al.*, 2002b and DePaola *et al.*, 2003a.

Table IV-1c. Summary of Criteria and Selection of Data to Define the Ratio of Pathogenic to Total *V. parahaemolyticus* (Vp) Levels in Oysters.

Study	Selection Criteria		Used in Harvest Module?
	Total and Pathogenic Vp Measured in Isolates?	Total and Pathogenic Vp Measured in Oysters?	
DePaola <i>et al.</i> , 2002	Yes	Yes	Yes
Kaufman <i>et al.</i> , 2003	Yes	Yes	Yes
DePaola <i>et al.</i> , 2000	Yes	Yes	No ^a
FDA/ISSC, 2000; Cook <i>et al.</i> , 2002a	Yes	No ^b	No
FDA/ISSC, 2001; Cook <i>et al.</i> , 2002b	Yes	Yes	No ^c
Thompson <i>et al.</i> , 1976	Yes	No	No ^d
Kaysner <i>et al.</i> , 1990	Yes	Yes	No ^d
DePaola <i>et al.</i> , 2003a	Yes	Yes	No ^e

^a The study was not used because it was conducted following outbreaks in 1997 and 1998 and therefore may not reflect typical levels.

^b Most but not all states analyzed each sample for both total and pathogenic *V. parahaemolyticus*.

^c The study was not used because this was the only identified study that included analysis of oysters at the time of retail and was needed to validate the model predictions for the level of *V. parahaemolyticus* in oysters after cold storage.

^d The study was not used because the data were provided as an aggregate number of TLH and TDH isolates over many samples rather than on a per sample basis.

^e The study was not used because the data were limited and possibly not representative of the entire Gulf Coast region.

Assumptions Made for Modeling the Harvest Module

- Individual oysters comprising a serving at time of consumption are harvested at the same time and location.
- Levels of *V. parahaemolyticus* in oysters (log basis) at the time of harvest are normally distributed with mean proportional to water temperature.
- The variability in water temperatures is adequately summarized by the mean and variance of daily noon-time temperatures at selected sites considered typical of each region/season.
- Pathogenesis is based on the presence of the most characterized virulence factor of the microorganism, thermostable direct hemolysin (TDH).
- Variation of the relative abundance of pathogenic versus total *V. parahaemolyticus* across collections of oysters is distributed as a Beta distribution.
- The relationship between pathogenic and total *V. parahaemolyticus* is temperature independent (i.e., percentage pathogenicity is constant throughout the year).
- The relationship between pathogenic and total *V. parahaemolyticus* is the same for the Gulf Coast, Northeast Atlantic, and Mid-Atlantic harvest regions.
- Intertidal harvesting consists of ~75% of Pacific Northwest harvest.

- For the Pacific Northwest (Intertidal) region, a range of exposures of between 4 to 8 hours before the oysters are collected was assumed for intertidal harvesting.

Modeling the Harvest Module

The various model inputs and output for the Harvest Module are illustrated in Figure IV-2 and discussed in detail below.

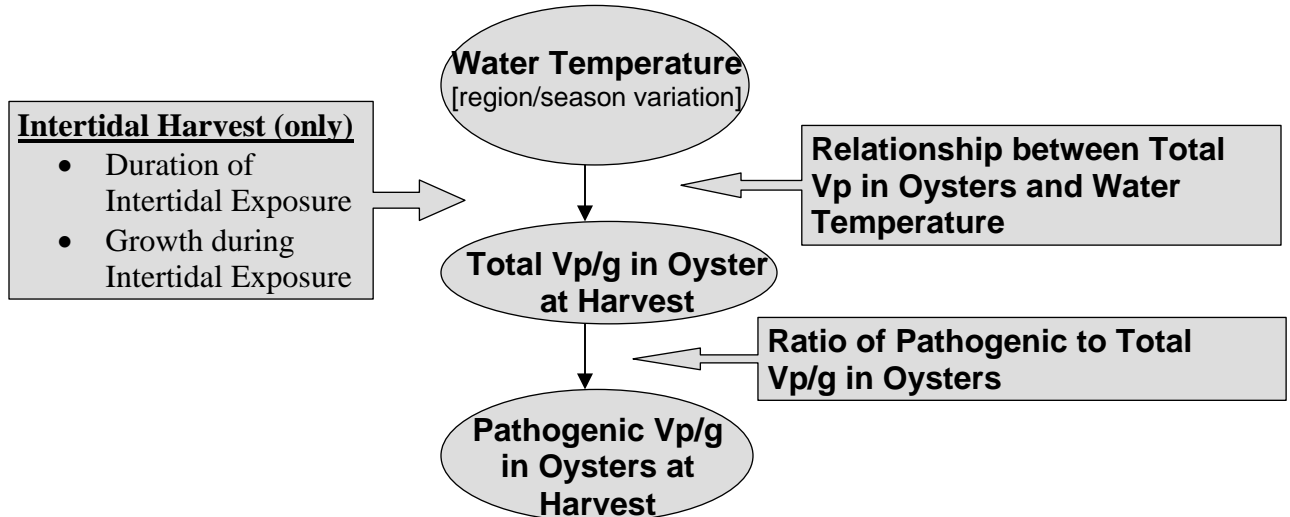


Figure IV-2. Schematic Depiction of the Harvest Module of the *Vibrio parahaemolyticus* (Vp) Exposure Assessment Model

Studies and Data Sources Used for the Harvest Module

- Water temperature: Data from the National Buoy Data Center (NBDC), 1984 to 1998 was used for all regions except the Pacific Northwest region. Data from the Washington State Department of Health (1999) were used for the Pacific Northwest region.
- The relationship between water temperature and levels of total *V. parahaemolyticus* in oysters: Data from FDA/ISSC, 2001 (data were also reported by Cook *et al.*, 2002b and DePaola *et al.*, 2003a) and DePaola *et al.* (1990) were used for all regions except the Pacific Northwest. Data from Washington State Department of Health (2000; 2001) were used for the Pacific Northwest.
- Ratio between pathogenic and total *V. parahaemolyticus* in oysters: Data from Kaufman *et al.* (2003) was used for the Gulf Coast, Northeast Atlantic, and Mid-Atlantic regions. Data from DePaola *et al.* (2002) was used for the Pacific Northwest region.
- Pacific Northwest Intertidal Harvest. See description of growth rate model in the Post-Harvest module.

Water Temperature Distributions

Regional and seasonal distributions of water temperatures were estimated based on accumulated records of coastal water buoys from the National Buoy Data Center (NBDC) for all regions except for the Pacific Northwest. Seasons were defined by calendar month; winter: January through March, spring: April through June, summer: July through September, and fall: October through December. The available data for most buoys contain hourly air and water temperatures from 1984 up to the present, with occasional data gaps due to instrumentation malfunction. Representative buoys were identified for the Gulf Coast, Mid-Atlantic and Northeast Atlantic regions. For each region a buoy site was selected for which both water and air temperature data were available because air temperature was identified as a relevant parameter needed with respect to post-harvest effects and examination of the NBDC data indicated a correlation between air and water temperature for shallow water areas.

For the Pacific Northwest, there were no buoys in the NBDC database that could be taken to be representative of the temperature conditions of the shallow water estuaries where oysters are harvested. Water temperature distributions for this region were therefore estimated based on temperature measurements taken during routine monitoring of selected oyster harvesting sites (Washington State Department of Health, 1999).

Based on the observation that oyster harvesting generally commences early in the morning and ends mid or late afternoon, the daily water temperature recorded at noon was taken to be representative of the average temperature determining *V. parahaemolyticus* densities at harvest. A single average daily temperature was used because examination of the NBDC data indicated that diurnal temperature variations were relatively minor relative to temperature variations occurring across different days or weeks. This is discussed in more detail in Appendix 5.

Within a given season, region, and year, the midday water temperature data from the NBDC buoys was generally found to be unimodal. For simplicity, a normal distribution was fit to the empirical water temperature data (for each region, season, and year). The mean (μ) and standard deviation (σ) of the distribution of water temperatures within any particular year for different region and season combinations are shown in Table IV-2. The extent of year-to-year variation of these distributions is summarized by the mean and the variance of the parameters μ and σ . The mean and variance of these parameters are denoted in the table as mean(μ), variance(μ), mean(σ) and variance(σ), respectively. The correlation between μ and σ is denoted by corr(μ , σ). A positive correlation between parameters μ and σ can be interpreted as indicating that when the mean water temperature is higher than normal the variation in temperatures from one day to the next is generally greater than that observed when the mean temperature is lower than normal. Similarly, a negative correlation summarizes the observation that temperatures are less variable when the mean water temperature is higher than normal.

Table IV-2. Summary Statistics of Midday Water Temperature Distributions for Different Regions and Seasons

Region	Statistics ^a	Water Temperature Distributions (°C)			
		Winter (Jan - March)	Spring (April - June)	Summer (July - September)	Fall (Oct - Dec)
Gulf Coast (Dauphin Island, AL buoy) ^b	mean(μ)	14.2	24.5	28.9	17.9
	mean(σ)	2.7	3.5	1.5	4.5
	variance(μ)	1.54	0.98	0.11	3.2
	variance(σ)	0.27	0.27	0.11	0.55
	corr(μ, σ)	-0.08	-0.55	-0.41	-0.53
Northeast Atlantic (Ambrose buoy, NY harbor) ^b	mean(μ)	4.51	12.0	20.7	12.0
	mean(σ)	1.23	4.2	1.34	3.37
	variance(μ)	1.04	0.74	0.86	0.73
	variance(σ)	0.23	0.34	0.22	0.36
	corr(μ, σ)	-0.14	0.57	-0.25	-0.08
Mid-Atlantic (Thomas Point Lighthouse buoy, Chesapeake Bay) ^b	mean(μ)	3.92	16.8	25.0	11.6
	mean(σ)	1.92	5.1	1.8	5.1
	variance(μ)	1.0	0.56	0.25	1.0
	variance(σ)	0.21	0.34	0.12	0.85
	corr(μ, σ)	-0.31	-0.16	0.47	-0.28
Pacific Northwest (Washington State) ^c	mean(μ)	8.1	13.7	17.4	10.7
	mean(σ)	1.62	2.4	2.4	2.8
	variance(μ)	0.76	1.0	0.60	0.16
	variance(σ)	0.13	0.24	0.16	0.13
	corr(μ, σ)	0.01	0.7	-0.13	0.36

^a μ and σ denote mean and standard deviation of within region/season temperature distribution, respectively; mean(μ), variance(σ), and corr(μ, σ) denote the mean, variance and correlation between the parameters μ and σ across different years.

^b Source of data: National Buoy Data Center (NBDC) <http://www.ndbc.noaa.gov/index.shtml>. NBDC measures surface water temperature (sensors are generally 1.0 to 1.5 meter deep).

^c Source of data: Washington State Department of Health (1999).

The NBDC buoy located at Dauphin Island, Alabama was chosen as representative of water temperatures for the Gulf Coast. This buoy has recorded water temperatures beginning in 1987. For the spring season, the distribution of midday water temperature was found to vary from year to year with an average mean of 24.5 °C (76.1 °F). The variance of the mean from one year to the next was 0.98, which corresponds to a standard deviation of 0.99 °C. Similarly, for the standard deviation of the within year temperature distributions, the central tendency across different years was an average of 3.5 °C with a

variance of 0.27, which corresponds to a standard deviation of 0.52 °C. The correlation between μ and σ was -0.55 indicating that the day-to-day temperatures were generally less variable when the overall mean temperature was higher than that of a typical year.

For the Pacific Northwest there were no near-shore NBDC buoys recording water temperatures that could be considered representative of oyster growing areas. Consequently, for this region, seasonal and year-to-year variations in water temperature distributions were developed based on compiled data from the Washington State Department of Health from 1988 through 1999. These water temperature data were recorded in association with collection of samples for monitoring of *Vibrio* species and fecal coliforms and are therefore directly representative of temperatures for oyster growing areas. Averages of water temperature were substituted when multiple measurements were recorded for any given day. Year-to-year variations in the water temperature distributions for the Pacific Northwest were developed in the same manner as that for the other regions.

Differences from one year to the next were evident for all regions and seasons. Therefore, the potential effect of year-to-year variation in the water temperature distributions was included in the model. First, the mean and the standard deviation of the parameters of the fitted normal distributions for each region/season combination were determined across all available years of data (see Table IV-2 and Appendix 5 for more details). The mean and standard deviation were then used to sample, assuming a normal distribution, a simulated set of 1,000 parameter values for each region/season combination. These sampled values were used to characterize the year-to-year variation of water temperature distributions in model uncertainty simulations. The simulated normal distributions used in model simulations were truncated at the observed upper and lower temperatures for each region/season combination.

Relationship Between Water Temperature and Total *Vibrio parahaemolyticus* Levels in Oysters

The relationship between total *V. parahaemolyticus* densities in oysters and water temperature was quantified using three comprehensive survey data sets: DePaola *et al.* (1990); FDA/ISSC (2001); and Washington State Department of Health (2000, 2001). These data sets were selected for quantitative modeling based on the criteria listed above (Table IV-1b).

Because different methodologies were used for enumeration in these three surveys (Table IV-3), the data sets were not pooled together. Instead, regression models were fit separately to each data set. A relatively large proportion of samples within the data sets had non-detectable levels of *V. parahaemolyticus*. In the DePaola *et al.* (1990) study, 26 of 61 oyster samples (43%) did not have detectable *V. parahaemolyticus* (the lower limit of detection is approximately 10 cfu/g). In the 2001 FDA/ISSC study (later published as Cook *et al.*, 2002b), 232 of 624 (37%) samples analyzed for total *V. parahaemolyticus* were found to have less than the limit of detection (10 cfu/g) and 93 of 262 (36%) oyster samples were less than the limit of detection (0.3 cfu/g) in the Washington State

monitoring data (Washington State Department of Health, 2000; 2001). For regression analysis, it was assumed that *V. parahaemolyticus* was present in these non-detect samples at levels less than the detection limit (i.e., the true density was below the limit of detection) but never zero (see discussion of Tobit regression below).

Table IV-3. Summary of Data Used for Modeling the Effect of Water Temperature on Total *Vibrio parahaemolyticus* Densities

Study	Region	Number Samples	Method of Isolation	Limit of Detection
DePaola <i>et al.</i> , 1990	Northeast Atlantic Mid-Atlantic Gulf Coast Pacific Northwest	61 ^a	Membrane filtration	10 cfu/g
FDA/ISSC, 2001/ Cook <i>et al.</i> , 2002b	Northeast Atlantic Mid-Atlantic Gulf Coast	624 ^b	Direct plating	10 cfu/g
Washington State Department of Health, 2000; 2001	Pacific Northwest	262 ^c	FDA-BAM (3-tube MPN)	0.3 cfu/g

^a Total of 65 oyster samples; 61 oyster samples with corresponding water temperature measurements.

^b Some samples were lost due to laboratory accidents; 671 samples collected, 656 samples analyzed and of those 624 were oyster samples.

^c Samples were collected over a period of multiple years.

Regression Analysis. Tobit regression is a maximum likelihood procedure for which the likelihood of the data reflects both the probability of obtaining non-detectable and detectable density levels. The influence of non-detectable outcomes is determined by the probability of the density in a sample falling below a fixed limit of detection. The Tobit regression method was used to avoid bias and underestimation of variance of the total predicted *V. parahaemolyticus* densities. For example, if the non-detectable values are replaced with zeros or with half the limit of detection and a regression line is fit to the data then the estimated relationship of total *V. parahaemolyticus* densities versus water temperature could be substantially biased towards higher or lower levels. Imputing the non-detectable values (such that the value is between zero and the non-detectable limit) rather than assume they are zero or half the limit of detection reduces the bias of the estimate. See Appendix 5 for details about the Tobit regression analysis procedures and results.

Plots of the best fitting regression line versus temperature and the associated 5th and 95th confidence intervals are shown in Figures IV-3 through IV-5 for each of the three data sets. In these figures, non-detectable *V. parahaemolyticus* levels were replaced with randomly imputed values (open circles) based on the maximum likelihood estimate (MLE) of the regression relationship. Regression analysis of the three data sets indicated that the effect of temperature on the mean log₁₀ total *V. parahaemolyticus* densities was approximately linear in the range of water temperatures sampled.

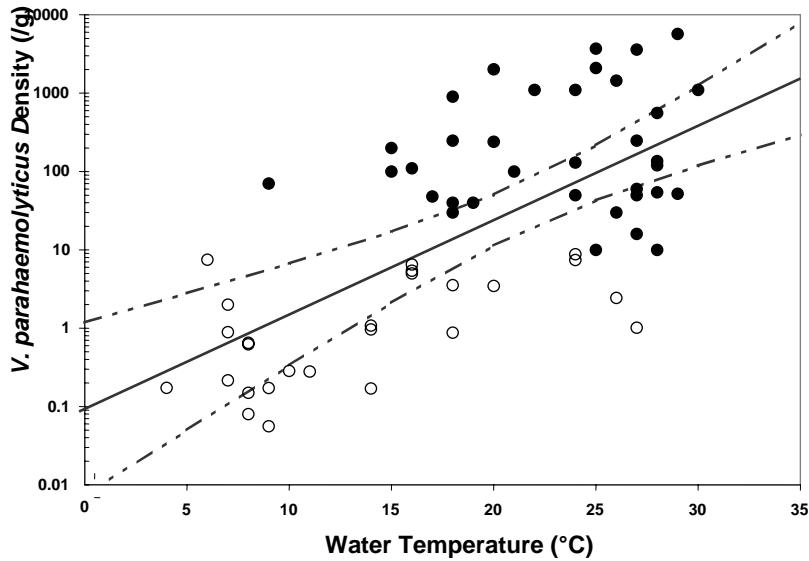


Figure IV-3. Tobit Regression Fit of *Vibrio parahaemolyticus* Densities in Oysters Versus Water Temperature Using the DePaola et al. (1990) Data Set
 [Solid line is the best estimate of the median *V. parahaemolyticus*/g. Dashed lines show the 5th and 95th % confidence limits. Closed circles are *V. parahaemolyticus* detectable values from DePaola *et al.*, 1990. Open circles are randomly imputed values for samples with densities less than the limit of detection (10 cfu/g).]

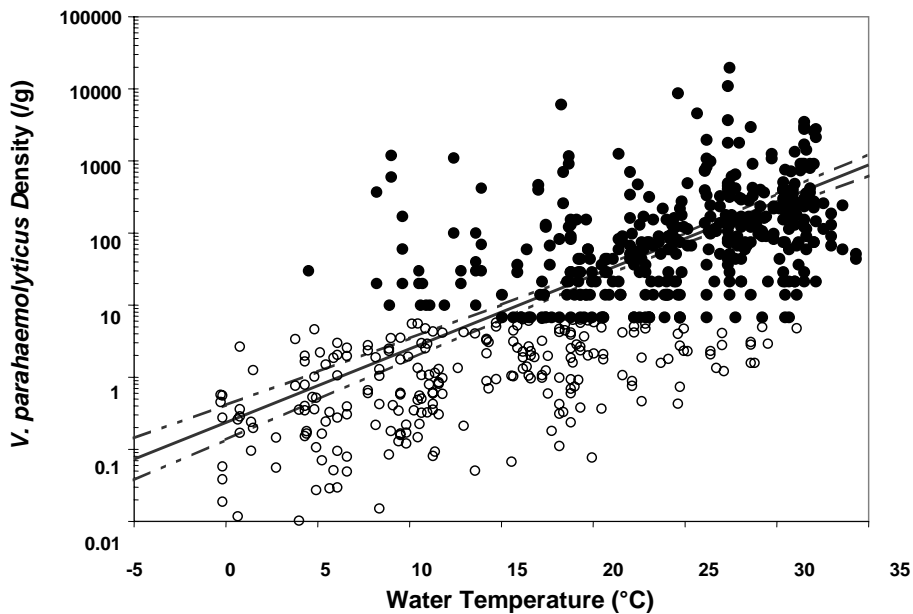


Figure IV-4. Tobit Regression Fit of the *Vibrio parahaemolyticus* Densities in Oysters Versus Water Temperature Using the FDA/ISSC (2001) Data Set
 [Solid line is the best estimate of the median *V. parahaemolyticus*/g. Dashed lines show the 5th and 95th % confidence limits. Closed circles are *V. parahaemolyticus* detectable values from FDA/ISSC, 2001. Open circles are randomly imputed values for samples with densities less than the limit of detection (10 cfu/g).]

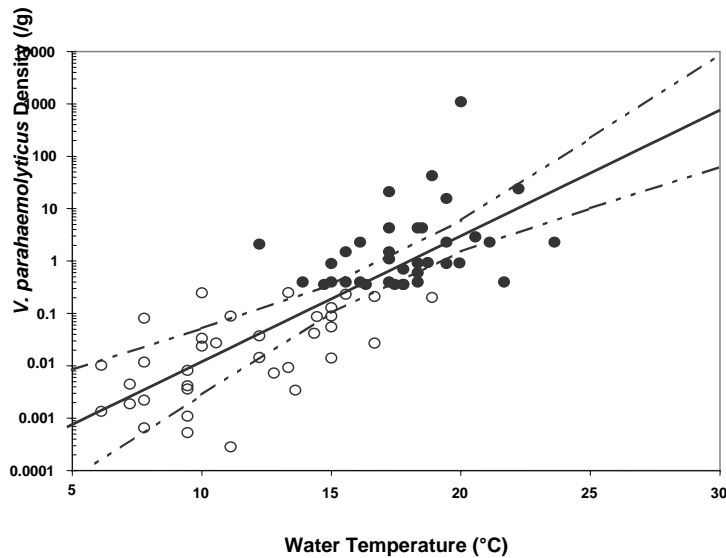


Figure IV-5. Tobit Regression Fit of the *Vibrio parahaemolyticus* Densities in Oysters Versus Water Temperature Using the State Department of Health (2000; 2001) Data Sets

[Solid line is the best estimate of the median *V. parahaemolyticus*/g. Dashed lines show the 5th and 95th % confidence limits. Closed circles are *V. parahaemolyticus* detectable values from Washington State Department of Health (2000; 2001). Open circles are randomly imputed values for samples with densities less than the limit of detection (0.3 cfu/g).]

In order to develop a more accurate predictive distribution for total *V. parahaemolyticus* density (cfu/g oyster) in harvest waters, the method error for the data described in Table IV-3 was estimated and then subtracted from the estimated variance about the regression fit to obtain an estimate of population variation. This correction is important to prevent an inappropriate over estimation of the variance of *V. parahaemolyticus* densities. See Appendix 5 for the determination of independent estimates of method error to correct the variances.

Uncertainty. The results of the Tobit regression analysis of the three data sets were used to generate 1,000 sets of parameters for the relationship of water temperature to total *V. parahaemolyticus* densities in oysters. These sets of regression parameters were used to represent uncertainty of the water temperature relationship and variance of total *V. parahaemolyticus* densities in the Monte Carlo simulations. For the Gulf Coast, Mid-Atlantic and Northeast Atlantic regions, the uncertainty from the regression analyses shown in Figures IV-3 and IV-4 were used. Approximately 500 sets of parameters from distributions of the model fits to these data sets were obtained and combined. The resulting 1,000 sets of parameters were used once for each of the 1,000 model simulations for these three regions. For the Pacific Northwest region the 1,000 parameters were obtained from the distribution shown in Figure IV-5.

The effect of regression parameter uncertainty was implemented in the risk assessment by using a multivariate normal approximation for parameter uncertainty for each of the three

data sets. Accounting for the effect of the uncertainty in the data sets was implemented in Monte Carlo simulations by generating a sample of 1,000 sets of parameters from the uncertainty distributions. Independent estimates of method error for each of the three data sets were then used to correct this additional variance in the observed data. See Appendix 5 for detailed discussion of how the regression parameter uncertainty was assessed based on a multivariate normal approximation.

Growth of *Vibrio parahaemolyticus* During Intertidal Exposure

A significant portion of the oysters in the Pacific Northwest are harvested when oyster reefs are exposed during the course of the tide cycle. Exposure to the air and radiative heating of oysters in bright sunlight can elevate oyster temperatures substantially above that of the water (and air) temperature. To model the effect of intertidal harvesting on *V. parahaemolyticus* densities in the Pacific Northwest, the effect of elevated oyster temperatures and duration of exposure during the collection process was modeled as a separate growth step occurring prior to that associated with transport of the harvest to processing facilities at ambient air temperature. The loglinear growth rate model described in the Post-Harvest module below was used.

To predict the growth of *V. parahaemolyticus* in intertidal harvested oysters prior to refrigeration, the growth rate model was applied twice. It was first applied to determine the extent of growth that corresponds to 4 to 8 hours of intertidal exposure and secondly to determine the extent of growth that occurs during subsequent transportation (1 hour).

The proportions of days that are cloudy, partly cloudy and sunny during the summer in the Pacific Northwest are about 33% each, respectively (National Weather Service, 2002). Given that the most significant elevation of oyster temperature is likely to occur during exposure under sunny conditions the recent studies of intertidal exposure in the Pacific Northwest (DePaola *et al.*, 2002; Herwig and Cheney, 2001), conducted over multiple sampling occasions, likely reflect the varying effects of sunny versus cloudy conditions. The range of oyster versus air temperature differences observed in these studies was 0 to 10°C. More definitive information is lacking and, based on the range of observations alone, a uniform distribution with a range of 0°C to 10°C was considered a reasonable representation of both the variability and uncertainty of the average difference in oyster versus ambient air temperature during periods of intertidal exposure. With respect to duration of exposure, oysters are typically collected by barge at the time of the incoming tide at the collection site. Consequently, the duration of exposure can be expected to vary as a consequence of the varying depth of the oyster reefs relative to the maximum tide height. Considering the likely range of depths of commercial reefs, a range of exposures of between 4 to 8 hours was assumed with all values within this range considered equally likely. The uniform distribution chosen represents uncertainty as well as the variability in the duration of exposure likely to occur.

Not all of the Pacific Northwest harvest is collected after intertidal exposure. A smaller, but still significant portion of the overall harvest is collected by dredging submerged oyster reefs and, consequently, for this portion of the harvest the densities at time of collection were modeled based on water temperature (i.e., without an intertidal growth

step), as was done for the other regions of the country where there is no intertidal harvesting. The estimate of the proportion of the Pacific Northwest harvest that is collected during intertidal cycles was obtained based on data for average shellstock harvest volume in four major harvest areas of Washington State from 1990 to 2000 (Kaysner, 2002) and expert opinion on the percentage of harvest that is collected intertidally in these selected areas. This combination of harvest data and expert opinion indicated that the overall statewide percentage of shellstock harvested after intertidal exposure is approximately 75% of the total harvest for all seasons. Since Washington State is the largest harvest area in the Pacific Northwest this statistic was considered representative of the region as a whole. Thus, the intertidal growth calculation described here was assumed to apply to 75% of the Pacific Northwest harvest.

Ratio of Pathogenic to Total *Vibrio parahaemolyticus* Levels in Oysters

Seven studies were identified which provide data on the relationship between total and pathogenic *V. parahaemolyticus* in oysters (Table IV-4). In these studies, samples were analyzed for pathogenic *V. parahaemolyticus* (TDH⁺). The microorganisms isolated from the TDH⁺ samples were further analyzed to determine the percentage of the total *V. parahaemolyticus* microorganisms in the oysters that are pathogenic. Differences were observed in the various United States regions with higher percent pathogenic values observed in the Pacific Northwest compared to the Gulf Coast and Atlantic regions.

Table IV-4. Estimates of Mean Pathogenic *Vibrio parahaemolyticus* as a Percentage of Total *Vibrio parahaemolyticus*

Oyster Samples		<i>Vibrio parahaemolyticus</i> Isolates			Region (Study)
Number Tested	Number Pathogenic ^a	Number Tested ^b	Number Pathogenic ^a	Pathogenic (%)	
153 ^c	ND ^d	2,218 (MPN)	4 KP+	0.18	Gulf Coast (Thompson and Vanderzant, 1976)
60	13	5,159 (DP)	44 TDH+	0.18 ^f	Gulf Coast (Kaufman <i>et al.</i> , 2003)
198	8	3,429 (DP)	9 TDH+	0.3	Gulf Coast, Mid-Atlantic, Northeast Atlantic (FDA/ISSC, 2000; Cook <i>et al.</i> , 2002a)
106	3	5,600 (MNP+DP)	16 TDH+	0.3	Texas (DePaola <i>et al.</i> , 2000)
156	34	6,018 (EB) 6,992 (DP)	46 31	0.76 0.44	Gulf Coast (DePaola <i>et al.</i> , 2003a) ^g
65	13	1,103 ^e (DP)	27 ^e	2.3 ^f	Pacific Northwest (DePaola <i>et al.</i> , 2002)
23	1	308 (MPN)	10 TDH+	3.2	Pacific Northwest (Kaysner <i>et al.</i> , 1990b)

^a Pathogenic is defined as a Kanagawa-positive (KP+) or thermostable direct hemolysin-positive (TDH+). TDH is a toxin produced by *V. parahaemolyticus* that lyses red blood cells in Wagatsuma agar. ^b Number of isolates tested. Test methods: EB=enrichment broth followed by streaking on agar; DP=direct plating; MPN=most probable number. ^c Samples included oysters, water and sediment samples. ^d ND = not determined. ^e Isolates obtained from 36 oyster samples collected at or “near” maximum intertidal exposure. ^f Estimated mean percentage pathogenic from fitted Beta distribution.

^g This is a subset of the Cook *et al.*, 2002a study.

Two studies, DePaola *et al.* (2002) and Kaufman *et al.* (2003) were selected as the most appropriate for estimating the distribution of pathogenic to total *V. parahaemolyticus* in oysters, based on the criteria described in Table IV-1c. The data from these two studies indicated that the number of pathogenic *V. parahaemolyticus* in sample portions was frequently non-detectable. In addition, high numbers of pathogenic microorganisms were sometimes observed in samples that had low counts of total *V. parahaemolyticus* in replicate samples. Some degree of variation is expected due to the natural processes of growth and competition between different strains of *V. parahaemolyticus* in the presence of other micro flora in the oysters. Additionally, the study by DePaola *et al.* (2003a) suggests that there may be some seasonal variation in the percentage of *V. parahaemolyticus* that are pathogenic. However, this finding has not been replicated in other studies. Accordingly, for the purpose of this risk assessment, the ratio between pathogenic and total *V. parahaemolyticus* densities was assumed to be temperature independent.

The studies representing different regions in the United States were analyzed separately. The study by DePaola *et al.*, (2002) was conducted in the Hood Canal area and represented the Pacific Northwest region. The study by Kaufman *et al.* (2003) was conducted in the Gulf Coast. It was assumed that the percentage pathogenic data from the Gulf Coast region can also be used to represent the Mid-Atlantic and Northeast Atlantic regions. This assumption was based on the data by Cook *et al.* (2002b) which showed that there was no apparent difference in the percentage of TDH⁺ *V. parahaemolyticus* in oyster samples among the Gulf Coast, Mid-Atlantic, and Northeast Atlantic regions.

Given the low densities of pathogenic *V. parahaemolyticus* in oysters and the resulting high frequency of non-detectable amounts in samples, the distributions of percentage pathogenic were estimated based on the assumption that pathogenic counts in sample portions were distributed according to a Beta-Binomial distribution. The Beta-Binomial distribution is a flexible two-parameter distribution commonly used to model variability of proportions (see Appendix 5 for additional information). In applying the Beta-Binomial distributional model to the Gulf Coast and Pacific Northwest data, the amount of pathogenic *V. parahaemolyticus* observed in a given sample portion is assumed to be binomially distributed with size parameter equal to the number of total *V. parahaemolyticus* expected in that sample volume. This is based on the number of total *V. parahaemolyticus* actually observed in the corresponding sample portion assayed for total *V. parahaemolyticus*. The probability parameter of the binomial distribution for pathogenic counts per sample is assumed to be randomly distributed according to a Beta distribution with unknown parameters α and β . The α and β parameters defining the distribution of percentage pathogenic were estimated based on the observed counts of total and pathogenic *V. parahaemolyticus* and sample volumes by maximizing the Beta-Binomial likelihood of the observed data. The resulting estimates of the mean of the distribution of percentage pathogenic (P) for the various harvest regions are given in Table IV-5. See Appendix 5 for details.

Table IV-5. Estimate of the Mean of Distributions of Percentage Pathogenic *Vibrio parahaemolyticus* in Oysters

Regions	α^a	β^a	ϕ^a	P ^a
Pacific Northwest^b	0.283	11.86	0.076	2.33% (1.05%, 5.47%)
Gulf Coast and Atlantic Regions^c	0.394	221	0.0045	0.18% (0.09%, 0.44%)

^a α and β denote the parameters, ϕ denotes the overdispersion and P denotes the average of the assumed Beta distribution with 5th and 95th percentile confidence intervals in parentheses. Values are the Maximum Likelihood Estimates of the Beta distribution parameters for the mean of the distributions of percentage pathogenic *Vibrio parahaemolyticus* in oysters.

^b Estimates were derived from the DePaola *et al.* (2002) study.

^c Estimates were derived from the Kaufman *et al.* (2003) study.

Uncertainty. The studies by Kaufman *et al.* (2003) and DePaola *et al.* (2002) provide information which is sufficient for estimation of the parameters for the Beta distribution of the percentage pathogenic *V. parahaemolyticus*. However, there is uncertainty associated with the estimates due to the limited sample sizes of the studies, particularly in regard to the volume of sample examined for pathogenic *V. parahaemolyticus*. There is also the possibility that the distribution of percentage pathogenic *V. parahaemolyticus* changes from one year to the next in response to changing environment conditions. In this regard, conditions in the Gulf Coast and Pacific Northwest during the summer of 2001 (when the two studies were conducted) appear to have been close to the norm. That is, the estimates of the mean percent pathogenic *V. parahaemolyticus* obtained on the basis of these studies are comparable to the estimates reported in Table IV-4 based on studies conducted in previous years. It is unknown at present the extent to which the distribution of percentage pathogenic may vary or how extreme (high or low) the mean and variance of the percent pathogenic *V. parahaemolyticus* distribution might fluctuate from one year to the next. In order to evaluate the effect of these uncertainties on the predicted illness rates, the uncertainty associated with the α and β parameter estimates was determined by using a parametric bootstrap procedure. See Appendix 5 for details.

For each region/season combination, the density of pathogenic *V. parahaemolyticus* at harvest was obtained by multiplying the density of total *V. parahaemolyticus* at harvest, as influenced by water temperature, with a value for the percentage of total *V. parahaemolyticus* that are pathogenic that was generated by a beta distribution with specific parameters. These parameters were derived to account for the uncertainty of what the actual percent pathogenic truly is by a multivariate analysis of the harvest data. Based on an analysis of the data, 1,000 plausible beta distribution parameters with an overall mean of 2.3 % was generated for the Pacific Northwest and 0.18% was generated for all other regions except the Pacific Northwest. These 1,000 plausible beta parameters were used once in the 1,000 simulations, but each set of parameters was used to generate 10,000 individual estimates of percent pathogenic during the model iterations.

Output of the Harvest Module

The output of the Harvest Module is the level of total and pathogenic *V. parahaemolyticus* in oysters at the time of harvest. For each region/season combination, the distribution of pathogenic *V. parahaemolyticus* at harvest was obtained by combining the distribution of total *V. parahaemolyticus* at harvest, as influenced by water temperature, with the appropriate distribution for the percentage of total *V. parahaemolyticus* that are pathogenic. Specific details of these calculations, the Monte Carlo methods used, and their implementation in @Risk (Palisade) based on the distributions and relationships as described above, can be found in Appendix 3.

Table IV-6 shows the mean and confidence intervals of the uncertainty distributions of the mean levels (i.e., the averages with respect to variability) of total and pathogenic *V. parahaemolyticus* at harvest for each of the 24 region/season combinations. The uncertainty in the mean estimates is also represented in Table IV-6 as the upper and lower bounds of the confidence limits (see discussion below). A comparison of mean total and pathogenic *V. parahaemolyticus* levels across these 24 region/season combinations indicates that, as expected, the Gulf Coast values are considerable higher than the other regions due to the warmer water temperatures in the Gulf. The levels of *V. parahaemolyticus* in the mid-Atlantic and Northeast Atlantic Summer are higher than those of the Pacific Northwest (when harvest occurs by dredging). Even during the summer, water temperatures in the Pacific Northwest are cooler (~11 °C), on average, than in the other Gulf and Atlantic regions. However, exposure to ambient temperatures for longer time periods, such as occurs during intertidal harvest in some Pacific Northwest areas, allows for additional growth of the microorganism, resulting in an increase in those levels to levels higher than for the mid- and Northeast Atlantic.

Table IV-6. Predicted Mean Levels of *Vibrio parahaemolyticus* per gram in Oysters at Harvest

Region	Season	Mean Total <i>V. parahaemolyticus</i> /g ^a	Mean Pathogenic <i>V. parahaemolyticus</i> /g ^a
Gulf Coast (Louisiana)	Winter	52 (18, 130)	0.087 (0.025, 0.22)
	Spring	940 (270, 3.1x10 ³)	1.6 (0.33, 5.4)
	Summer	2.1x10 ³ (630, 7.3x10 ³)	3.6 (0.74, 12)
	Fall	220 (61, 640)	0.38 (0.077, 1.2)
Gulf Coast (Non-Louisiana) ^b	Winter	52 (18, 130)	0.093 (0.025, 0.23)
	Spring	940 (280, 3.1x10 ³)	1.6 (0.32, 5.2)
	Summer	2.1x10 ³ (630, 7.7x10 ³)	3.6 (0.73, 12)
	Fall	220 (62, 600)	0.38 (0.077, 1.1)
Mid-Atlantic	Winter	3.5 (0.73, 8.7)	0.006 (0.001, 0.014)
	Spring	200 (67, 580)	0.33 (0.084, 1.0)
	Summer	780 (230, 2.2x10 ³)	1.3 (0.28, 3.9)
	Fall	51 (17, 140)	0.087 (0.023, 0.23)
Northeast Atlantic	Winter	3.7 (0.83, 8.7)	0.0064 (0.0012, 0.016)
	Spring	42 (15, 110)	0.07 (0.019, 0.18)
	Summer	230 (83, 590)	0.39 (0.10, 1.1)
	Fall	33 (13, 81)	0.057 (0.016, 0.15)
Pacific Northwest (Dredged) ^c	Winter	0.019 (0.0028, 0.056)	0.0004 (0.0001, 0.0014)
	Spring	0.81 (0.12, 2.3)	0.019 (0.0019, 0.054)
	Summer	5.0 (1.3, 14)	0.12 (0.022, 0.34)
	Fall	0.15 (0.05, 0.30)	0.0034 (0.0008, 0.0081)
Pacific Northwest (Intertidal) ^d	Winter	0.039 (0.0047, 0.12)	0.001 (0.0001, 0.0031)
	Spring	61 (0.86, 290)	1.4 (0.017, 6.1)
	Summer	650 (51, 2.6x10 ³)	15 (0.87, 63)
	Fall	2.3 (0.24, 6.9)	0.051 (0.004, 0.15)

^a Values in parentheses are the 5th and 95th percentiles of the uncertainty distribution. Values rounded to 2 significant digits.

^b Note: the values for Louisiana and non-Louisiana areas are similar because the water temperature is similar for these regions. Differences in the Gulf Coast states occur in the post-harvest portion of the model (See Table IV-11).

^c Represent harvest conditions when oyster reefs are submerged.

^d Represent harvest conditions during intertidal exposure.

Uncertainty. The output of the model simulations is a two-dimensional variability and uncertainty distribution for each region/season combination. At fixed values of the uncertainty parameters, the resulting one-dimensional distributions represent model predictions of the intrinsic variation of *V. parahaemolyticus* densities at time of harvest (i.e., variation from one collection of oysters to the next), conditional on the values of the uncertainty parameters. These variability distributions were found to be positively skewed (i.e., close to lognormal) suggesting that the variability of total *V. parahaemolyticus*/g at fixed temperature dominates the effects of variations of temperature (within each region/season).

It should be noted that, while the ratio of pathogenic to total *V. parahaemolyticus* values are close to the mean of the percent pathogenic distribution (as estimated and discussed above) the values do not match precisely because of the random approximation inherent to the Monte Carlo simulation (Appendix 3). The width of the confidence intervals gives an indication of the uncertainty of the predictions with an approximate 10-fold to 20-fold range, depending upon the region/season and the output variable.

It is also worth noting that the variability of pathogenic *V. parahaemolyticus*/g is greater than that of total *V. parahaemolyticus*/g. This is a consequence of the fact that, for pathogenic *V. parahaemolyticus*/g, there is the added effect of the variability of the percent pathogenic from one collection of oysters to the next. An appropriate summary of these two-dimensional distributions of the output variables is the one-dimensional uncertainty distribution of the mean of the variability distribution(s). Although other statistics and percentiles of the variability distributions have relevance with respect to the extremes of exposure that may occur on the individual level, it is the mean of the variability distributions that is the single most relevant measure of population exposure and hence the most pertinent for comparisons across different region and season categories.

Post-Harvest Module

The Post-Harvest Module predicts the effects of typical industry practices on *V. parahaemolyticus* densities in oysters during transportation, distribution and storage from harvest through retail. Factors that influence the levels of pathogenic *V. parahaemolyticus* in oysters (i.e., growth or die-off) include: ambient air temperatures at time of harvest; time from harvest until the oysters are placed under refrigeration; time it takes the oysters to cool once under refrigeration, and length of refrigeration time until consumption.

Growth and Survival. The growth and survival of *V. parahaemolyticus* in shellstock oysters has been studied. Cook and Ruple (1989) reported that levels of *V. parahaemolyticus* increase at temperatures above 10 °C, but in most cases did not detect an increase during storage at 10 °C. After one day of storage at either 22 °C or 30 °C the levels of *V. parahaemolyticus* were 2 to 3 orders of magnitude higher than those at harvest. Gooch *et al.* (2002) reported a 50-fold increase in *V. parahaemolyticus* levels after storage at 26 °C for 10 hours and a 790-fold increase after 24 hours. After refrigeration at 3 °C for approximately 14 days a 6-fold decrease in the levels was observed. The results from these studies indicate that *V. parahaemolyticus* can grow rapidly in unrefrigerated oysters.

Data Selection and Criteria for the Post-Harvest Module

The selection of data for use in the Post-Harvest Module considered the availability of data and limitations of the data sources. Model inputs (i.e., data or assumptions) included the following.

- To calculate the growth of *V. parahaemolyticus* in oysters from harvest to initial refrigeration, model inputs were needed for the duration of harvest, time-to-refrigeration, oyster temperature, and growth rate. Air temperature was used as a surrogate to estimate oyster temperature.
- To calculate the growth of *V. parahaemolyticus* in oysters from initial refrigeration until cooled to a no-growth temperature, model inputs were needed for the cooldown time and growth rate during cooling.
- To calculate the levels of *V. parahaemolyticus* in oysters from refrigeration to retail, model inputs were needed for the die-off rate and duration of cold storage.

Data were generally not available for the temperature of oysters after harvest. It was assumed that the temperature of oysters would equilibrate with the air temperature. Therefore, the air temperature data from the comprehensive NBDC database were used for each region/season combination. All identified studies were used in the model to provide information for time from harvest to refrigeration, growth/decline rate of *V. parahaemolyticus* in oysters during storage, and storage time between refrigeration and consumption.

Assumptions for the Post-Harvest Module

- The growth and survival of pathogenic *V. parahaemolyticus* in harvested oysters is the same as total *V. parahaemolyticus*.
- The relative growth rate of total *V. parahaemolyticus* in oysters versus broth culture conditions is temperature independent.
- Oysters equilibrate rapidly with that of ambient temperature after harvest and prior to refrigeration; ambient air temperature is a surrogate for oyster meat temperature. For Pacific Northwest (Intertidal) region, oyster temperature is greater than air temperature because of the effect of direct sunlight.
- Air temperature at noon is representative of the environmental temperature that oysters are subject to after harvest and prior to refrigeration. (This assumption does not apply to the Pacific Northwest (Intertidal) region.)
- Water activity of oysters does not vary substantially.
- NSSP guidelines for the maximum time that oysters can remain unrefrigerated after harvest are never exceeded.
- The extent of growth occurring over time at a given average temperature and predicted maximal growth rate is assumed to follow a simple three-phase loglinear model with no lag phase (Buchanan *et al.*, 1997).
- Value for the maximal density at all temperatures approaches a plateau of approximately 10^6 total *V. parahaemolyticus* per gram after 24 hours (Gooch *et al.*, 1999; 2002). [Note: To ensure that levels of pathogenic *V. parahaemolyticus* do not exceed the value equivalent to 10^6 total *V. parahaemolyticus*, the

simulation model was run separately, but in parallel for total and pathogenic *V. parahaemolyticus* (see Appendix 3).]

- Oysters are harvested uniformly from the start of the harvest up to one hour prior to conclusion of the harvesting operation. (This assumption does not apply to the Pacific Northwest (Intertidal) region.)
- The duration of time until oysters reach “no-growth” temperature after being placed under refrigeration varies uniformly between 1 and 10 hours.
- Once “no-growth” temperature is attained no further growth occurs during storage and transport through the retail market.
- No temperature abuse or mishandling occurs at retail, eating establishments, or as a result of consumer behavior.

Modeling the Post-Harvest Module

The various model inputs and output for the Post-Harvest Module are illustrated in Figure IV-6 and discussed in detail below.

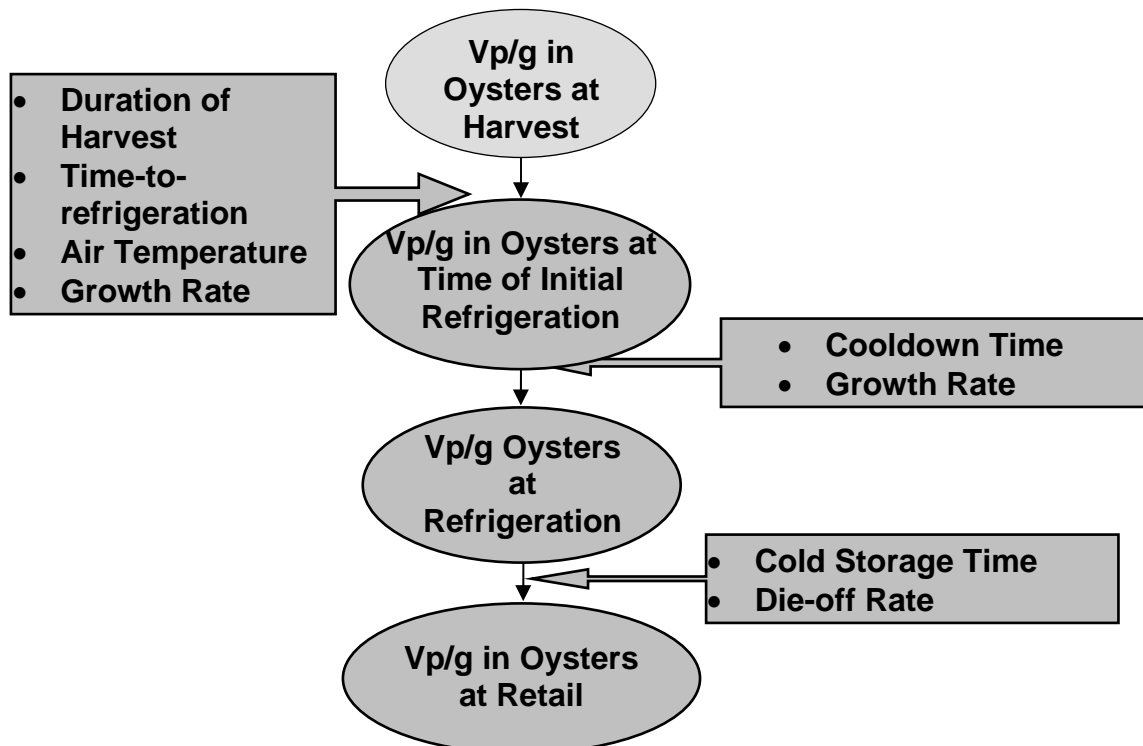


Figure IV-6. Schematic Depiction of the Post-Harvest Module of the *Vibrio parahaemolyticus* Exposure Assessment Model

[Vp/g is *Vibrio parahaemolyticus* per gram oyster. Levels of total and pathogenic *V. parahaemolyticus* were simulated by the model separately and in parallel.]

Studies and Data Sources Used for the Post-Harvest Module

- **Growth rate of *V. parahaemolyticus***: The growth rate was based on estimates obtained from Miles *et al.*, 1997 and Gooch *et al.*, 2002.
- **Time from harvest to refrigeration**: Information from a 1997 GCSL survey was used to estimate the duration of harvesting operations under current industry practices (Gulf Coast Seafood Laboratory, 1997) for the Gulf Coast States. The Gulf Coast practices were assumed to be representative of the Pacific Northwest, Mid-Atlantic, and Northeast Atlantic regions.
- **Oyster Temperature Distributions**: Air temperature data from the National Buoy Data Center (NBDC) were used as a surrogate for oyster temperature for all regions with the exception of the Pacific Northwest intertidal. For intertidal harvesting, oyster temperature was based on NBDC air temperature, oyster versus air temperature differences (DePaola *et al.*, 2002; Herwig and Cheney, 2001), and the National Weather Service (NWS, 1999) data on the proportion of days that are cloudy, partly cloudy and sunny.
- **Die-off rate during cold storage**: Data (a point estimate) from Gooch *et al.* (2002) were used for all regions and seasons.
- **Cold storage time**: Data from Cook *et al.* (2002a) (originally reported as FDA/ISSC, 2000) were used for all regions and seasons.

Growth of *Vibrio parahaemolyticus* from Harvest to First Refrigeration

The extent of growth that occurs during the period of time from harvest until the time that oysters are first placed under refrigeration is determined by four factors:

- the duration of harvest,
- the growth rate of *V. parahaemolyticus* as a function of air temperature,
- the temperature of oyster meat following harvest, and
- the length of time held unrefrigerated.

Additionally, for the Pacific Northwest, *V. parahaemolyticus* densities at time of harvest are influenced by whether or not oysters are collected intertidally.

Growth Rate Model

Gooch *et al.* (2002) is the only study identified which observed the post-harvest growth in oysters and it was limited to only one temperature (26 °C). Therefore, a model of *V. parahaemolyticus* growth in microbiological broth medium was used (Miles *et al.*, 1997) to predict growth of *V. parahaemolyticus* in oysters at a range of temperatures. The predictions of this model were adjusted to predict the growth rate of total *V. parahaemolyticus* in oysters. An upper limit of 10^6 was set for the maximum density of total *V. parahaemolyticus* in oysters. Based on a study by Cook (2002a), the growth and survival of pathogenic and total *V. parahaemolyticus* in oysters after harvest were

considered to be the same. Cook (2002a) reported that the presence of the *tdh* gene that codes for pathogenicity does not alter the growth rate of *V. parahaemolyticus* under typical temperature conditions.

Miles *et al.* (1997) studied the growth rate of four strains of *V. parahaemolyticus* in broth cultures at different temperatures and water activities. For each combination of temperature and water activity, the extent of bacterial growth observed was modeled using the Gompertz function. This is a sigmoid growth curve with a growth rate (slope) that increases up to a maximum rate (μ_m) and then falls to zero as the bacterial population reaches a steady state. A plot of the resulting model prediction for μ_m as a function of temperature is a unimodal function with a maximum value and no growth rate outside of the predicted range of temperatures favorable for growth.

It was assumed that water activity of oysters does not vary substantially with a nominal value equal to the optimal value of 0.985 predicted to occur under broth culture conditions. At this water activity, the predicted growth rate in broth at 26 °C (78.8 °F) is 0.84- \log_{10} per hour, which is approximately a 7-fold increase in density per hour. This is approximately four times greater than the rate of growth observed for *V. parahaemolyticus* in oysters held at 26 °C (78.8 °F) (Gooch *et al.*, 2002).

Therefore, for the risk assessment model, the predictions of the growth rate in broth cultures were divided by a growth rate factor. This factor was estimated based on Gooch *et al.*, (2002) experimental data, but to account for uncertainty, a triangle distribution with a range of 3 to 5 and mean of 4 was used in the model.

After transfer of an inoculum to different medium or environmental conditions there is typically a demonstrable lag phase during which time the bacterial population adapts to different environmental conditions and growth is sub optimal. This lag phase is commonly modeled by a sigmoid growth function such as the logistic or Gompertz. However, a sigmoid growth function (e.g., Gompertz) is not an appropriate model for growth of *V. parahaemolyticus* in oysters after harvesting, as changes in environment are typically gradual and do not arrest the growth rate and induce a lag phase. Consequently, the extent of growth occurring over time at a given average temperature was assumed to follow a simple three-phase loglinear model with no lag phase (Buchanan *et al.*, 1997). This model is of the form:

$$\log_{10}(N(t)) = \min\{\log_{10}(N(0)) + \mu_m * t, A\}$$

where $N(0)$ refers to bacterial density at harvest, $N(t)$ refers to the bacterial density at a given time (t) post-harvest, A is the logarithm of the maximum attainable density of *V. parahaemolyticus* in oysters, and the parameter μ_m (the maximal growth rate) is a function of ambient temperature. At 26 °C, the density of *V. parahaemolyticus* in oysters was observed to approach a plateau of approximately 6.0- \log_{10} per gram after 24 hours (Gooch *et al.*, 1999; 2002). This value was assumed for the maximal density (A) at all temperatures. Figure IV-7 shows the predictions (mean) of the \log_{10} increase in *V. parahaemolyticus* density from an initial level of 1,000/g as a function of time for three ambient temperatures, 20, 26 and 32 °C (68, 78.8, and 89.6 °F).

Oyster Temperatures

Ideally, the average temperature of oyster meat would be used to determine the growth rate parameter (μ_m) in the above equation. This temperature varies due to the temperature of both the air and water at the time of oyster harvest. The temperature of the oyster meat after harvest can be reasonably expected to equilibrate to that of the air although this may be modified somewhat by evaporative cooling and the extent to which oysters are properly shaded from direct sunlight aboard ship. This expectation was confirmed by warming/cooling experiments using a temperature probe, which indicated that individual oysters equilibrate rapidly to air temperature (i.e., <30 minutes) from initially wide temperature differences. When oysters were placed in a sack the rate of equilibration was observed to be slower (i.e., ~2 hours) and complete equilibration did not occur due to the effect of evaporative cooling (Cook, 2001). However, it was assumed that the temperature of oyster meat equilibrates rapidly with that of the ambient air. Therefore air temperature was used as a surrogate for oyster meat temperature for oysters harvested by dredging. For oysters harvested in intertidal areas, additional growth of *V. parahaemolyticus* was considered (see section titled, "Growth of *Vibrio parahaemolyticus* During Intertidal Exposure" in the Harvest Module section).

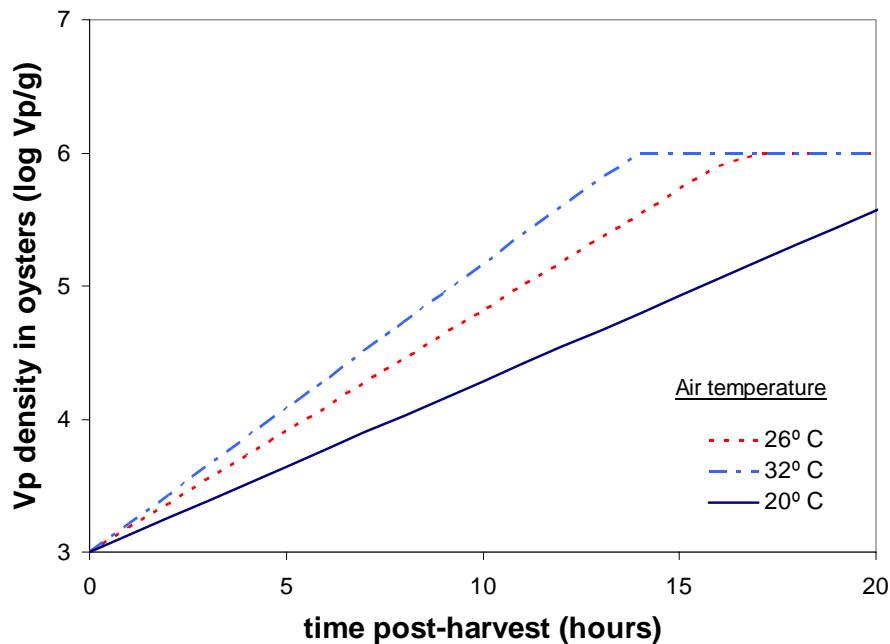


Figure IV-7. Predicted Mean Loglinear Growth of *Vibrio parahaemolyticus* in Oysters from an Initial Density of 1,000 (3-log_{10}) *Vibrio parahaemolyticus* per gram as a Function of Ambient Air Temperature

Air Temperature Distributions

Air temperature data were used as a surrogate for oyster temperature data because of limited data of the temperatures in oysters under different environmental conditions. For all regions except the Pacific Northwest (Intertidal), ambient air temperature data recorded at midday from the near-shore NBDC (National Buoy Data Center; <http://www.ndbc.noaa.gov/index.shtml>) buoys were used for this purpose. Examination of water and air temperatures obtained from the NBDC database show a strong correlation between water and air temperature. This correlation has been incorporated into the model by using the distribution of the difference in water temperature versus air temperature. The temperature difference distributions along with the water temperature distributions (from the Harvest Module) are used in the Post-Harvest Module simulations to predict air temperature. The difference in air and water temperature was found to be well characterized by a normal distribution. The parameters for the normal distribution were different for each region/season combination (see Appendix 3 for link to spreadsheets for this information). The distributions of difference in air temperature versus water temperature were obtained by pooling the data available for each near-shore buoy across all available years. The mean and standard deviation of these distributions are shown in Table IV-7.

Table IV-7. Mean Differences between Air and Water Temperature Distributions from Various Regions at Midday

Region (Buoy Location)	Mean of the Differences Between Air and Water Temperature (° C) Distributions ^a			
	Winter (Jan-March)	Spring (April-June)	Summer (July-Sept)	Fall (Oct-Dec)
Northeast Atlantic (Ambrose buoy, NY harbor)	-2.6 (5.0)	2.2 (3.2)	0.52 (2.7)	-3.2 (4.2)
Mid-Atlantic (Thomas Point Lighthouse buoy, Chesapeake Bay, MD)	-0.25 (4.0)	0.54 (2.9)	-1.4 (2.1)	-2.1 (3.1)
Gulf Coast (Dauphin Island, AL buoy)	-1.07 (3.3)	-1.24 (1.63)	-1.66 (1.33)	-1.62 (3.3)
Pacific Northwest (NOAA buoy on north end of Puget Sound, WA)	-1.6 (1.8)	1.3 (1.3)	1.3 (1.5)	-0.8 (2.0)

^a Value in parenthesis is the standard deviation for the mean.

Source of data NBDC; available at <http://www.ndbc.noaa.gov/index.shtml>

Distribution of Time Oysters are Unrefrigerated

For oysters harvested by dredging, the distribution of the length of time that oysters are held unrefrigerated was inferred based on the distribution of duration of daily oyster

harvesting operations (i.e., the combination of harvesting and transportation time). The distribution of time that oysters are unrefrigerated was obtained by assuming that oysters are collected uniformly from the start of the harvest up to one hour prior to conclusion of the harvesting operation when oysters are landed and placed in cold storage. An additional hour was assumed to be representative of the duration of transportation time to the processing facility, although this may vary somewhat for different harvesting regions. The derived distribution for time unrefrigerated reflects the fact that oysters collected at the start of the harvesting operation are exposed to ambient air temperatures for a longer period of time than those collected towards the end of harvesting operations. Consequently the mean time that oysters remain unrefrigerated is much less than the maximum duration of harvesting might suggest.

Information from a 1997 GCSL survey was used to estimate the duration of harvesting operations under current industry practices (GCSL, 1997). The survey was conducted in several Gulf Coast states during the fall of two successive years; one season prior to initiation of the NSSP time-to-refrigeration requirements (for states whose product has been confirmed as the source of two or more *V. vulnificus* illnesses), and then the following year after implementation. Duration of harvest was reported to be longer in Louisiana than in Florida and Texas, during both years. This probably reflects more remote oyster harvesting areas in Louisiana relative to other states on the Gulf Coast. Also, the duration of harvesting operations was reported to be shorter after the implementation of the NSSP guidelines due to compliance of the harvesters with the new requirements that took effect in 1996.

Data on the duration of harvesting during seasons other than the fall were not obtained during the 1997 GCSL survey. However, given the water temperature thresholds at which the NSSP time-to-refrigeration requirements are specified to be in effect, duration of harvesting during the spring and summer can be reasonably inferred to be similar to that reported during the fall. Therefore, the current duration of harvesting in the Gulf Coast during the spring, summer and fall was assumed to be equal to that reported in the 1997 GCSL survey during the fall of 1996, when the NSSP time-to-refrigeration requirements were in effect. The current duration of harvesting during the winter was assumed to be equal to the duration of harvesting that was reported prior to the implementation of the NSSP guidelines (fall of 1995) because, when cooler water conditions prevail, the NSSP requirements are not as stringent. A distinction between Louisiana and the rest of the Gulf Coast states was made based on the apparent differences in the reported durations of harvesting in the 1997 GCSL survey. Louisiana represents roughly half of the Gulf Coast harvest.

No data were identified for the duration of harvesting operations in regions other than the Gulf Coast. Consequently, estimates for other regions were inferred based on selected states included in the 1997 GCSL survey. The practices of Florida and Texas were assumed to be representative of the Pacific Northwest, Mid-Atlantic, and Northeast Atlantic regions. In the absence of conflicting information, the longer (pre-1996) reported harvesting durations were taken to be appropriate for all seasons, since

temperature thresholds at which more stringent time-to-refrigeration requirements would take effect would not commonly be exceeded outside of the Gulf Coast.

Table IV-8 shows the minimum, maximum and the most likely durations of oyster harvesting that have been inferred to apply for each of the different regions and seasons based on the 1997 GCSL survey data. Beta-PERT distributions were fit to these data to obtain smooth and continuous estimates of the distributions of the harvest durations. A Beta-PERT distribution is commonly used to infer a continuous distribution when the available data or expert opinion identifies only the range and most likely value of the parameter to be modeled. Figure IV-8 shows an example Beta-PERT distribution with minimum of 2, maximum of 11 and mode of 8 hours.

Table IV-8. Duration of Oyster Harvesting Operation for Each Region and Season Combination

Location	Distribution	Duration of Harvest (hours) ^a			
		Winter (Jan-March)	Spring (April-June)	Summer (July-Sept)	Fall (Oct-Dec)
Gulf Coast (Louisiana)	Maximum	13	11	11	13
	Minimum	7	5	5	7
	Mode	12	9	9	12
Gulf Coast (Non-Louisiana)	Maximum	11	10	10	10
	Minimum	2	3	3	3
	Mode	8	7	7	7
Northeast Atlantic	Maximum	11	11	11	11
	Minimum	2	2	2	2
	Mode	8	8	8	8
Mid-Atlantic	Maximum	11	11	11	11
	Minimum	2	2	2	2
	Mode	8	8	8	8
Pacific Northwest (Dredged)	Maximum	11	11	11	11
	Minimum	2	2	2	2
	Mode	8	8	8	8
Pacific Northwest (Intertidal) ^b	Maximum	11	11	11	11
	Minimum	2	2	2	2
	Mode	8	8	8	8

^a Data Source: GCSL (1997) survey responses.

^b For the intertidal harvest, the duration of intertidal exposure of 4 to 8 hours is a component of the harvesting duration and a maximum of 11 hours harvest duration is still assumed to apply (Appendix 5).

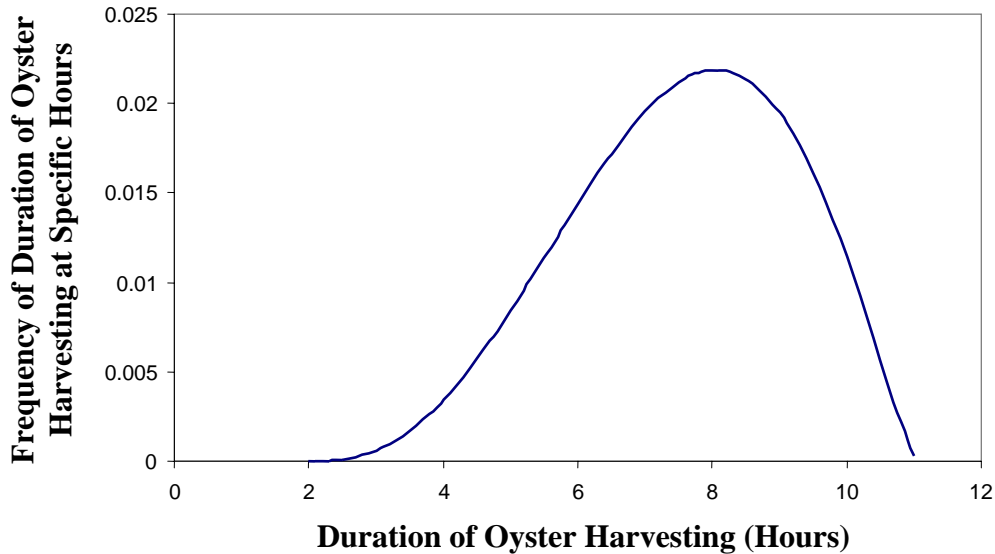


Figure IV-8. Example Beta-PERT Probability Density Distribution for Duration of Oyster Harvesting

Growth of *Vibrio parahaemolyticus* During Cooldown

Vibrio parahaemolyticus will continue to grow in oysters after they are placed under refrigeration until the temperature of the oyster tissue falls below a certain threshold (e.g. 8 °C) (46.4 °F) (Cook and Ruple, 1989). The time it takes for oysters to cool once under refrigeration is presumably quite variable depending on efficiency of the cooler, quantity of oysters to be cooled and their arrangement in the cooler. Data on cooling rates of commercial oyster shellstock could not be located. Preliminary GCSL experiments with a single in-shell oyster at 30 °C (86 °F) in which a temperature probe was inserted into its tissue indicated a cooling rate of approximately 0.5 °C (0.9 °F)/min when placed into a 3 °C (37.4 °F) cooler (DePaola, 1999). However, 24 oysters in an uninsulated plastic container required approximately 7 hours to drop from 26 °C (78.8 °F) to 3 °C (37.4 °F). In another GCSL study, one bushel of commercial size oysters (>3" hinge to bill) contained in a burlap sack was tempered to 25 °C. Using thermocouples inserted in oysters at different depths of the bushel, the investigator found that the oyster on the bottom of the sack cooled to 10 °C in 1.9 hr. (Contact with the cold floor of the cooler probably hastened its cooling.) The oysters in the center of the sack required 2.1 and 2.6 hr. to cool to 10 °C. The oyster in the top of the sack cooled in 2.2 hr. The single oyster outside the sack cooled to 10 °C in 0.3 hr (Cook, 2002b).

These data suggest considerable variability in the cooling rate depending upon the load and/or configuration of the oysters to be cooled. The cooling rate would also depend on the temperature of the cooler, which is likely to vary (FDA/ISSC, 2000). The distribution of cooler temperatures/efficiencies in the industry (e.g., both wholesale and retail establishments) is an uncertainty impacting the estimation of an appropriate distribution for the cooldown time. Based on this observation, a rectangular distribution between 1 and 10 hours was used for the cooldown time to represent both the variability (e.g., due to

load and/or configuration of oysters in a cooler) and the uncertainty inherent due to lack of knowledge concerning cooler temperatures and typical loading conditions.

As oysters cool down to storage temperatures the growth rate of *V. parahaemolyticus* slows with the declining temperature of the oyster tissue. At the start of the cooldown period, when oysters are first placed under refrigeration, the growth rate is still equal to the initial rate as determined by ambient air temperature. Assuming that no appreciable temperature abuse occurs after oysters have been placed in cold storage, further growth stops at the end of the cooldown period when oysters have reached a no-growth storage temperature. Beyond these reasonable assumptions little data are available as to the shape of the cooling curve, which is likely to depend on the loading and/or configuration of oysters in the cooler and the cooler temperature. Both of these factors are likely to vary under actual industry practice. Given this identified uncertainty, it was assumed that during the period of cooldown, the growth rate of *V. parahaemolyticus* drops linearly down to zero. This assumption may overestimate the growth that occurs if the temperature equilibration follows an exponential law (i.e., Newton’s Law of Cooling). However, typical loading and configuration of oysters in sacks stacked on pallets can be reasonably expected to reduce convective flow of chilled air and thereby slow equilibration of oysters to the cooler temperature (Schwarz, 2003b). Thus an exponential cooling rate was considered unlikely with respect to most of the harvest. Given the assumption of a linear cooling curve, a discrete approximation was used to model the amount of growth occurring during cooldown. Conditional on the duration of the cooldown period, the extent of growth during each hour of the cooldown period was approximated as an average growth rate during that hour times a duration of one hour. These average growth rates were determined by the duration of the cooldown period, the growth rate prior to refrigeration (i.e., as determined by the ambient air temperature for a given oyster lot), and the assumed linearity of the cooling curve. These calculations of average growth rate per hour consistent with the linear cooldown rate assumption are illustrated in the Table IV-9, where, for example, it takes T hours for a particular oyster lot to reach cooler temperature.

Table IV-9. Discrete Approximation of Variation in the Growth Rate of *Vibrio parahaemolyticus* during a Cooldown Period of T Hours

Hour of the Cooldown Period	Average Growth Rate (Log ₁₀ /hr) during the Hour of Cooldown ^a
1	$\frac{(T + 1) - 1}{T} \mu_m$
2	$\frac{(T + 1) - 2}{T} \mu_m$
3	$\frac{(T + 1) - 3}{T} \mu_m$
T	$\frac{(T + 1) - T}{T} \mu_m$
T+1	0

^a T=hours of cooldown period; μ_m =growth rate, at a given air temperature.

The total additional growth was then obtained as the sum of these values over the cooldown period subject to the restriction that the maximum density of 6.0-log₁₀ per gram could not be exceeded. Specifically, the potential amount of additional growth is the sum of the growth over the T hours:

$$\begin{aligned} \sum_{k=1}^T \mu_m * \frac{(T+1) - k}{T} &= \mu_m * \left[(T+1) - \frac{1}{T} \sum_{k=1}^T k \right] \\ &= \mu_m * \left[(T+1) - \frac{T+1}{2} \right] \\ &= \mu_m * \frac{T+1}{2} \end{aligned}$$

and this amount of additional growth is truncated by the assumption of a maximum density according to the following formula:

$$\min\left(\mu_m * \frac{T+1}{2}, 6 - \log_{10} N\right)$$

where N represents the density of *V. parahaemolyticus* at the time of first refrigeration and A is the maximum attainable density (6-log₁₀ per gram). Since the cooldown time T is a random variable with a mean of 5.5 hours, the average extent of growth is 3.25* μ_m in the absence of the truncation effect, where μ_m is the maximal growth rate determined by ambient air temperature at time of harvest. Thus, for an initial growth rate of 0.19-log₁₀ per hour (i.e., at 26 °C), the average growth occurring during cooldown is approximately 0.6-log₁₀ when densities at time of first refrigeration are generally below the maximum density, as is typically the case.

Change in Levels of *Vibrio parahaemolyticus* During Cold Storage

Gooch *et al.* (2002) showed that in oysters, *V. parahaemolyticus* levels declined 6-fold (0.8-log₁₀ cfu/g) when stored 14 to 17 days at 3 °C. This average rate of change was used as a point estimate of the rate of decline considered typical of refrigerated oysters in the marketplace, although some error may be introduced because commercial oysters are typically stored at higher temperatures (5-10 °C). This observation is supported by analysis of *V. parahaemolyticus* levels in retail oysters sampled from commercial establishments which suggests a decline of 0.04-log₁₀ cfu/g per day (FDA/ISSC, 2000; Cook *et al.*, 2002a). Both estimates are potentially biased to over predicting the extent of decline due to the fact that chill-stressed *V. parahaemolyticus* may not have been recovered by the methods used in these studies. However, in the Gooch *et al.* study, one of the enumeration methods used employed a repair step in a medium containing magnesium, which has been shown to increase recovery of chill-stressed cells. This method did not result in higher *V. parahaemolyticus* counts after refrigeration than the

other measurement methods that were used. Therefore, the potential bias due to the effect of chill-stress was considered negligible. The estimate of the storage effect based on the Gooch *et al.* study was considered the more reliable estimate because the study was conducted under controlled conditions. The estimate based on the ISSC/FDA retail study is potentially confounded and/or biased by factors other than storage time.

Cold Storage Time

Data from the ISSC/FDA retail study for the time between harvest and sample collection were assumed to be a reliable estimate for the length of refrigeration time (Cook *et al.*, 2002a). Summary statistics on the storage time for samples obtained during the study are shown in Table IV-10. A small degree of error may be introduced by assuming that these data are representative of storage time in so far as samples were generally collected on Monday or Tuesday and most servings are consumed in restaurants on weekends. Since this was a year long nationwide survey, the mean of 7.7 days and range of 1 to 21 days was assumed to be representative of all seasons and regions. A Beta-PERT distribution was utilized based on these statistics to infer the range and magnitude of variation expected to occur in the duration of storage time.

Table IV-10. Cold Storage Time between First Refrigeration and Retail

Storage Time Distribution	Local (days)^a	Non-Local (days)^b	Overall (days)^c
Minimum	1	2	1
Maximum	20	21	21
Mean	6.3	9.9	7.7
Most Likely	6	5	6

Source of data: FDA/ISSC, 2000 and Cook *et al.*, 2002a

^a Local consumption refers to oysters that were harvested and consumed in the same region.

^b Non-local consumption refers to oysters that were harvested, transported to another region, and then consumed.

^c Overall refers the total of all oysters; consumed both locally and non-locally.

The effect of storage was modeled by combining the distribution of storage times with the point estimate of the rate of change in *V. parahaemolyticus* levels per day. Thus, it is assumed that storage temperatures are always below the “no-growth” temperature for *V. parahaemolyticus*. The effect of this assumption is to likely underestimate the variance of the change in *V. parahaemolyticus* densities. During the FDA/ISSC retail study 25% of coolers were found to be >5.5 °C (42° F) and 2.5 % were >10°C (50 °F) at the time of sample collection (FDA/ISSC, 2000; Cook *et al.*, 2002a). A report by the FDA Retail Food Program Steering Committee suggests that 34% of "seafood retailers" practice improper storage conditions, i.e., temperatures >5.5 °C (FDA Retail Food Program Steering Committee, 2000). These estimates of deviation from compliance are relatively consistent and suggest that it is possible that *V. parahaemolyticus* levels increase in stored oysters. However, the ISSC/FDA retail study data indicate an overall average decrease in *V. parahaemolyticus* levels during storage. The rate of decrease would be

anticipated to be higher and the effect less variable if the 5.5 °C standard was consistently maintained.

Output of the Post-Harvest Module

The output of the Post-Harvest module, like that of the Harvest Module, is a two-dimensional variability and uncertainty distribution for each of a set of selected output variables and for each region/season combination. The output variables of interest for the Post-Harvest Module include the levels (i.e., densities) of total and pathogenic *V. parahaemolyticus* in oysters at the time of consumption. As discussed previously with respect to output of the Harvest module, the most pertinent summary of the two-dimensional variability and uncertainty distributions is the one-dimensional uncertainty distribution of the average levels (i.e., the averages over variability).

Table IV-11 shows the predicted levels of total and pathogenic *V. parahaemolyticus* in oysters post-harvest. The post-harvest results, in comparison to those shown in Table IV-6 for at-harvest, are indicative of the nominal effects of current post-harvest handling and processing practices on the potential for growth of *V. parahaemolyticus* in oysters. *Vibrio parahaemolyticus* levels post harvest are highest in the Louisiana and non-Louisiana Gulf Coast regions as expected, because the levels at harvest were the highest and ambient temperature is much higher in this region than in the other regions, allowing for more growth. The levels in the Louisiana Gulf Coast region are much higher than those in the non-Louisiana Gulf Coast region reflecting the longer time-to-refrigeration data used in the model for the Louisiana oyster harvest.

Table IV-11. Predicted Mean Levels of Total and Pathogenic *Vibrio parahaemolyticus* per Gram in Oysters Post-Harvest

Region	Season	Mean Total <i>V. parahaemolyticus</i> ^a	Mean Pathogenic <i>V. parahaemolyticus</i> ^a
Gulf Coast (Louisiana)	Winter	290 (30, 920)	0.48 (0.04, 1.6)
	Spring	2.3x10 ⁴ (8.5x10 ³ , 4.3x10 ⁴)	39 (12, 88)
	Summer	6.0x10 ⁴ (2.7x10 ⁴ , 1.1x10 ⁵)	100 (37, 220)
	Fall	5.7x10 ³ (1.3x10 ³ , 1.4x10 ⁴)	10 (1.8, 25)
Gulf Coast (Non-Louisiana)	Winter	130 (19, 430)	0.23 (0.026, 0.80)
	Spring	1.6x10 ⁴ (5.7x10 ³ , 3.3x10 ⁴)	28 (7.6, 65)
	Summer	4.2x10 ⁴ (1.8x10 ⁴ , 8.2x10 ⁴)	73 (24, 160)
	Fall	2.5x10 ³ (440, 6.6x10 ³)	4.4 (0.64, 12)
Mid-Atlantic	Winter	1.4 (0.29, 3.6)	2.4x10 ⁻³ (4.0x10 ⁻⁴ , 5.8x10 ⁻³)
	Spring	4.2x10 ³ (1.2x10 ³ , 9.3x10 ³)	7.3 (1.7, 18)
	Summer	1.2x10 ⁴ (2.7x10 ³ , 3.1x10 ⁴)	21 (3.8, 54)
	Fall	310 (23, 990)	0.54 (0.035, 2.0)
Northeast Atlantic	Winter	1.5 (0.31, 3.4)	2.5x10 ⁻³ (4.0x10 ⁻⁴ , 6.3x10 ⁻³)
	Spring	510 (51, 1.7x10 ³)	0.88 (0.063, 3.0)
	Summer	2.5x10 ³ (500, 6.8x10 ³)	4.3 (0.68, 12)
	Fall	52 (9.5, 160)	0.088 (0.012, 0.29)
Pacific Northwest (Dredged) ^b	Winter	8.0x10 ⁻³ (1.1x10 ⁻³ , 0.024)	1.9x10 ⁻⁴ (2.0x10 ⁻⁵ , 6.0x10 ⁻⁴)
	Spring	9.1 (0.11, 43)	0.22 (2.0x10 ⁻³ , 0.87)
	Summer	100 (6.3, 430)	2.3 (0.10, 11)
	Fall	0.23 (0.037, 0.67)	6.0x10 ⁻³ (6.0x10 ⁻⁴ , 0.018)
Pacific Northwest (Intertidal) ^c	Winter	0.017 (1.9x10 ⁻³ , 0.056)	4.0x10 ⁻⁴ (3.0x10 ⁻⁵ , 1.4x10 ⁻³)
	Spring	150 (0.66, 780)	3.7 (0.014, 19)
	Summer	1.7x10 ³ (120, 6.1x10 ³)	38 (2.0, 140)
	Fall	3.9 (0.15, 17)	0.086 (3.0x10 ⁻³ , 0.30)

^a Values in the parentheses are the 5th and 95th percentiles of uncertainty distributions. Values rounded to 2 significant digits.

^b Represents harvest conditions where oyster reefs are submerged.

^c Represents harvest conditions (i.e., higher oyster temperature and longer duration) during the intertidal exposure.

Consumption Module

The Consumption Module estimates the levels of pathogenic *V. parahaemolyticus* in a single serving of an oyster meal. The quantity and weight of oysters consumed per serving and the density of pathogenic *V. parahaemolyticus*/g shellfish at consumption are included in the modeling of this module. The determination of the number of raw oyster servings per annum is also discussed in this chapter and is used in the risk characterization portion of the model to calculate the illnesses per annum from the model-predicted illnesses per serving. Because raw oysters are infrequently consumed in the United States, the number of raw oyster servings was derived using the amount of oyster landings reported by the National Marine Fisheries Service (NMFS) for each region season, the mean weight of oysters per serving, and the likely amount of the harvest that is consumed raw.

Consumption was restricted in scope to domestically harvested product because most United States raw consumption is associated with domestically harvested oysters. Total United States imports of live oysters (which may then be consumed raw) have averaged approximately 3.5 million pounds (meat weight) per year from 1991 to 1998 (Hardesty, 2001). This corresponds to approximately 10% of the average yearly United States domestic harvest volume as reported by the National Marine Fisheries Service (NMFS) from 1990 to 1998. Most of these imported live oysters are from Canada (British Columbia and Prince Edward Island) and are of relatively low risk in consideration of generally cooler water temperatures of northern harvest areas. Although some confirmed United States illnesses have been traced back to imported oysters from Canadian harvest areas (i.e., in the Pacific Northwest), the relative number is very small and hence there is little bias associated with excluding imported oysters from the assessment.

United States exports of domestically harvested oysters generally account for less than 10% of the total United States harvest volume in any given year (Muth *et al.*, 2000; Hardesty, 2001). While oyster landing statistics reported to the NMFS include that intended for both domestic and export markets, the reported landings themselves are likely to be somewhat lower than actual landings (Muth *et al.*, 2000) and therefore there is little bias in assuming that reported landings of oysters to the NMFS provide a reasonable estimate of total domestically produced oyster harvest available for domestic consumption.

Data Selection and Criteria for the Consumption Module

The selection of data for use in the Consumption Module considered the availability of data and limitations of the data sources. Data used in the model included the following:

- the number of oysters consumed per serving, and
- the weight of oyster meats.

Number of Oysters Consumed per Serving. The criteria used to select the data used to estimate the distribution of the number of raw oysters consumed per serving is provided in Table IV-12. A nationally representative survey with a large number of raw oyster consumers would be preferable. However, because the best available national survey included a small number of oyster consumers, a regional survey was selected.

Weight of Oyster Meats. Only one large, nationally representative study was identified.

Table IV-12. Summary of Criteria and Selection of Data Used for the Number of Oysters per Serving

Study	Criteria		Used in Consumption Module?
	Nationally Representative?	Large Number of Oyster Consumers? ^a	
USDA CSFII (1992)	Yes	No (6 individuals)	No
Degner and Petrone, 1994	No (Florida)	Yes (306 individuals)	Yes

^a The number of oyster consumers in the study sample relates to the implied accuracy of the data.

Assumptions for the Consumption Module

- The consumption patterns by immunocompromised and healthy populations are the same.
- The percentage of raw oyster consumption does not vary by region or season.
- All *V. parahaemolyticus* illnesses are associated with consumption of domestic oysters (i.e., the impact of imported oysters on total illnesses was not evaluated).
- Raw oyster consumption patterns in Florida are representative for the United States

Modeling the Consumption Module

Distributions of doses of pathogenic *V. parahaemolyticus* ingested with oyster servings were obtained by combining predicted distributions of pathogenic *V. parahaemolyticus* per gram with estimated distributions for the number of oysters per serving and the mean weight of individual oysters as shown in Figure IV-9.

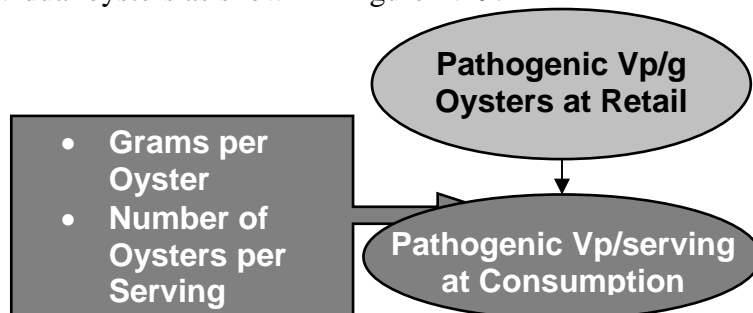


Figure IV-9. Schematic Depiction of the Consumption Module of the *Vibrio parahaemolyticus* Exposure Assessment Model

Studies and Data Sources Used for the Consumption Module

- Number of raw oysters consumed per serving: Data from a regional telephone survey, conducted by the Florida Agricultural Market Research Center, University of Florida (Degner and Petrone, 1994) was used to estimate the distribution of the

- number of oysters/serving. This estimated distribution was used for all regions and seasons.
- Oyster meat weight: Data from the ISSC/FDA retail study (FDA/ISSC, 2000; DePaola, 2002) were used to estimate the distribution of the average gram weight of oysters in a serving at the time of consumption. This estimated distribution was used for all regions and seasons. Data from Kaufman *et al.* (2003) were used to adjust the reported oyster weights from the ISSC/FDA study for the weight of the mantle fluid.

Number of Raw Oysters per Serving

Data from a regional telephone survey, conducted by the Florida Agricultural Market Research Center, University of Florida (Degner and Petrone, 1994) was used to determine the number of oysters consumed per serving. The survey was conducted during April and May of 1994. It included 1,012 adults in seven metropolitan areas in north and central Florida. Three hundred and six of the respondents reporting raw oyster consumption at least once in the previous year provided self-reported or recall information as to the number of oysters that they typically consumed per serving. These data were used as an estimate of the distribution of number of oysters per serving. The empirical distribution of the survey data is shown in Figure IV-10. The most typical serving sizes reported by the respondents were 6, 12 and 24 oysters, with 12 being the most frequent.

The Florida survey data was assumed to apply nationwide. Potentially, this may be biased somewhat with respect to the number of oysters per serving on the national level since the consumption survey was conducted in a region which is not necessarily representative of the entire country. Also, the survey was conducted in 1994 and even though consumption behavior may be changing from year to year, the estimated distribution of oysters per serving was assumed to apply to current consumption behavior. The magnitude of these potential biases is expected to be small relative to other identified uncertainties.

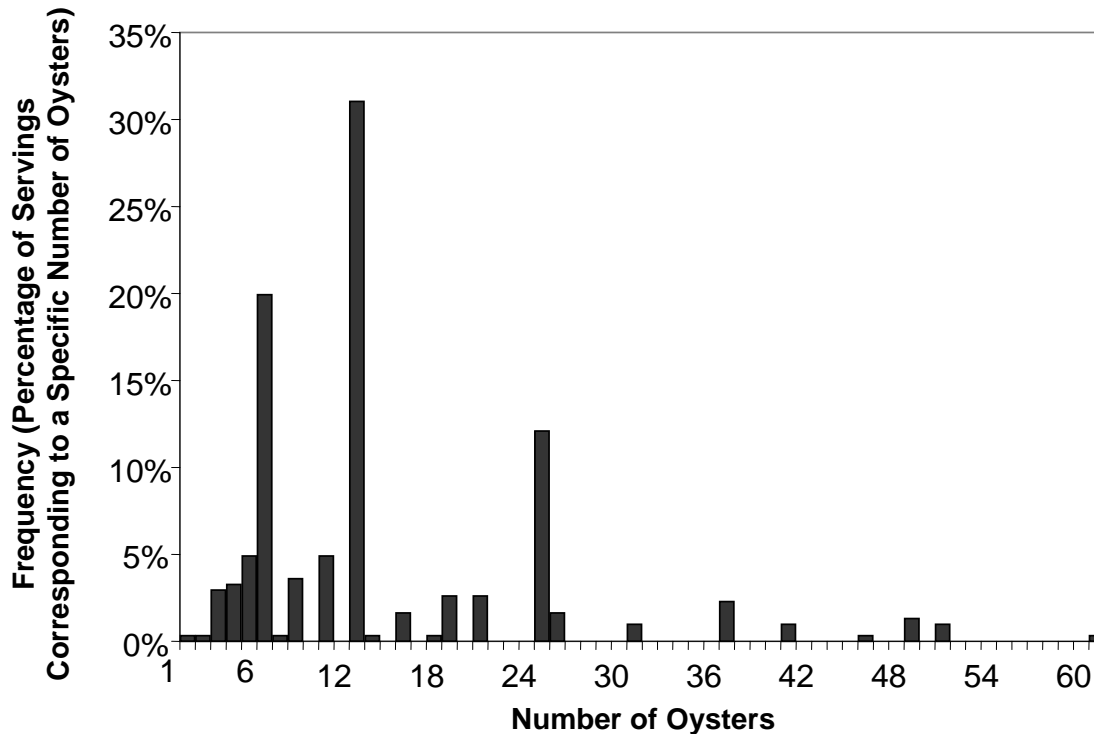


Figure IV-10. Self-reported Frequency of Number of Oysters Consumed per Serving (University of Florida Consumption Survey) (Degner and Petrone, 1994).

Oyster Meat Weight

The ISSC/FDA retail data (FDA/ISSC, 2000; DePaola, 2002) was used to estimate the gram weight of oysters consumed per serving. In this study, oyster weights were taken for 339 of the 370 samples collected from wholesale and retail locations. Samples generally consisted of 12 oysters (range, 4 to 15) and this included both the oyster meat and the mantle fluid. The average oyster weight per sample (meat and mantle fluid) was calculated by dividing the total gram weight by the number of oysters in the sample. The resulting distribution of average oyster weight per sample was found to be positively skewed (Appendix 5, Figure A5-11). This is likely because the oyster samples collected from retail establishments were harvested from many different growing areas; the Gulf Coast, Mid-Atlantic, Northeast Atlantic and Pacific Northwest regions were all equally represented.

Although there were some apparent differences in the mean oyster weight distribution by region and season of harvest, the differences were not large. A single estimate of the distribution of average gram weight per oyster based on pooling all of the data was considered appropriate and this estimate was assumed to apply to oysters harvested from all regions and seasons. A lognormal distribution was fit to the observed average oyster weight data in order to obtain a smooth estimate of the average oyster weight, rather than using the empirical distribution of the data. The maximum likelihood estimates obtained corresponded to a geometric mean average oyster weight of 15.2 grams and a geometric standard deviation of 1.4 grams.

Since the samples in the retail study were a combination of both oyster meat and mantle fluid a correction is needed to infer the average meat weight per oyster. Mantle fluid is typically not consumed. Based on mantle fluid versus meat weight measurements of individual Gulf Coast oysters collected during the Kaufman *et al.* (2003) study and the weight of oysters at retail (DePaola, 2002), approximately 90% of the total oyster weight is the meat weight. Therefore, the average oyster weight distribution was multiplied by this average percentage to obtain a distribution of the average meat weight per oyster.

Oyster Meat Weight per Serving

The total gram weight of oyster meat consumed per serving was obtained as the combination of the distribution of the number of oysters consumed and the distribution of the average meat weight per oyster at retail. The distribution of total consumption per serving was truncated at less than 10 grams or more than 2,000 grams because consumption outside these levels is unlikely. The best estimate of the mean meat weight per serving was approximately 200 grams.

Number of Raw Oyster Servings per Annum

The total annual number of servings consumed was estimated using data on the total landings of oysters, the mean weight of oysters per serving, and the likely amount of the total harvest that is consumed raw. Industry estimates suggest that approximately 50% of the Gulf Coast harvest is consumed raw (Muth *et al.*, 2000). This estimate was assumed to apply for each region/ season combination. The total amount (weight) of oysters harvested from different regions and seasons in the United States was obtained from the National Marine Fisheries Service (NMFS). For this risk assessment, the average NMFS landings data from 1990 to 1998 were used as shown in Table IV-13.

Table IV-13. National Marine Fisheries Service (NMFS) Average Yearly Oyster Landings from 1990 to 1998

Harvest Location	Oyster Meats Harvested (pounds) ^a				Total
	Winter (Jan - March)	Spring (April - June)	Summer (July - Sept)	Fall (Oct - Dec)	
Gulf Coast					
Louisiana	2,751,000	2,630,000	2,854,000	2,769,000	11,004,000
Non-Louisiana	96,000	1,393,000	847,000	2,358,000	6,694,000
Total	4,848,000	4,023,000	3,701,000	5,127,000	17,699,000
Atlantic					
Northeast	2,112,000	714,000	676,000	3,710,000	7,212,000
Mid-Atlantic	946,000	125,000	66,000	1,492,000	2,629,000
Pacific					
Northwest	2,402,000	1,682,000	1,379,000	3,181,000	8,644,000
Total	10,308,000	6,544,000	5,822,000	13,509,000	36,183,000

Source of data: <http://www.nmfs.noaa.gov/>

^a 1 pound= approximately 0.4536 kilograms

Total landings across different regions and seasons vary from year-to-year, presumably due to the influence of numerous factors (e.g. closures due to water quality, market forces). Although some year-to-year trends and fluctuations are evident in the oyster landings data, these year-to-year differences are generally less than 25% of the overall average oyster landing for the identified period from 1990 to 1998. This is a relatively small variation relative to other identified modeling uncertainties impacting risk characterization.

The total amount of oyster meat consumed equals the sum of the amounts in each serving consumed. Thus, the total number of servings can be estimated using the following equation:

$$\sum_{k=1}^N S_k = N * E[S] = f * L$$

where N denotes the total number of servings, S_k denotes amount of meat weight consumed in each of the N servings, $E[S]$ denotes the average of the S_k , f denotes the percentage of the total landed oyster meat weight that is consumed raw, and L denotes the total weight of oyster meat landed (i.e., for a given region and season combination). This equation was used to solve for N , the total number of servings, for each region/season combination.

Table IV-14 provides the calculated number of raw oyster servings for each region/season combination. The total annual number of raw oyster servings is approximately 40 million (i.e., $N = [(0.5 \times 16,400,000 \text{ kg})/0.2 \text{ kg}]$). In this calculation, the total landings (L), from Table IV-14, is approximately 36 million pounds (16 million kg). The mean meat weight per serving ($E[S]$) is estimated as 200 grams (based on the ISSC/FDA retail study) and the percentage of total landed oyster meat weight consumed raw (f) is assumed to be 50%.

Assuming that children do not eat raw oysters and the adult U.S. population is approximately 200 million, the annual consumption rate is approximately 0.2 servings per adult per year ($40/200 = 0.2$). This consumption rate was calculated. This consumption rate is consistent with the estimate of 0.0005 servings per day or 0.18 servings per person per year based on the 1989-1992 CFSII survey data. It should be noted that regional consumption rates are likely. For example, the consumption rate reported in the Florida consumer survey (Degner and Petrone, 1994) is considerably higher (5.2 servings per year) than the national estimates described above (approximately 0.2 servings per year).

Table IV-14. Annual Number of Raw Oyster Servings Used in the Model for Each Region and Season Combination

Harvest Location	Average Number of Raw Oyster Servings ^a				Total
	Winter (Jan - March)	Spring (April - June)	Summer (July - Sept)	Fall (Oct - Dec)	
Gulf Coast (Louisiana)	3,100,000	3,000,000	3,200,000	3,100,000	12,400,000
Gulf Coast (Non-Louisiana)	2,700,000	1,600,000	960,000	2,700,000	7,960,000
Atlantic Northeast	2,400,000	810,000	770,000	4,200,000	8,180,000
Mid-Atlantic	1,100,000	140,000	75,000	1,700,000	3,015,000
Pacific Northwest (dredged)	680,000	480,000	390,000	900,000	2,450,000
Pacific Northwest (intertidal)	2,000,000	1,400,000	1,200,000	2,700,000	7,300,000
Total	11,980,000	7,430,000	6,595,000	15,300,000	41,000,000

^a Calculated using the oyster landings provided by <http://www.nmfs.noaa.gov/>.

Output of the Consumption Module

The output of the Consumption Module is the level of pathogenic *V. parahaemolyticus* associated with typical serving sizes. The output of the simulation consists of a two-dimensional variability and uncertainty distribution or, alternatively, a sequence of variability distributions indexed by selected sets of uncertainty parameters. An appropriate summary of this two-dimensional variability and uncertainty distributions is the one-dimensional uncertainty distribution of the mean of the variability distribution(s).

Table IV-15 shows the predicted mean levels of pathogenic *V. parahaemolyticus* at consumption. As would be expected, the relative level of exposure for the different region/season combinations at consumption should be no different from the levels at post-harvest; consumption levels are derived from the post-harvest levels and the serving size and it is the same average (200 g) for all region/season combinations. The mean levels of pathogenic *V. parahaemolyticus* per serving are higher at time of consumption for the Gulf Coast (Louisiana and non-Louisiana) compared to the other regions. The highest levels are attributed to the Gulf Coast (Louisiana) region.

Table IV-15. Predicted Mean Levels of Total and Pathogenic *Vibrio parahaemolyticus* per Serving of Oysters at Consumption

Region	Season	Total <i>V. parahaemolyticus</i> per Serving ^a	Mean Pathogenic <i>V. parahaemolyticus</i> per Serving ^a
Gulf Coast (Louisiana)	Winter	5.8×10 ⁴ (6.0×10 ³ , 1.8×10 ⁵)	98 (8.1, 330)
	Spring	4.6×10 ⁶ (1.7×10 ⁶ , 8.7×10 ⁶)	7.9×10 ³ (2.3×10 ³ , 1.8×10 ⁴)
	Summer	1.2×10 ⁷ (5.5×10 ⁶ , 2.2×10 ⁷)	2.1×10 ⁴ (7.5×10 ³ , 4.4×10 ⁴)
	Fall	1.2×10 ⁶ (2.6×10 ⁵ , 2.8×10 ⁶)	2.0×10 ³ (320, 5.1×10 ³)
Gulf Coast (Non-Louisiana)	Winter	2.7×10 ⁴ (3.8×10 ³ , 8.7×10 ⁴)	47 (5.1, 160)
	Spring	3.2×10 ⁶ (1.2×10 ⁶ , 6.6×10 ⁶)	5.6×10 ³ (1.5×10 ³ , 1.3×10 ⁴)
	Summer	8.5×10 ⁶ (3.6×10 ⁶ , 1.7×10 ⁷)	1.5×10 ⁴ (4.9×10 ³ , 3.2×10 ⁴)
	Fall	5.0×10 ⁵ (9.0×10 ⁴ , 1.3×10 ⁶)	880 (110, 2.5×10 ³)
Mid-Atlantic	Winter	280 (59, 720)	0.48 (0.09, 1.2)
	Spring	8.5×10 ⁵ (2.5×10 ⁵ , 1.9×10 ⁶)	1.5×10 ³ (330, 3.5×10 ³)
	Summer	2.5×10 ⁶ (5.4×10 ⁵ , 6.3×10 ⁶)	4.3×10 ³ (750, 1.1×10 ⁴)
	Fall	6.2×10 ⁴ (4.6×10 ³ , 2.0×10 ⁵)	110 (7.1, 410)
Northeast Atlantic	Winter	300 (63,690)	0.5 (0.09, 1.2)
	Spring	1×10 ⁵ (1×10 ⁴ , 3.4×10 ⁵)	180 (12, 620)
	Summer	5×10 ⁵ (1×10 ⁵ , 1.4×10 ⁶)	860 (130, 2.6×10 ³)
	Fall	1×10 ⁴ (1.9×10 ³ , 3.2×10 ⁴)	17 (2.4, 57)
Pacific Northwest (Dredged)^b	Winter	1.6 (0.22, 4.9)	0.04 (0.00, 0.12)
	Spring	1.9×10 ³ (2.3, 8.7×10 ³)	42 (0.4, 160)
	Summer	2.1×10 ⁴ (1.3×10 ³ , 8.7×10 ⁴)	460 (21, 2.1×10 ³)
	Fall	47 (7.5, 140)	1.2 (0.12, 3.6)
Pacific Northwest (Intertidal)^c	Winter	3.4 (0.38, 11)	0.08 (0.01, 0.28)
	Spring	3.0×10 ⁴ (130, 1.6×10 ⁵)	740 (2.6, 3.7×10 ⁴)
	Summer	3.3×10 ⁵ (2.4×10 ⁴ , 1.2×10 ⁶)	7.5×10 ³ (370, 3.0×10 ⁴)
	Fall	800 (31, 3.5×10 ³)	17 (0.50, 74)

^a Values in parentheses are the 5th and 95th percentiles of the uncertainty distributions. Values rounded to 2 significant digits.

^b Average levels when oyster reefs are submerged.

^c Average levels after intertidal exposure.

V. RISK CHARACTERIZATION

The Risk Characterization component of the risk assessment is the integration of the Exposure Assessment and Dose-Response models. It provides estimates of the probability of illness and the overall annual illness burden attributed to consumption of oysters harboring pathogenic *V. parahaemolyticus* given current harvesting practices for each of the 24 region/season combinations. The influence of variability and uncertainty factors on the predicted risk were evaluated using statistical analyses. The risk assessment results were validated using data not included in the model.

Simulations

Figure V-1 shows a schematic representation of all the parameters used in the simulation for each module and how the output of a module becomes an input for the following module. The probable numbers of illnesses were simulated separately for 24 region/season combinations. The predictions of illnesses were determined by the predicted distributions of the amount of pathogenic *V. parahaemolyticus* consumed and the dose-response relationship. Throughout the simulations, the uncertainty and variability was propagated through the various events along the pathway from harvest to consumption.

The calculations were performed by the Monte Carlo method of re-sampling from specified input distributions and appropriately combining the sampled values to generate the corresponding output distributions. In order to include the uncertainty and variability (as appropriate) for each model input, a total of 1,000 simulations were run for each region/season combination. Within each simulation there were 10,000 iterations which represent individual servings of raw oysters. Due to the relatively large number of servings consumed within each of the region/season combinations, the numbers of illnesses were determined by multiplying the mean predicted risk per serving by the number of servings consumed. Additional details of the model are given in Appendix 3. A web address is also provided in Appendix 3, where a worksheet can be found which shows the different formulae, parameters and method of implementation of the Monte Carlo simulations.

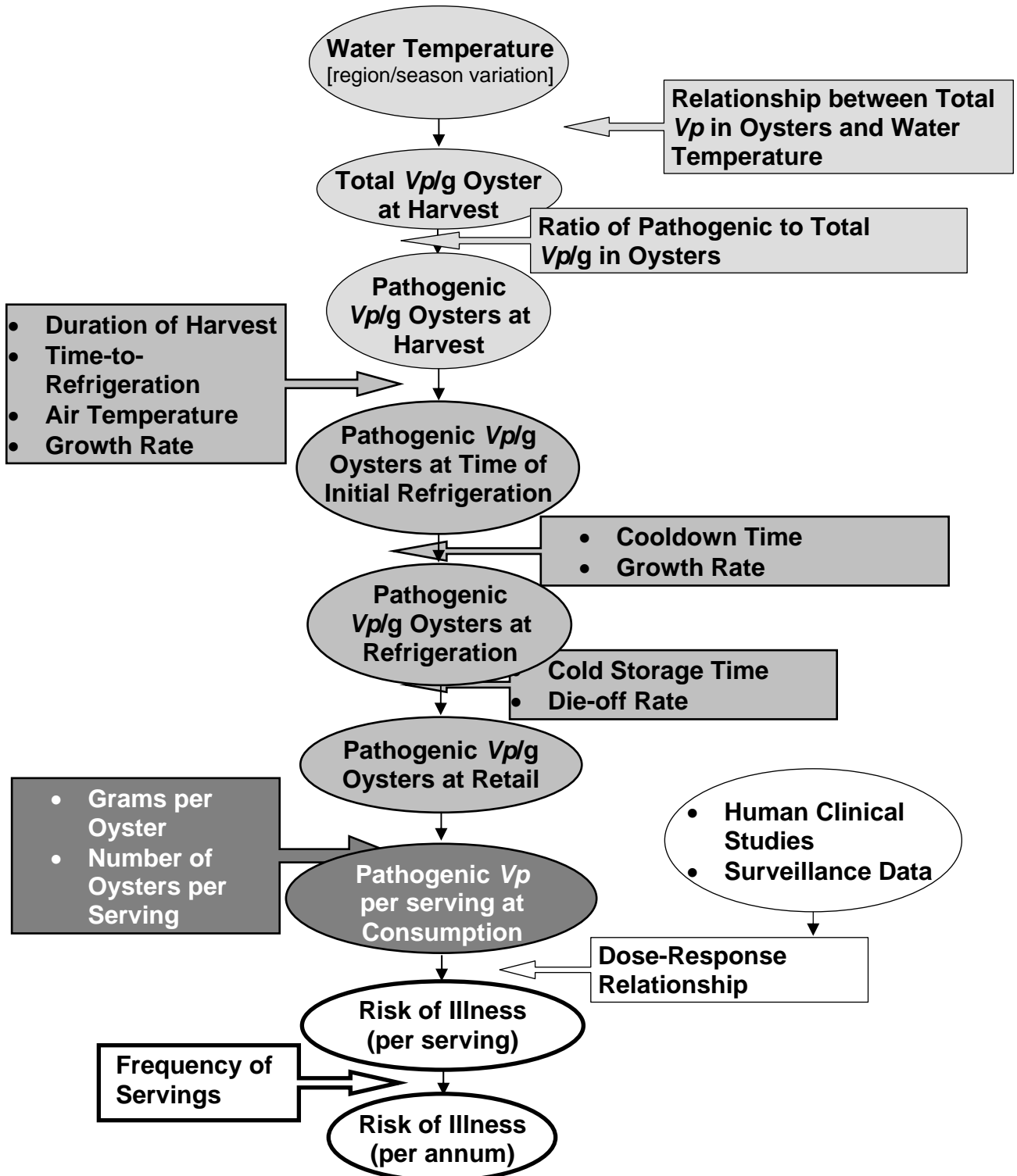


Figure V-1. Schematic Representation of the *Vibrio parahaemolyticus* Risk Assessment Model

[The light grey boxes with black lettering show the Harvest Module, the gray boxes with black lettering show the Post-Harvest Module, the dark grey boxes with white lettering show the Consumption Module, the white boxes with black lettering show the Dose-Response model, and the white boxes with dark black outline show the Risk Characterization.]

Predicted Illness Burden

Risk per Serving

The “risk per serving” is the risk of an individual becoming ill (gastroenteritis alone or gastroenteritis followed by septicemia) when they consume a single serving of oysters. The predicted mean risk per serving for each region/season combination is shown in Table V-1. The predicted risk per serving is highest for the Gulf Coast (Louisiana) region and lowest for Pacific Northwest (dredged). Within a region, the risk per serving is highest for the warmer seasons (summer and spring) and lowest for the cooler seasons (fall and winter). For example, for the Northeast Atlantic, the risk per serving in the winter is approximately 1×10^{-8} meaning only one illness in every 100 million servings. For this same region, the risk per serving in the summer is approximately 3 orders of magnitude higher (one illness in every 100,000 servings).

Risk per Annum

The “risk per annum” is the predicted number of illnesses (gastroenteritis alone or gastroenteritis followed by septicemia) in the United States each year. The predicted mean risk per annum for each region/season combination is shown in Table V-2. The Gulf Coast accounts for approximately 92% (~2,600) of the predicted number of illnesses per year. The Gulf Coast (Louisiana) alone accounts for approximately 73% of predicted illnesses per year. The low numbers of illnesses predicted for the Northeast Atlantic and Mid-Atlantic oyster harvests are attributable to both the colder water temperatures and the relatively smaller harvest from these regions during the warm summer months.

Severity of Illness

The predicted number of cases of septicemia was determined for the total United States population as shown in Table V-3. The number of predicted cases of septicemia was estimated by multiplying the mean number of predicted illnesses (Table V-2) by the probability of gastroenteritis progressing to septicemia (0.0023). The derivation of the probability of gastroenteritis progressing to septicemia was described in Chapter III: Hazard Characterization (Table III-4). Most of the cases of illness are predicted to be associated with the Gulf Coast region oyster harvest and this is also the region associated with the highest number of cases of septicemia.

Table V-1. Predicted Mean Risk per Serving Associated with the Consumption of *Vibrio parahaemolyticus* in Raw Oysters

Region	Mean Risk per Serving ^a				
	Summer (July to September)	Fall (October to December)	Winter (January to March)	Spring (April to June)	Total ^b
Gulf Coast (Louisiana)	4.4x10 ⁻⁴ (3.4x10 ⁻⁵ , 1.4x10 ⁻³)	4.3x10 ⁻⁵ (2.1x10 ⁻⁶ , 1.5x10 ⁻⁴)	2.1x10 ⁻⁶ (5.2x10 ⁻⁸ , 8.3x10 ⁻⁶)	1.7x10 ⁻⁴ (1.2x10 ⁻⁵ , 5.4x10 ⁻⁴)	6.6x10 ⁻⁴
Gulf Coast (Non-Louisiana)^c	3.1x10 ⁻⁴ (2.3x10 ⁻⁵ , 1.0x10 ⁻³)	1.9x10 ⁻⁵ (7.4x10 ⁻⁷ , 6.6x10 ⁻⁵)	1.1x10 ⁻⁶ (3.1x10 ⁻⁸ , 4.2x10 ⁻⁶)	1.2x10 ⁻⁴ (8.3x10 ⁻⁶ , 3.9x10 ⁻⁴)	4.5x10 ⁻⁴
Mid-Atlantic	9.2x10 ⁻⁵ (4.9x10 ⁻⁶ , 3.3x10 ⁻⁴)	2.2x10 ⁻⁶ (4.9x10 ⁻⁸ , 1.0x10 ⁻⁵)	1.1x10 ⁻⁸ (4.9x10 ⁻¹⁰ , 3.8x10 ⁻⁸)	3.1x10 ⁻⁵ (1.8x10 ⁻⁶ , 1.1x10 ⁻⁴)	1.3x10 ⁻⁴
Northeast Atlantic	1.8x10 ⁻⁵ (8.4x10 ⁻⁷ , 6.9x10 ⁻⁵)	4.0x10 ⁻⁷ (1.2x10 ⁻⁸ , 1.6x10 ⁻⁶)	1.1x10 ⁻⁸ (4.9x10 ⁻¹⁰ , 3.5x10 ⁻⁸)	3.6x10 ⁻⁶ (8.4x10 ⁻⁸ , 1.5x10 ⁻⁵)	2.2x10 ⁻⁵
Pacific Northwest (Dredged)^d	1.0x10 ⁻⁵ (1.6x10 ⁻⁷ , 4.2x10 ⁻⁵)	2.6x10 ⁻⁸ (6.9x10 ⁻¹⁰ , 9.5x10 ⁻⁸)	8.1x10 ⁻¹⁰ (3.2x10 ⁻¹¹ , 3.2x10 ⁻⁹)	8.7x10 ⁻⁷ (4x10 ⁻⁹ , 3.1x10 ⁻⁶)	1.1x10 ⁻⁵
Pacific Northwest (Intertidal)^d	1.4x10 ⁻⁴ (3.2x10 ⁻⁶ , 6.2x10 ⁻⁴)	3.9x10 ⁻⁷ (3.1x10 ⁻⁹ , 1.6x10 ⁻⁶)	1.7x10 ⁻⁹ (5.5x10 ⁻¹¹ , 6.5x10 ⁻⁹)	1.3x10 ⁻⁵ (2.3x10 ⁻⁸ , 5.8x10 ⁻⁵)	1.5x10 ⁻⁴

^a Risk per serving refers to the predicted risk of an individual becoming ill (gastroenteritis alone or gastroenteritis followed by septicemia) when they consume a single serving of raw oysters. Values in parentheses are the 5th and 95th percentiles of the uncertainty distribution. Values rounded to 2 significant digits.

^bNote: This value is the total mean predicted risk per serving, it is the rate of illness occurring of individuals who consume a single serving of oysters from the regional harvest in each of the four seasons.

^c Includes oysters harvested from Florida, Mississippi, Texas, and Alabama. The time from harvest to refrigeration in these states is typically shorter than for Louisiana.

^dOysters harvested using intertidal methods are typically exposed to higher temperature for longer times before refrigeration compared with dredged methods.

Table V-2. Predicted Annual Number of Illnesses Associated with the Consumption of *Vibrio parahaemolyticus* in Raw Oysters

Region	Mean Annual Number of Illnesses ^a				
	Summer (July to Sept)	Fall (October to December)	Winter (January to March)	Spring (April to June)	Total
Gulf Coast (Louisiana)	1406 (109, 4435)	132 (6, 468)	7 (0.2, 26)	505 (36, 1624)	2,050
Gulf Coast (Non-Louisiana)^b	299 (22, 985)	51 (2, 180)	3 (<0.1, 11)	193 (13, 631)	546
Mid-Atlantic	7 (0.36, 25)	4 (<0.1, 17)	<0.1 (<0.01, <0.1)	4 (0.2, 15)	15
Northeast Atlantic	14 (0.6, 53)	2 (0.1, 7)	<0.1 (<0.01, <0.1)	3 (<0.1, 12)	19
Pacific Northwest (Dredged)	4 (<0.1, 16)	<0.1 (<0.01, <0.1)	<0.1 (0, <0.01)	0.42 (<0.1, 2)	4
Pacific Northwest (Intertidal)^c	173 (4, 750)	1 (0.01, 4)	<0.01 (<0.01, 0.01)	18 (<0.1, 81)	192
TOTAL	1,903	190	10	723	2826

^a Mean annual number illnesses refers to predicted annual number of illnesses (gastroenteritis alone or gastroenteritis followed by septicemia) in the United States each year. Values in parentheses are the 5th and 95th percentiles of the uncertainty distribution. Note: Actual values for the illness predictions are provided in Appendix 7.

^b Includes oysters harvested from Florida, Mississippi, Texas, and Alabama. The typical time from harvest to refrigeration of oysters for these states is shorter than for Louisiana.

^c Oysters harvested using intertidal methods are typically exposed to higher temperature for longer times before refrigeration compared with dredged methods.

Table V-3. Predicted Mean Number of Cases of *Vibrio parahaemolyticus* Septicemia Associated with the Consumption of Raw Oysters

Region	Mean Annual Cases of Septicemia ^a				Total
	Summer (July to Sept)	Fall (October to December)	Winter (January to March)	Spring (April to June)	
Gulf Coast (Louisiana)	3	<1	<1	1	4
Gulf Coast (Non-Louisiana) ^b	<1	<1	<1	<1	1
Mid-Atlantic	<1	<1	<1	<1	<1
Northeast Atlantic	<1	<1	<1	<1	<1
Pacific Northwest (Intertidal) ^c	<1	<1	<1	<1	<1
Pacific Northwest (Dredged) ^c	<1	<1	<1	<1	<1
TOTAL	4	<1	<1	2	7

^a Calculated by multiplying the estimated probability of septicemia (0.0023; Table III-4) by the mean predicted number of illnesses (Table V-2). Note: Actual values for septicemia cases shown as <1 are provided in Appendix 7.

^b Includes oysters harvested from Florida, Mississippi, Texas, and Alabama. The typical time from harvest to refrigeration of oysters for these states is shorter than for Louisiana.

^c Oysters harvested using intertidal methods are exposed to higher temperature for longer times before refrigeration compared with dredged methods.

Uncertainty Distributions of Predicted Illness

The uncertainty of the predicted number of annual *V. parahaemolyticus* illnesses was analyzed for each region/season combination. The shape of the distribution is a consequence of model uncertainties based on 1,000 simulations. The predicted number of illnesses is greatly affected by the combination of the multiple uncertainties of all the inputs used in the model.

Figure V-2 provides an example uncertainty distribution for the Mid-Atlantic region for the spring and summer harvest seasons. The shape of the distribution is representative of each of the region/season combinations. In this example, 22% of the time (i.e., 220 of 1,000 simulations) the model predicted that approximately 8 illnesses each year were attributable to the Mid-Atlantic Summer harvest. Uncertainty distributions for the remaining region/season combinations are found in Appendix 8.

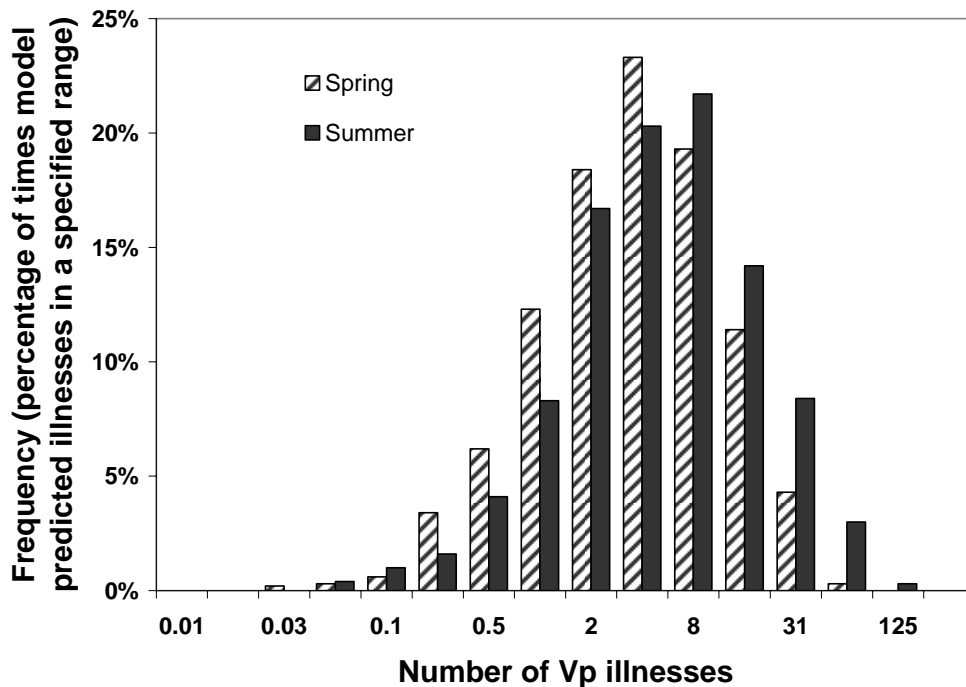


Figure V-2. Uncertainty Distribution of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Mid-Atlantic Harvests

Sensitivity Analysis

Statistical methods were applied to the model results for each region/season combination to identify and quantify the relative importance of both uncertainty and variability factors. These methods were applied to assess the importance of uncertainty and variability factors separately. Sensitivity analysis methods applicable to the context of food safety risk assessment models (Patil and Frey, 2004; Saltelli *et al.*, 2000; Frey *et al.*, 2004) were evaluated and the appropriate methods were selected for the analyses.

In this risk assessment, a distinction was made between model parameters that are uncertain versus those that represent “true” variability. As previously stated, within each of the 1,000 simulations of the model, there are 10,000 iterations which represent individual oyster servings. All values generated within an iteration of the model are variability factors. Uncertainties do not change within an iteration but do differ for each simulation. Two examples are provided below to illustrate the difference in variability and uncertainty as applied in the model.

- **Example 1.** For all regions (except Pacific Northwest Dredged), the time that oysters are unrefrigerated is determined by a random selection of a number between one and the maximum time the boat is on the water. Within each

iteration of a model simulation, a different value is selected for the time the oysters are unrefrigerated.

- Example 2. The model component used to predict *V. parahaemolyticus* growth rate was estimated from growth data in a laboratory culture. The growth rate expected in oysters is less certain because it was only measured at one temperature and was substantially different from that in the laboratory culture. Consequently, a distribution of uncertainty for the relative growth rate in oysters versus laboratory culture was specified with a mean equal to the observed ratio of growth rate at that one temperature. The value sampled from this specified uncertainty distribution is the same for each iteration but a new value within the distribution is selected for each of the 1,000 simulations.

The overall model was structured to separate variability and uncertainty factors to the maximum extent practical. The distinction between these two types of factors was maintained in sensitivity analyses of model simulation output because the principle effect of uncertainty is to shift the mean of the variability distributions of the predicted risk per serving. In contrast, variability factors affect the risk associated with individual servings as a consequence of *V. parahaemolyticus* levels varying from one harvest lot to the next, even when all uncertainty parameters are fixed.

A “segmented” approach was used for this risk assessment in that each of the 24 region/season combinations were simulated and analyzed separately. This approach was adopted as an effective means for specifying the diversity that exist in oyster harvesting practices and climatic conditions among the different regions. However, as a consequence of the segmented approach factors that affect risk have the potential to vary more strongly across different region/season combinations than within each region/season combination. This implies that evaluation of results for any particular region/season combination can not be inferred to apply directly to the aggregate of all 24 region/season categories.

Water temperature is the factor whose importance is most obscured by the segmented modeling approach. Within each region/season combination, the variation and impact of differing levels of water temperature is relatively minor in comparison to that of other model factors. However, this is not true across region/season categories. In fact, the wide variation of water temperature across different regions and seasons was one of the primary reasons for defining the various regions and seasons selected for the model. Across these region/season categories, changes in risk are strongly related to changes in water temperature as shown in Figure V-3.

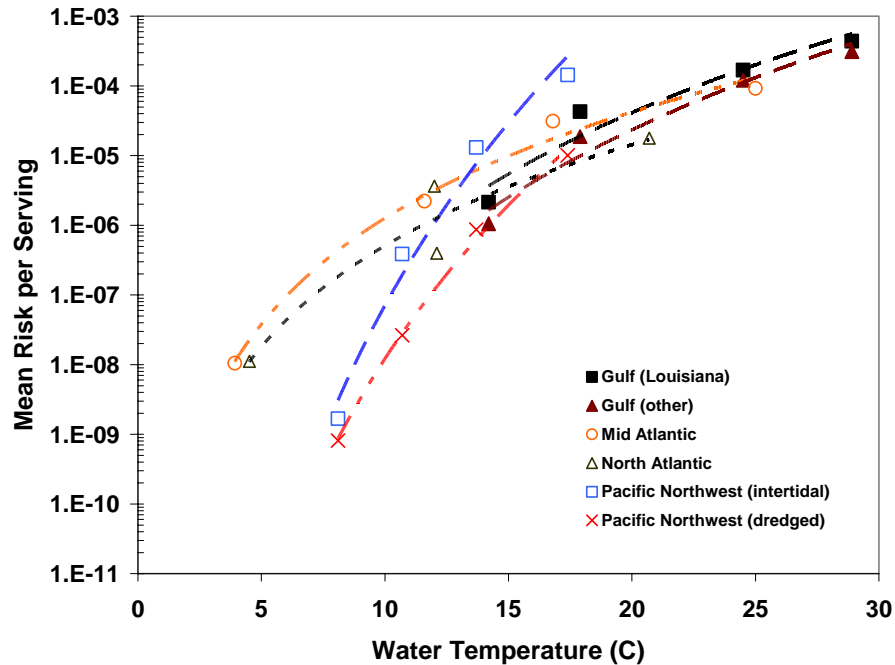


Figure V-3. Influence of Water Temperature on Variation of Mean Risk per Serving for Each Region

Sensitivity Analysis of Variability

A tornado plot is a convenient means of graphically depicting which factors in a model are the most influential. This type of plot is a graph of the correlations between the model output (i.e., risk) and various input factors (e.g., levels of *V. parahaemolyticus* in oysters at harvest). The graph is called a "tornado plot" because of the tornado-like appearance of the graph when factors are arrayed from most influential at the top to least influential at the bottom. It should be noted however, that factors with strong negative correlation are observed at the bottom of the plot, even though they may be more influential than a factor with a moderate positive correlation.

For this risk assessment, Pearson correlation between the model output and input factors was considered an appropriate correlation measure for the tornado plots. Although use of rank correlation is also applicable and potentially more robust than the Pearson correlation, care must be taken in interpretation of results obtained after rank transformation. The influence of factors which influence the output by way of interactions may not be appropriately identified when rank correlation is used (Saltelli and Sobol, 1995).

To ascertain the influence or importance of variability factors, Pearson correlations between the log risk/serving and selected inputs were calculated. Correlation against

risk/serving is not appropriate because it is not normally distributed. Next, a mean correlation was obtained by taking the average over uncertainty samples. The tornado plots for each region/season combination are provided in Appendix 8. Several example graphs are provided below (Figures V-4 to V-7).

Table V-4 provides a summary of the tornado plot analyses of model variability factors. The most influential factor is the level of total *V. parahaemolyticus* in oysters at the time of harvest. It ranks highest for all region/seasons except for the Pacific Northwest winter harvests, where the ratio of pathogenic to total *V. parahaemolyticus* (% pathogenic) in oysters ranks highest. In general, the second most influential factor is the percentage pathogenic *V. parahaemolyticus* in oysters at harvest. Air temperature is another highly influential factor for most regions and seasons. It often ranks as the second most influential factor (see Table V-4 and Appendix 8). This is not surprising because the potential growth of *V. parahaemolyticus* in oysters during the time from harvest to refrigeration is a function of the ambient air temperature at the time of harvest and the length of time oysters are unrefrigerated. *Vibrio parahaemolyticus* will multiply in oysters until adequately chilled.

For the Pacific Northwest (Intertidal) harvest (Figures V-6 and V-7), the influence of oyster temperature and intertidal exposure time were also evaluated. For this region (and method of harvest) higher levels of risk per serving are associated with oysters that have been collected on warm sunny days leading to higher oyster temperatures and more *V. parahaemolyticus* growth during intertidal exposure. The lower rank of importance of the percentage of total *V. parahaemolyticus* that are pathogenic for this region and harvest type may be attributed to the relatively stronger influence of air (and oyster) temperature. The magnitude of the correlation of percentage pathogenic with risk per serving for this region is still comparable with that of the other regions such as the Gulf Coast or Mid-Atlantic. Intertidal exposure time is much less influential than other factors. This is attributable to the relatively narrow range of variation of this factor in comparison to that of other factors.

The other variability factors analyzed have significant effects, but to a lesser extent. In the Gulf Coast (Louisiana) and other “warm” regions, the time-to-refrigeration was generally the third most important influential factor affecting risk of illness. Serving size (number of oysters consumed) was another influential factor; the more oysters an individual consumes, the more likely it is that the person could become ill. Not surprisingly, conditions that foster the growth of *V. parahaemolyticus* within the oyster (length of time oysters are unrefrigerated, time it takes to cool down the oysters, water and air temperature) are all positively associated with the risk of illness. Since the levels of *V. parahaemolyticus* decrease during cold storage, the length of time the oysters are refrigerated is negatively correlated with the risk and that factor points the opposite direction on the tornado plot.

Table V-4. Variability Factors from Tornado Plots for Each Region and Season Combination

Season	Variability Factors in Order of Importance ^a					
	Gulf Coast (Louisiana)	Gulf Coast (Non-Louisiana)	Mid-Atlantic	Northeast Atlantic	Pacific Northwest (Dredged)	Pacific Northwest (Intertidal)
Summer	Log ₁₀ VP % path time unrefrig air temp g consumed cooldown	Log ₁₀ VP % path air temp time unrefrig cooldown g consumed	Log ₁₀ VP % path air temp time unrefrig g consumed cooldown	Log ₁₀ VP % path air temp g consumed time unrefrig cooldown	Log ₁₀ VP % path air temp g consumed time unrefrig cooldown	Log ₁₀ VP Air temp Oyster temp % path g consumed intertidal time cooldown time unrefrig
Fall	Log ₁₀ VP Air temp % path g consumed time unrefrig cooldown	Log ₁₀ VP Air temp % path g consumed time unrefrig cooldown	Log ₁₀ VP Air temp % path g consumed time unrefrig cooldown	Log ₁₀ VP % path air temp g consumed time unrefrig cooldown	Log ₁₀ VP % path air temp g consumed time unrefrig cooldown	Log ₁₀ VP Air temp % path oyster temp g consumed intertidal time cooldown time unrefrig
Winter	Log ₁₀ VP % path air temp g consumed time unrefrig cooldown	Log ₁₀ VP % path air temp g consumed time unrefrig cooldown	Log ₁₀ VP % path g consumed air temp cooldown time unrefrig	Log ₁₀ VP % path g consumed air temp cooldown time unrefrig	% path log ₁₀ VP g consumed air temp cooldown time unrefrig	% path log ₁₀ VP air temp oyster temp g consumed intertidal time cooldown time unrefrig
Spring	Log ₁₀ VP Air temp % path time unrefrig g consumed cooldown	Log ₁₀ VP Air temp %path time unrefrig g consumed cooldown	Log ₁₀ VP Air temp %path g consumed time unrefrig cooldown	Log ₁₀ VP Air temp %path g consumed time unrefrig cooldown	Log ₁₀ VP %path air temp g consumed time unrefrig cooldown	Log ₁₀ VP Air temp Oyster temp g consumed intertidal time cooldown

^aLog₁₀ VP = log₁₀ *V. parahaemolyticus* in oysters at harvest; % path= ratio of pathogenic to total *V. parahaemolyticus* in oysters at harvest; time unrefrig= time between harvest and refrigeration of oysters; air temp= ambient air temperature (used to determine oyster temperature after harvest); g consumed= grams of oysters consumed per serving; cooldown= time required for oyster to cool to no-growth temperature for *V. parahaemolyticus*; oyster temp= temperature of oysters during intertidal exposure; intertidal time= duration of time that intertidally-collected oysters are exposed prior to collection.

Note: Negatively correlated factors are not included in this table. For the actual tornado plots for each region/season combination see Appendix 8.

V. RISK CHARACTERIZATION

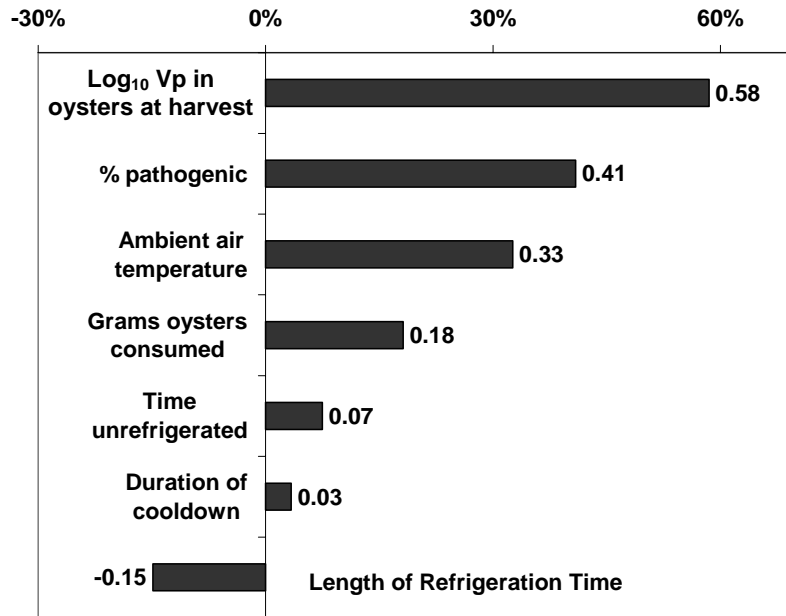


Figure V-4. Tornado Plot of Influential Variability Factors on *Vibrio parahaemolyticus* (Vp) Illness per Serving of Raw Oysters for the Gulf Coast (Louisiana) Winter Harvest

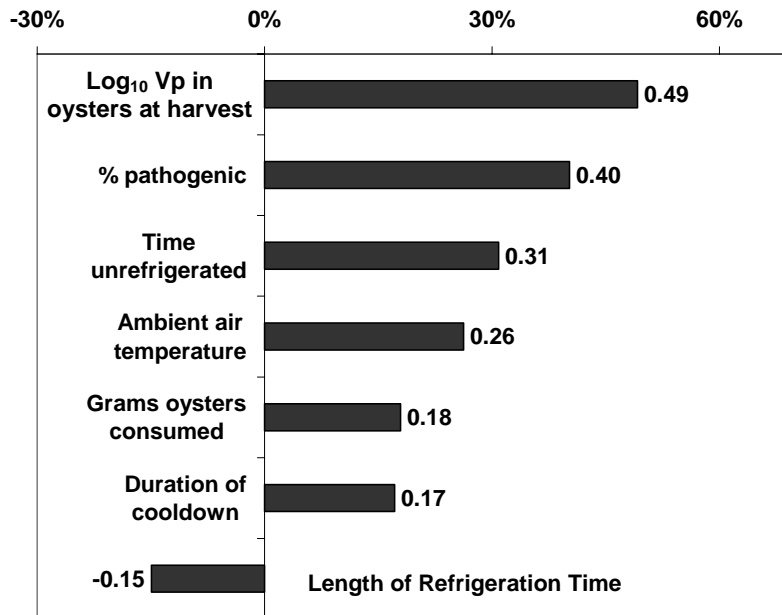


Figure V-5. Tornado Plot of Influential Variability Factors of *Vibrio parahaemolyticus* (Vp) Illness per Serving of Raw Oysters for the Gulf Coast (Louisiana) Summer Harvest

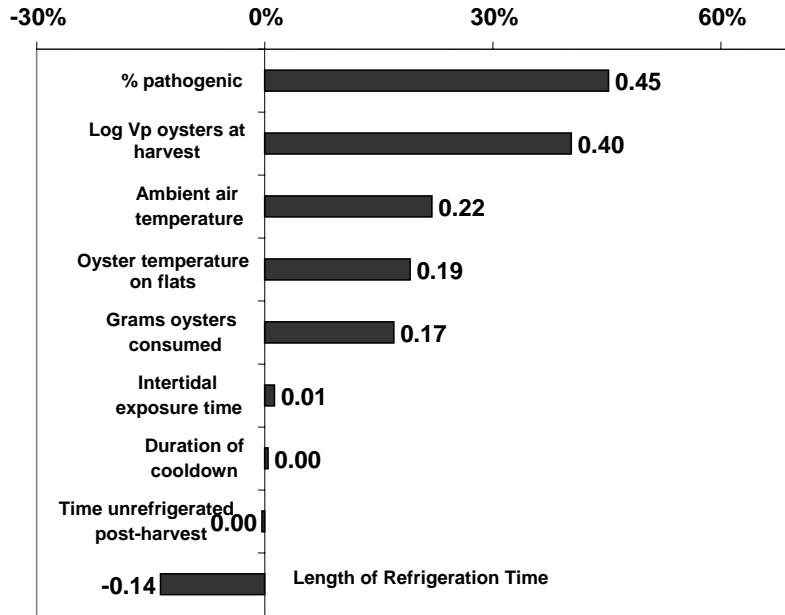


Figure V-6. Tornado Plot of Influential Variability Factors on *Vibrio parahaemolyticus* (Vp) Illness per Serving of Raw Oysters for the Pacific Northwest Coast (Intertidal) Spring Harvest

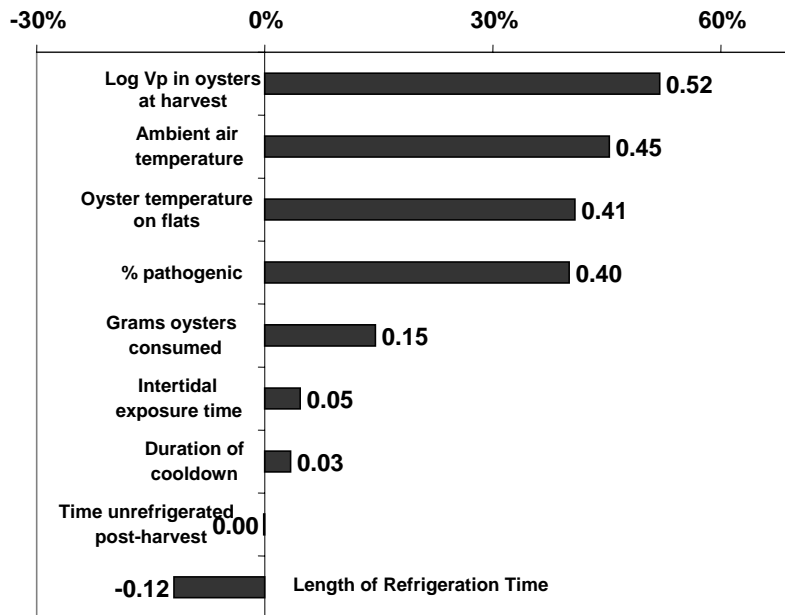


Figure V-7. Tornado Plot of Influential Variability Factors on *Vibrio parahaemolyticus* (Vp) Illness per Serving of Raw Oysters for the Pacific Northwest Coast (Intertidal) Winter Harvest

A potential deficiency associated with Tornado plots (i.e., pairwise correlations) as a sensitivity measure is that the importance of the factors is evaluated one at a time. Correlation between input factors themselves can confound the interpretation of importance in a Tornado plot. Therefore, to confirm and substantiate the results, a variance-based method of sensitivity analysis was also applied to two selected region/season combinations. The results of this analysis for the Gulf Coast (Louisiana)/Summer and Pacific Northwest (Intertidal)/Summer region/season combinations is given in Appendix 6. The results are generally consistent with the ranking of importance shown in Table V-4.

The correlation between predicted risk per serving and total *V. parahaemolyticus* density at the time of harvest for the Gulf Coast (Louisiana) summer harvest is shown in Figure V-8. While the correlation is high, indicating that *V. parahaemolyticus* levels at the time of harvest are an important indicator of risk, there is substantial variation (of risk) at any particular harvest level due to the influence of other factors. This illustrates that the usefulness of any indicator as a means to mitigate risk depends on the extent to which the factor can be controlled and this should be considered when assessing the value of identifying a factor with high influence. Additionally, it should be noted that this relatively high degree of importance in regard to indication of risk per serving does not necessarily equate with the most practical or economical avenues of mitigation. See “Chapter VI: What-If Scenarios” for information on the impact of various mitigation strategies on the predicted risk.

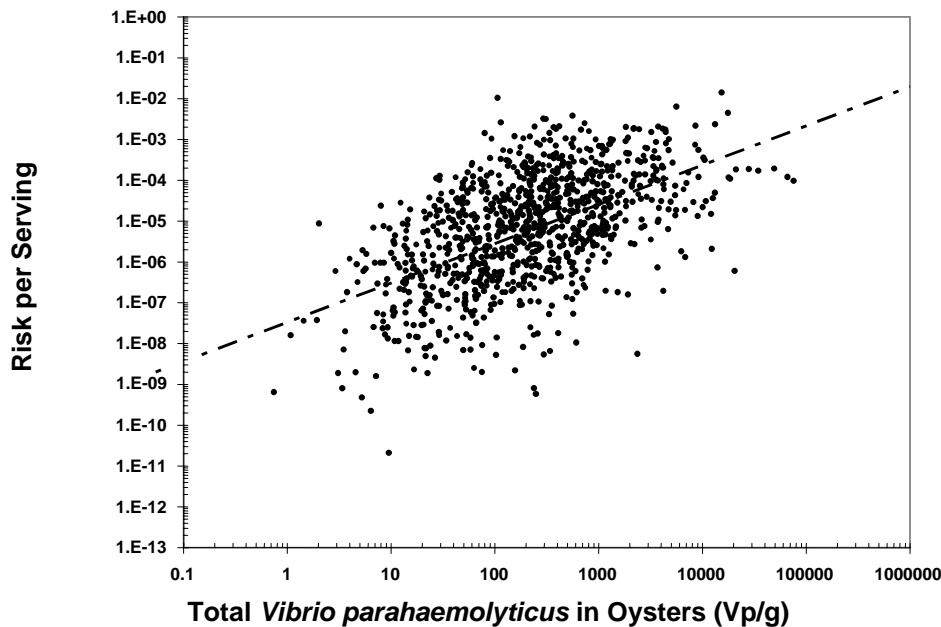


Figure V-8. Correlation of Risk per Serving and Total *Vibrio parahaemolyticus* in Oysters at Harvest for the Gulf Coast (Louisiana) Summer

[Individual simulation results are represented by a single dot. The dotted line is the least squares regression line fit to the simulation output.]

Sensitivity Analysis of Uncertainty Factors

Simulations were performed to examine the influence of uncertainty factors on the predicted risk estimates. Five uncertainty factors were evaluated:

- (1) the growth rate of *V. parahaemolyticus* in oysters,
- (2) the ratio of number of pathogenic to total *V. parahaemolyticus* in oysters,
- (3) the year-to-year variation of water temperature distributions,
- (4) the prediction of total *V. parahaemolyticus* (based on water temperature), and
- (5) the Beta-Poisson dose-response model.

One measure of sensitivity (or importance) of these factors is the reduction in the variance of the uncertainty distribution of the mean risk per serving when each factor is held fixed to its nominal or mean level. If a factor has a substantial contribution to the overall uncertainty of the risk (i.e., is important), then there is a large reduction in the variance of the uncertainty distribution when the factor is held at a fixed level. This is most effectively summarized as the percentage reduction in the variance relative to that of the baseline uncertainty distribution of mean risk per serving (Saltelli *et al.*, 2000). Thus, the importance (i.e., the percentage reduction in variance) is calculated according to the following formula.

$$\text{importance of the } i^{\text{th}} \text{ factor} = \frac{\text{Var}(\text{risk}) - \text{Var}(\text{risk} \mid \text{no variation of the } i^{\text{th}} \text{ factor})}{\text{Var}(\text{risk})}$$

where $\text{Var}(\text{risk})$ denotes the unconditional variance of the uncertainty distribution of mean risk per serving and $\text{Var}(\text{risk} \mid \text{no variation of the } i^{\text{th}} \text{ factor})$ denotes the conditional variance when one factor (the i^{th}) is fixed.

As an example, this measure of importance was applied to rank the importance of the five selected uncertainty factors on predictions for the Gulf Coast (Louisiana)/Summer harvest. This region/season combination was selected because it represents the largest number of predicted illnesses. To estimate the conditional variances of the uncertainty distributions of mean risk per serving, 1,000 Monte Carlo simulations were performed for each of five model input factors. In each of these simulations, one of the five factors was fixed and the others were allowed to vary (as in the baseline model). The unconditional variance was also obtained based on a set of 1,000 Monte Carlo simulations in which all five factors were allowed to vary (as in the baseline model). The results of these simulations and the associated estimates of importance are summarized in Table V-5.

Table V-5. Importance of Selected Uncertainty Factors Based on Reduction in the Variance of the Uncertainty Distribution of the Mean Risk per Serving for the Gulf Coast (Louisiana) Summer Harvest

Uncertainty Factor	Conditional Variance ^a	Importance ^b
Dose-response model	5.23x10 ⁻⁸	75.4%
Percentage pathogenic	1.77x10 ⁻⁷	16.5%
Growth rate in oysters	1.83x10 ⁻⁷	13.8%
Relationship of total <i>V. parahaemolyticus</i> levels and water temperature	1.89x10 ⁻⁷	11.1%
Year-to-year water temperature variation	2.08x10 ⁻⁷	2.0%

^a Conditional variance refers to the variance of the uncertainty distribution of the mean risk per serving conditional on the specified uncertainty factors being fixed to nominal (mean) values, one at a time.

^b Importance is based on a comparison to an unconditional variance of 2.12x10⁻⁷ for the distribution of mean risk per serving from a simulation in which all uncertainty factors vary.

As shown in Table V-5, of the five uncertainty factors evaluated, the Beta-Poisson Dose-Response model ranks as the most important factor and has a substantial contribution (approximately 75% importance) to the uncertainty in the predicted mean risk per serving. The relative abundance of pathogenic strains in oysters and the growth rate of *V. parahaemolyticus* in oysters also contribute to the uncertainty in the results but to lesser degrees (i.e., approximately 14% and 16% importance each). The year-to-year variation of water temperature distributions ranks as the least important contributor to the uncertainty in the model results. In particular, the year-to-year variation in water temperature is extremely low. This reflects the fact that no appreciable year-to-year differences in Gulf Coast/Summer region water temperatures were evident in the NBDC data. This does not, however, necessarily imply that year-to-year variations of water temperature are equally inconsequential during other Gulf Coast seasons or in other regions. The importance of year-to-year variations of water temperature for other region/season combinations may vary somewhat, particularly for seasons during which the weather is more variable (e.g., spring and fall).

With respect to influence of dose-response uncertainty on the uncertainty of predicted mean risk per serving, it is worth noting, based on the model specification, that this is a reflection of parameter uncertainty of the Beta-Poisson model. Important sources of uncertainty that were not included in this assessment include those associated with the extrapolation of observed response at high doses to predicted response at low doses (i.e., model selection uncertainty). However, because a primary goal of the risk assessment was to evaluate the relative impact of different region/season combinations and to develop information on the impact of different intervention strategies, uncertainties associated with the dose-response model do not adversely impact the usefulness of the risk assessment.

An alternative method of importance assessment for these uncertainty parameters is to estimate the relative proportion of the variance of the uncertainty distribution of mean

risk per serving explained by each uncertainty parameter in a regression-based approach (i.e., a “variance reduction” measure based on an approximating regression fit of model simulation output). The results of such an analysis (see Appendix 6) were found to be generally consistent with the ranking of importance as shown in Table V-5.

Model Validation

The model was evaluated by comparing model output predictions to similar data that were not used in the model. The exposure predictions were validated using data on the levels of total *V. parahaemolyticus* in oysters. Two evaluations were performed, one based on the ISSC/FDA retail survey and the other based on data collected by the Washington State Department of Health. These data were compared to model predictions to assess the appropriateness of the model with respect to the Harvest and Post-Harvest Modules.

Validation of the overall risk estimates requires detailed data on the number of illnesses associated with consumption of oysters harvested from the various regions and seasons. Such data are very limited and are, to an unknown degree, confounded. An attempt to evaluate the model in this manner was undertaken using data reported to the CDC on *V. parahaemolyticus* infections. These data were compared to the model’s seasonal and regional predictions of illnesses. The number of *V. parahaemolyticus* cases predicted by the model was also compared qualitatively with preliminary data on the number of *V. parahaemolyticus* cases observed in the different provinces of Canada.

Validation of Predicted Levels of *Vibrio parahaemolyticus* in Oysters at Time of Consumption

A collaborative survey of *Vibrio parahaemolyticus* densities in oysters at the retail level (i.e., restaurants, oyster bars, wholesalers) was conducted by the ISSC and FDA in 1998 and 1999 (FDA/ISSC, 2000; Cook *et al.*, 2002a). Oyster samples were collected from selected states in the Pacific, Gulf Coast, Mid-Atlantic, and Northeast Atlantic regions. The samples were enumerated by an MPN method (Cook *et al.*, 2002a). A relatively high proportion of the non-Gulf Coast samples had non-detectable levels. To adjust for the varying proportion of non-detectable *V. parahaemolyticus* across the different regions and seasons, estimated means were obtained by fitting a Tobit regression to the data with different harvest region and season combinations as a predictor variable. The variance about the group means was assumed to be the same across different regions and seasons, since no data were available to assume otherwise. The limit of detection varied somewhat from sample to sample but was generally 0.18 MPN/g.

Comparison of estimates of mean and standard deviation of \log_{10} total *V. parahaemolyticus* densities from the ISSC/FDA study versus model predictions are shown in Figures V-9 through V-12 for the Gulf Coast (Louisiana), Gulf Coast (non-Louisiana), Mid-Atlantic, and Pacific Northwest (dredged and intertidal) regions. The data for the Northeast Atlantic region were not included in the analysis because the data set contained only few samples with detectable levels of *V. parahaemolyticus*. The estimates of the means based on ISSC/FDA data compare well with those predicted by

the model. In particular, model predictions of mean \log_{10} densities are in good agreement with ISSC/FDA data for all regions during the summer when the risk of illness is highest.

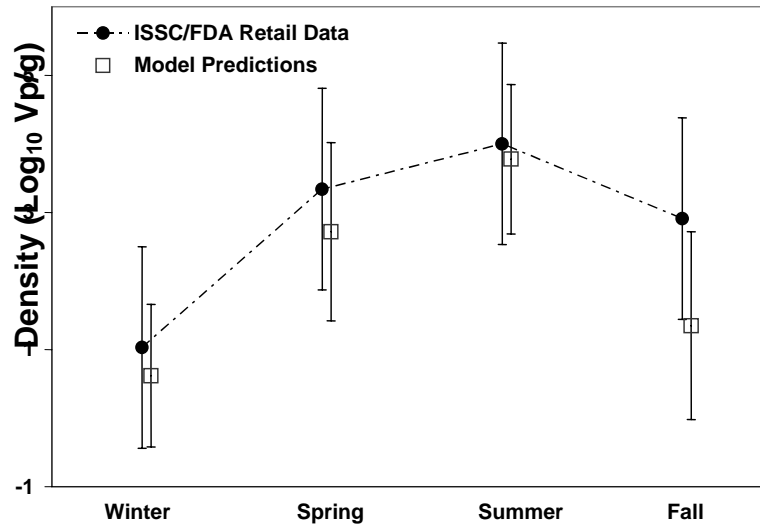


Figure V-9. Observed \log_{10} Density of Total *Vibrio parahaemolyticus* at Retail (Cook et al., 2002a) Compared to Model Predictions for the Gulf Coast (Louisiana)

Harvest [The error bars indicate one standard deviation above and below either the model predictions (square boxes) or observed values (filled circles).]

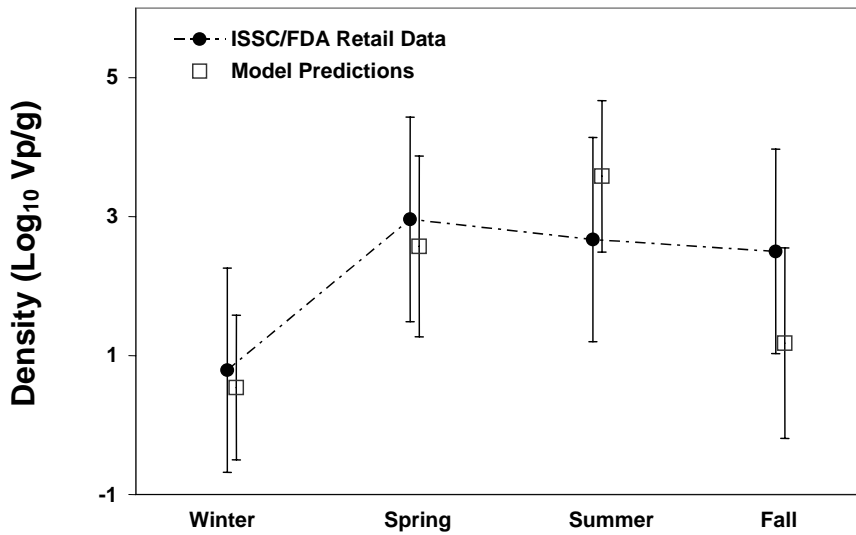


Figure V-10. Observed \log_{10} Density of Total *Vibrio parahaemolyticus* at Retail (Cook et al., 2002a) Compared to Model Predictions for the Gulf Coast (non-Louisiana)

Harvest [The error bars indicate one standard deviation above and below either the model predictions (square boxes) or observed values (filled circles).]

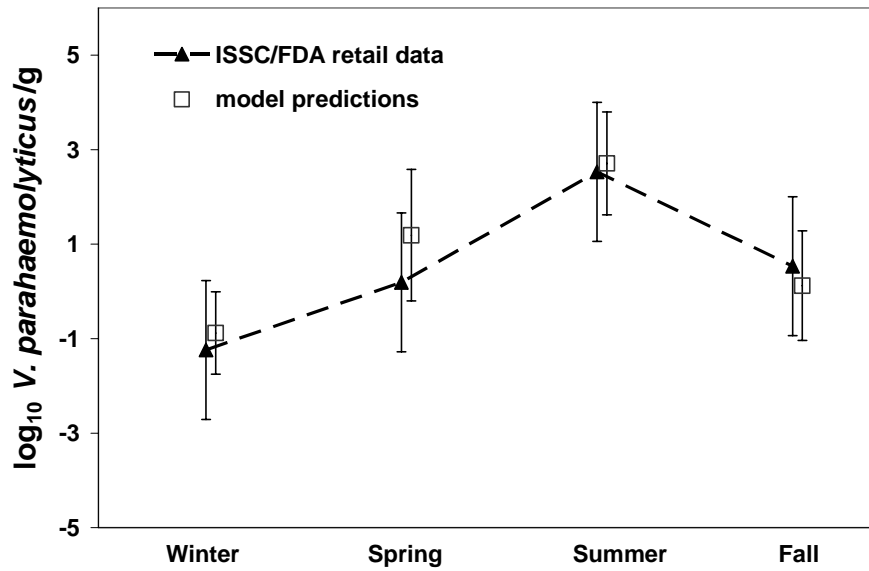


Figure V-11. Observed log₁₀ Density of Total *Vibrio parahaemolyticus* at Retail (Cook *et al.*, 2002a) Compared to Model Predictions for the Mid-Atlantic Coast Harvest [The error bars indicate one standard deviation above and below either the model predictions (square boxes) or observed values (filled triangles).]

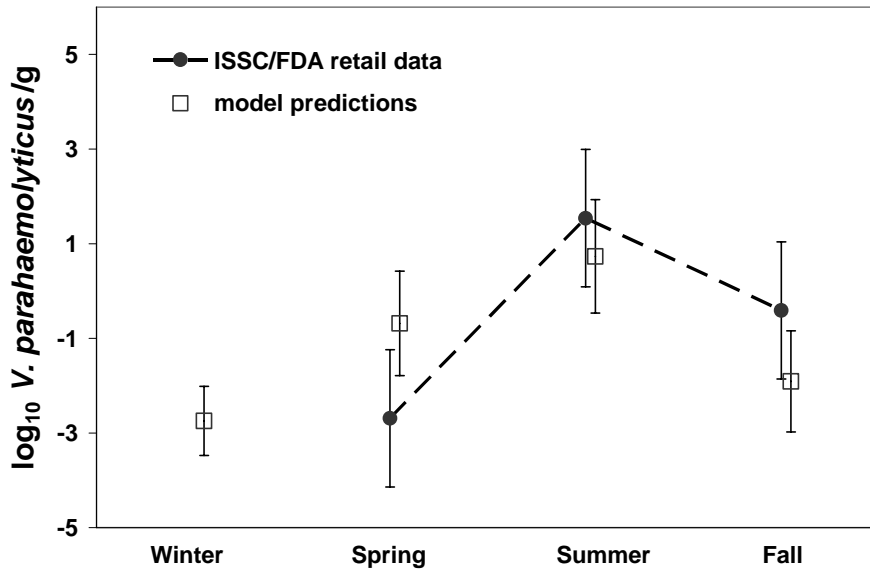


Figure V-12. Observed log₁₀ Density of Total *Vibrio parahaemolyticus* at Retail (Cook *et al.*, 2002a) Compared to Model Predictions for the Pacific Northwest (Dredged and Intertidal) Region [The error bars indicate one standard deviation above and below either the model predictions (square boxes) or observed values (filled circles).]

It should be noted that although the model predictions of the mean \log_{10} densities vary from year to year based on environmental conditions; the ISSC/FDA data were collected from a single year. Therefore, differences in the model predictions and the ISSC/FDA estimates would be expected. For example, for the Gulf Coast (Figures V-9 and V-10), model predictions of mean \log_{10} densities in the fall are somewhat lower than those obtained by the ISSC/FDA study. With regard to this discrepancy, water temperature measurements indicate that the fall season of 1998, corresponding to the time of ISSC/FDA sampling, was somewhat warmer than usual. Warmer temperatures allow more *V. parahaemolyticus* growth in oysters. The model was run to account for the higher temperatures for that year. Based on water temperature data from Weeks Bay, AL (NOAA, 2001), the mean daily water temperature in the fall of 1998 in the Gulf Coast region (Louisiana and non-Louisiana) was calculated to be 23 °C (e.g., approximately 5 °C warmer than typical fall mean daily water temperature of 17.8 °C). As shown in Figure V-13, using the warmer water temperature data from 1998, the model predicts higher numbers of *V. parahaemolyticus* for the fall harvest and the values are similar to the ISSC/FDA retail study observed data. Therefore, this analysis, using a specific year's data, supports the validation and predictive capabilities of the model.

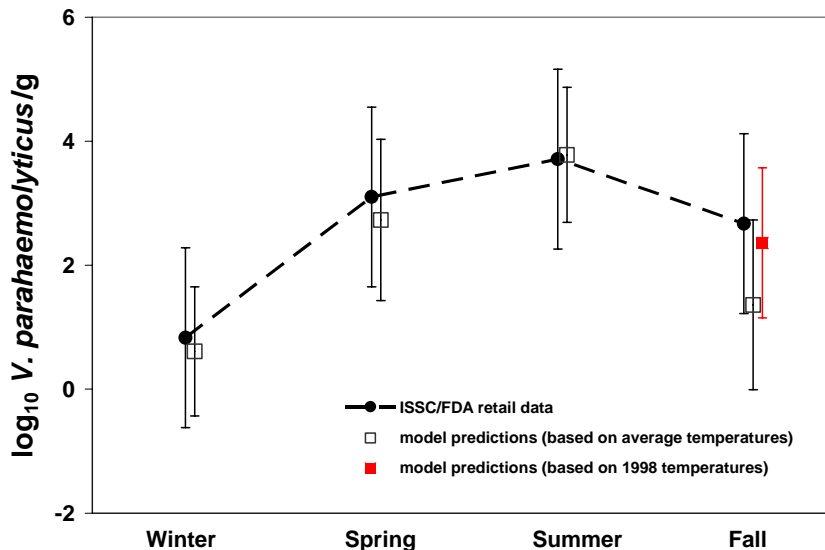


Figure V-13. Observed \log_{10} Density of Total *Vibrio parahaemolyticus* at Retail (Cook *et al.*, 2002a) Compared to Model Predictions for the Gulf Coast (Louisiana and non-Louisiana) Based on 1998 Fall Temperature [The error bars indicate one standard deviation above and below either the model predictions using average temperatures (open square boxes) model prediction using only 1998 temperature data (filled square box) or observed values (filled circles).]

An additional validation was conducted for the Pacific Northwest (Intertidal) region using data collected from the intertidal areas of Hood Canal and South Puget Sound (Washington State Department of Health, 2001). This subset of the Washington State monitoring data was not used in the model. Comparison of the model predictions of intertidal “at-harvest” levels with the observed levels is shown in Figure V-14. The model-predicted mean \log_{10} densities are similar to the regression-based estimate of the seasonal means. The results of a similar survey of *V. parahaemolyticus* levels in oysters harvested in the Vancouver area indicated a similar pattern as that observed in Washington State and predicted by the model (Buenaventura *et al.*, 2004; Bannerjee and Farber, 2005).

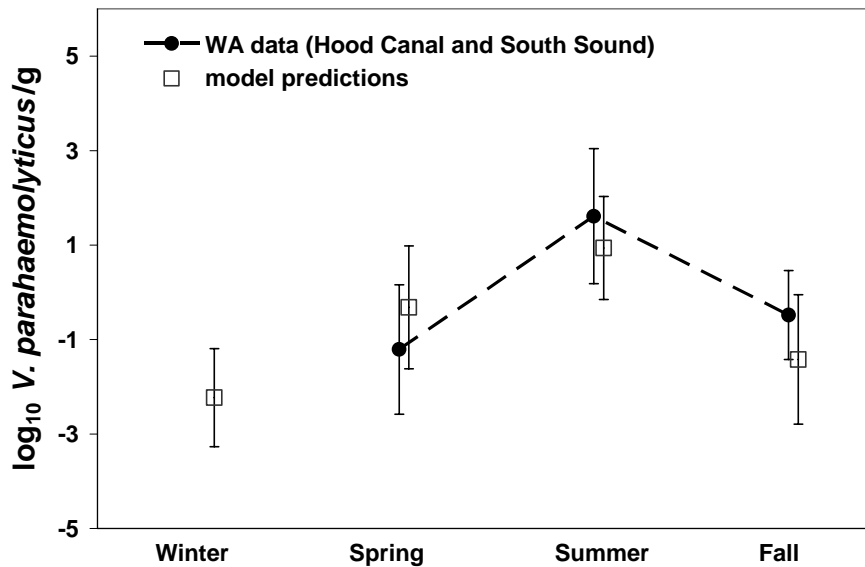


Figure V-14. Observed \log_{10} Density of Total *Vibrio parahaemolyticus* for the Pacific Northwest (Intertidal) Region (Washington State Department of Health, 2001) Compared to Model Predictions

[The error bars indicate one standard deviation above and below either the model predictions (square boxes) or observed values (filled circles).]

Based on the close agreement between model-predicted *V. parahaemolyticus* densities and observed densities at retail, the exposure assessment portion of the model is considered to be validated.

Comparison of Model-Predicted *Vibrio parahaemolyticus* Illnesses and Surveillance Data

Surveillance data collected by CDC were compared to the model predictions in an attempt to validate the risk characterization portion of the model (also see Appendix 9). The comparison took into account the intrinsic difference in what the two systems (i.e., analysis of surveillance data versus model predictions) measure. The risk assessment model predicts illness associated with oysters **harvested** from a given region. Surveillance data, however, provide an estimate of illnesses **reported** within a region, regardless of the source of the oyster.

For reporting of a *V. parahaemolyticus* illness to appear in the CDC database, the following chain of events must occur:

- a patient must seek medical attention;
- a physician must order analysis of a clinical specimen;
- the clinical laboratory must have and use the test materials and procedures specific to *V. parahaemolyticus*;
- the results of a positive clinical sample must be reported to the State Epidemiologist; and
- the State Epidemiologist must report the positive finding to CDC.

There are several potential confounding factors with the CDC surveillance data which present difficulties in using the surveillance data to validate the model predictions for harvest regions. First of all, CDC recognizes that there may be under diagnosing and underreporting of *V. parahaemolyticus* cases on a national basis. Therefore, the CDC includes an uncertainty factor of 20; the estimated total number of cases is equal to 20 times the reported cases (Mead *et al.*, 1999). However, it is unknown the extent of possible differences in reporting efficiencies from state-to-state. Secondly, in only a small fraction (~10%) of the reported cases was it possible to definitively determine the source of the oysters that caused illness and attribute it to a particular region. There are also state-to-state differences in case follow up (traceback) procedures. These uncertainties associated with the surveillance data complicate the direct use of available CDC data to validate the regional model predictions of illness.

The model predictions and the surveillance data estimates indicate similar trends in seasonal illnesses, with higher numbers associated with warmer months, fewer illnesses in cooler months, and the lowest number of illnesses in the winter. However, the model predictions of the number of *V. parahaemolyticus* illnesses in the winter were relatively low compared with the number of infections estimated from reported cases by the CDC. It is possible that the divergence between the CDC surveillance data and the predicted values reflect the existence of additional factors related to post-retail handling or consumption patterns of raw oysters during the winter months that have not been previously recognized and thus not incorporated into the model. Any consideration of such factors would require more sophisticated epidemiological investigations than those that are currently being performed. Alternatively, the differential could reflect the substantial uncertainty associated with the estimates derived from surveillance data.

As described above, the exposure assessment portion of the model is validated. However, the confounding factors and uncertainty associated with the surveillance data precluded validation of the risk characterization portion of the assessment. It is important to note that regardless of where the illnesses are reported and where the oysters were harvested, reducing exposure to *Vibrio parahaemolyticus* reduces the risk of illness. Various mitigations and control measures were evaluated and the effectiveness for different regions and seasons were determined as described in the next chapter, “What-If Scenarios.” The validation of the exposure assessment provides a high degree of confidence that the impact of the various mitigation strategies considered would provide the risk reduction profile indicated in the “what-if” scenarios.

VI. WHAT-IF SCENARIOS

One of the benefits of performing a quantitative product pathway risk assessment is that the model can be used to estimate the likely impact of intervention strategies on the predicted number of illnesses. The impact of different harvesting methods, season (i.e., water and air temperatures), time until refrigeration, and length of storage before consumption were included in the baseline model. By changing one or more of the input parameters and measuring the resulting change in the model outputs, the likely impact of new or different processing procedures or regulatory actions can be evaluated. These changes to the baseline model are commonly referred to as conducting “what-if” scenarios.

The what-if scenarios evaluated include the following:

- reducing levels of *V. parahaemolyticus* levels in oysters (representing various post-harvest mitigation controls)
- reducing time-to-refrigeration
- re-submersion of intertidally harvested oysters
- sample-based control plans

Mitigation Strategies

Strategies to reduce levels of *V. parahaemolyticus* in oysters after harvest include those associated with post-harvest treatments including immediate refrigeration, freezing, mild heating, and ultra high pressure. These procedures have varying degrees of effectiveness in reducing levels of *V. parahaemolyticus* in oysters. Potential mitigation strategies are summarized in Table VI-1 and described in greater detail below.

Table VI-1. Summary of Mitigation Strategies and Typical Effectiveness in Reducing Levels of *Vibrio parahaemolyticus* in Oysters

Mitigation	Description	Log ₁₀ Reduction ^a
Irradiation	Exposure of oysters to up to 3 kGy Cobalt-60 gamma radiation	6
Ultra high pressure	Treatment of oysters with high pressure such as 345 MPa for 30 seconds	6
Hot water/cold shock	Oysters are heated (hot water pasteurization) to 50°C and held for 10 minutes followed by cold shock	5
Mild heat	Oysters are heated to 50°C and held for 5 minutes	≥4.5
Freezing	Rapid freezing and frozen storage (35 days at -20°C)	2
Immediate refrigeration	Placing oysters under refrigeration immediately after removal from the water at harvest	≤1
Relaying	Transfer of oysters to “clean” growing areas for various lengths of time	<1
Depuration	Transfer of oysters (various lengths of time) to tanks containing seawater treated with UV light to inactivate bacteria.	0 to 2

^a These log reductions are based on studies described in this chapter and are specific to *Vibrio parahaemolyticus* but may not necessarily apply to 03:K6. Individual processors would need to conduct validation studies for their particular processing to measure log reduction under those specific conditions.

Irradiation. Gamma irradiation was investigated as an alternative post harvest treatment (PHT) for raw shell stock oysters (Andrews *et al.*, 2002). Live oysters, with naturally incurred and artificially inoculated *Vibrios*, were exposed to 0-3 kGy dose Cobalt-60 gamma radiation. *Vibrio parahaemolyticus* TX03:K6 required 1.0 kGy to reduce the level of the microorganism in oysters to non detectable levels (a 6-log₁₀ reduction). *Vibrio vulnificus* required 0.75 kGy to achieve a similar reduction. Sensory quality was maintained with irradiation exposure up to 1.5 kGy. Higher exposure levels affected the mortality of the oyster.

Hydrostatic Pressure. Inactivation of pathogenic microorganisms by high hydrostatic pressure was first demonstrated by Hite (1899). High hydrostatic pressure has been shown to be lethal to *V. parahaemolyticus* when suspended in various liquid media (Styles *et al.*, 1991; Berlin *et al.*, 1999). Styles *et al.* (1991) reported D-values of 5.1 min and 4.0 min for *V. parahaemolyticus* cells treated with 170 MPa at 23 °C (73.4 °F) in PBS and clam juice, respectively. Berlin *et al.* (1999) treated various pathogenic *Vibrio* species (approximately 10⁷ cfu/g) including *V. parahaemolyticus* with 200 to 300 MPa at 25 °C (77 °F) in artificial seawater and reported that all strains tested were below detectable levels after 15 minutes at 250 MPa and 5 minutes at 300 MPa. A similar response was observed with oyster homogenates. Viable but non-culturable (VBNC) *V. parahaemolyticus* cells appeared to be more resistant than culturable *V. parahaemolyticus* but these differences were not statistically significant. At least a 5 to 6-log₁₀ decrease in

the level of *V. parahaemolyticus* in oysters was observed by Calik *et al.* (2002) depending on the time and pressure applied to oysters. After treatment for 30 seconds at 345 MPa, there was a 6- \log_{10} reduction in the level of *V. parahaemolyticus* resulting in <10 CFU/ml. After 10 min at 240 MPa, the levels in the oysters ranged from <10 cfu/ml to ~30 cfu/ml (Calik *et al.*, 2002). *Vibrio parahaemolyticus* strains vary in their resistance to high pressure; with serotype O3:K6 strains being more resistant than other pathogenic strains (Cook, 2003). For serotype O3:K6, the average reduction was approximately 6- \log_{10} after 5 minutes at 250 MPa in PBS with a range of 5- \log_{10} to >9.6- \log_{10} . For other (non-O3:K6) pathogenic strains, the average \log_{10} reduction under the same conditions was ~12- \log_{10} reduction with a range of 9.6- \log_{10} to >15- \log_{10} .

Hot Water Pasteurization Followed by Cold Shock. The use of hot water pasteurization followed by cold shock has been reported to be effective in eliminating environmental strains of *V. vulnificus* and *V. parahaemolyticus* from naturally and artificially infected raw oysters (Andrews *et al.*, 2000). More recently this hot water/cold shock process was performed on *V. parahaemolyticus* O3:K6 (Andrews *et al.*, 2003). The investigators found that a 5- \log_{10} reduction in the levels of environmental strains was achieved by heating oysters until an internal temperature of 50 °C had been reached and then holding them at that temperature for 10 minutes. The total process time, including the “come-up” time, was 18 minutes. The oysters had to be held at 50 °C for 12 minutes, which resulted in a total treatment time of 22 minutes, to achieve similar reductions with O3:K6 strains (Andrews *et al.*, 2003).

Mild Heat Treatment. Cook and Ruple (1992) observed a 6- \log_{10} reduction of *V. vulnificus* levels when shucked oysters were heated to an internal temperature of 50 °C (122 °F) for 5 minutes. *Vibrio parahaemolyticus* and *V. vulnificus* have been reported to have similar sensitivity to heat (Cook, 1999; Cook, 2002c). Other studies have shown that a 4.5 to 6- \log_{10} (1,000,000-fold) reduction of *V. parahaemolyticus* densities could be expected by treating shucked oysters for 5 minutes at 50 °C (122° F) (Cook, 1999; Cook, 2002c). However, these studies observed that there is substantial variability in heat resistance among different strains. For example, when strains of serotype O3:K6 in phosphate buffered saline solution (PBS) were subjected to a mild heat treatment, there was a ~2- \log_{10} reduction. However, when non O3:K6 pathogenic strains were treated similarly a much greater reduction (~6- \log_{10}) was observed (Cook, 2002c).

Freezing. A two-phase inactivation occurs when *V. parahaemolyticus* are frozen; the effect of an initial cold shock followed by further declines during frozen storage conditions (Johnson and Liston, 1973; Cook, 1999). Estimates of the effect of cold shock and frozen storage conditions were determined by performing a regression analysis on data reported by Johnson and Liston (1973). Based on such an analysis, freezing combined with frozen storage for 30 days at -30 °C (-22 °F) and -15 °C (5 °F) is projected to result in a 1.2 and 1.6- \log_{10} reduction of *V. parahaemolyticus* numbers in oysters, respectively. A similar decline (2 to 3- \log_{10}) of *V. parahaemolyticus* (natural population and dosed with pathogenic O3:K6 serotype) was observed in oysters frozen 35 days at -20 °C (-4 °F) (Cook, 1999). In this study, oysters with high natural levels of TDH-negative *V. parahaemolyticus* were dosed with high levels of TDH+ *V.*

parahaemolyticus (O3:K6) and then frozen. Based on these studies, freezing combined with frozen storage for 30 days would be expected to produce approximately a 2- \log_{10} reduction of pathogenic *V. parahaemolyticus*. Both pathogenic strains (TDH-positive) and non-pathogenic (TDH-negative) *V. parahaemolyticus* respond similarly to freezing (Cook, 1999).

Immediate refrigeration. Gooch *et al.* (2002) found that the levels of *V. parahaemolyticus* in oysters increase with the length of time oysters are left unrefrigerated (26 °C) after harvest. That is, the levels can increase at least 50-fold in the warmer months when left at ambient temperatures for 10 h after harvest. Levels can in fact approach 10^5 to 10^7 viable cells (Cook and Ruple, 1989). However, since the levels of *V. parahaemolyticus* in freshly harvested oysters are generally low and growth does not occur at or below 10 °C, cooling oysters to that temperature soon after harvest will reduce any potential for bacterial growth. Furthermore, once the oysters are refrigerated, the levels decrease after prolonged refrigeration (six-fold after 14 days) (Gooch *et al.*, 2002). A reduction in the extent of growth of up to 50-fold in *V. parahaemolyticus* densities could be achieved by immediate cooling depending on the initial *V. parahaemolyticus* levels, ambient air temperature and time-to-refrigeration (Cook and Ruple, 1989; Gooch *et al.*, 2002). The extent of reduction of *V. parahaemolyticus* in oysters by immediate refrigeration is variable and approximately 1- \log_{10} reduction. Immediate cooling would involve icing or otherwise refrigerating oyster shellstock immediately upon harvest.

Relaying. Relaying is the process by which shellfish are cleansed by transferring them to “clean” shellfish growing areas. It has been used most commonly with shellfish harvested from water having marginal bacteriological quality. There is little information available on this approach in relation to reducing the levels of *V. parahaemolyticus*. Relaying is not likely to have a significant impact since *V. parahaemolyticus* is ubiquitous in estuarine environments. Son and Fleet (1980) demonstrated a decrease from 18 *V. parahaemolyticus*/g to < 5 *V. parahaemolyticus*/g in relayed oysters after 6 days.

Depuration. In the United States, depuration is conducted exclusively with UV light disinfection (Richards, 1988). There is a broad spectrum of conditions under which shellfish are depurated. Optimal times, temperatures and salinities for effective depuration vary among shellfish species. Depuration has been generally reported to have no significant effect on decreasing the level of *Vibrio* spp. in naturally infected oysters or clams, and these microbes may even multiply in depurating shellfish, tank water, and plumbing systems (Eyles and Davey, 1984; Greenberg and Duboise, 1981). However, a 1- \log_{10} reduction of *V. parahaemolyticus* was observed in the hardshell clam, *Mercinaria mercinaria*, after 72 h of depuration at room temperature (Greenberg and Duboise, 1981), and >2- \log_{10} reduction at 15 °C (59 °F) (Greenberg *et al.*, 1982). Son and Fleet (1980) observed a 5- \log_{10} reduction in lab-infected oysters (from 9×10^7 to 8×10^2 within 72 h).

Mitigations Scenarios

Reducing Levels of *Vibrio parahaemolyticus* in Oysters

The impact of post-harvest mitigations that reduce levels of pathogenic *V. parahaemolyticus* in oysters was evaluated. The reduction levels, representing the range of potential mitigation controls, were as follows.

- approximately 1- \log_{10} reduction (e.g., immediate refrigeration)
- 2- \log_{10} reduction (e.g., freezing)
- 4.5- \log_{10} reduction (e.g., mild heat treatment, ultra high pressure or irradiation).

As shown in Figure VI-1, these mitigations would be implemented post-harvest and at different steps in the sequence of events occurring from harvest to retail. For example, immediate refrigeration would occur on the boat, immediately after harvest and freezing would occur prior to storage.

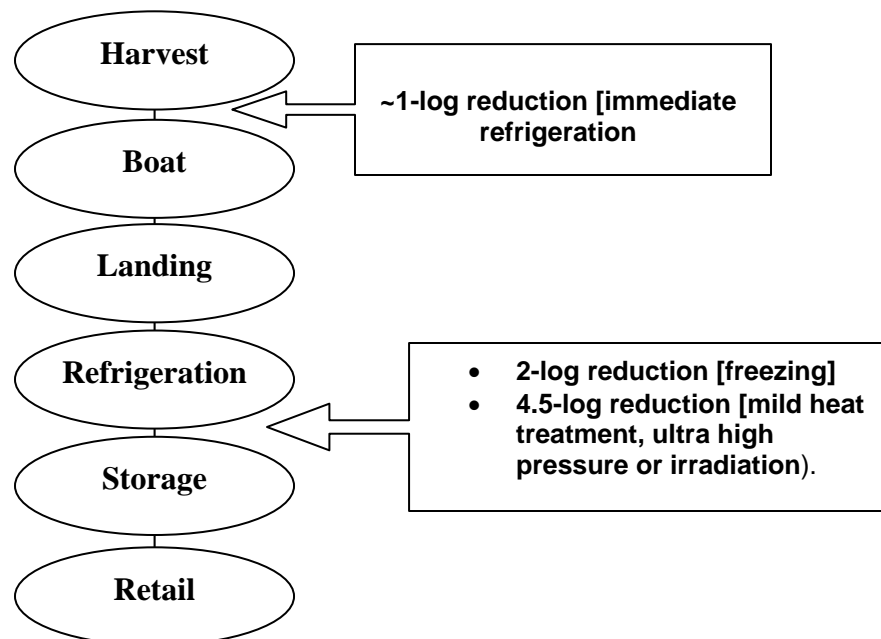


Figure VI-1. Schematic Representation from Harvest to Retail Showing Steps at which Evaluated Mitigations Occur

Immediate refrigeration was modeled by assuming that oysters would be cooled to no growth temperatures immediately following harvest. Assuming that this mitigation practice was followed without exception, post-harvest growth of *V. parahaemolyticus* in oysters would occur only during the period of cooldown required for the oyster meat to reach no growth temperatures. The time unrefrigerated was assumed to be zero and growth was considered to occur only during the cooldown period. The distribution of

cooldown duration was assumed to be the same as that specified with respect to the baseline assessment.

The potential effects of mild heat treatment, irradiation, and/or high hydrostatic pressure and that of freezing were modeled by reducing the density predictions of the baseline model (i.e., no mitigation, at retail) downward by factors of 4.5- \log_{10} and 2- \log_{10} , respectively. These effects correspond to dividing predicted total and pathogenic densities per gram by 31,623 and 100 for the 4.5- \log_{10} and 2- \log_{10} reductions, respectively. Implicitly, it was assumed that the effect of treatment on \log_{10} *V. parahaemolyticus* densities is uniform with no induced change in the variance of \log_{10} densities. If the variance of \log_{10} densities actually increases after mitigation, even as the mean \log_{10} density is decreased by the specified amount, then the potential degree of risk reduction is overstated.

The results of these “what-if” scenarios are summarized by harvest region in Table VI-2. See Appendix 6 for the results for each of the 24 region/season combinations. All three types of mitigation strategies were found to have a substantial effect on the probable number of illnesses likely to occur in comparison to the baseline (no mitigation). The scenarios indicate that implementing a mitigation that reduces *V. parahaemolyticus* levels in oysters after harvest by 4.5- \log_{10} would be expected to reduce the number of predicted illnesses to less than one per year for all regions and that immediate refrigeration would be expected to reduce the number of predicted illnesses by about 90%.

Table VI-2. Predicted Mean Number of Illnesses per Annum from Reduction of Levels of Pathogenic *Vibrio parahaemolyticus* in Oysters

Region	Season	Predicted Mean Number of Illnesses per Annum ^a			
		Baseline	Immediate Refrigeration ^b	2-log ₁₀ Reduction ^c	4.5-log ₁₀ Reduction ^d
Gulf Coast (Louisiana)	Spring	505	54	5.2	<1.0
	Summer	1,406	139	15	<1.0
	Fall	132	8.8	1.3	<1.0
	Winter	6.7	<1.0	<1.0	<1.0
Gulf Coast (Non-Louisiana)	Spring	193	29	2.0	<1.0
	Summer	299	42	3.1	<1.0
	Fall	51	7.7	<1.0	<1.0
	Winter	2.9	<1.0	<1.0	<1.0
Mid-Atlantic	Spring	4.4	<1.0	<1.0	<1.0
	Summer	6.9	<1.0	<1.0	<1.0
	Fall	3.8	<1.0	<1.0	<1.0
	Winter	<1.0	<1.0	<1.0	<1.0
Northeast Atlantic	Spring	3.0	<1.0	<1.0	<1.0
	Summer	14	1.7	<1.0	<1.0
	Fall	1.7	<1.0	<1.0	<1.0
	Winter	<1.0	<1.0	<1.0	<1.0
Pacific Northwest (Dredged)	Spring	<1.0	<1.0	<1.0	<1.0
	Summer	3.9	<1.0	<1.0	<1.0
	Fall	<1.0	<1.0	<1.0	<1.0
	Winter	<1.0	<1.0	<1.0	<1.0
Pacific Northwest (Intertidal)	Spring	18	10	<1.0	<1.0
	Summer	173	96	2.1	<1.0
	Fall	1.0	<1.0	<1.0	<1.0
	Winter	<1.0	<1.0	<1.0	<1.0

^aValues rounded to significant digits. See Appendix 7 for actual values of numbers presented as <1.0.

^b Represents conventional cooling immediately after harvest; the effectiveness of varies both regionally and seasonally and is typically approximately 1-log reduction.

^c Represents any process which reduces levels of *Vibrio parahaemolyticus* in oysters 2-log, e.g., freezing.

^d Represents any process which reduces levels of *Vibrio parahaemolyticus* in oysters 4.5-log, e.g., mild heat treatment, irradiation, or ultra high hydrostatic pressure.

The uncertainty in the estimates is shown in Figure VI-2, using the Gulf Coast summer harvest as an example. Although the distribution of predicted illness is reduced substantially under these mitigations, the variance of the predicted number of illnesses (compared to the baseline) remains relatively unchanged. This is a consequence of the effect of specified model uncertainties, particularly with respect to the dose-response, growth rate and the percentage of total *V. parahaemolyticus* that are pathogenic.

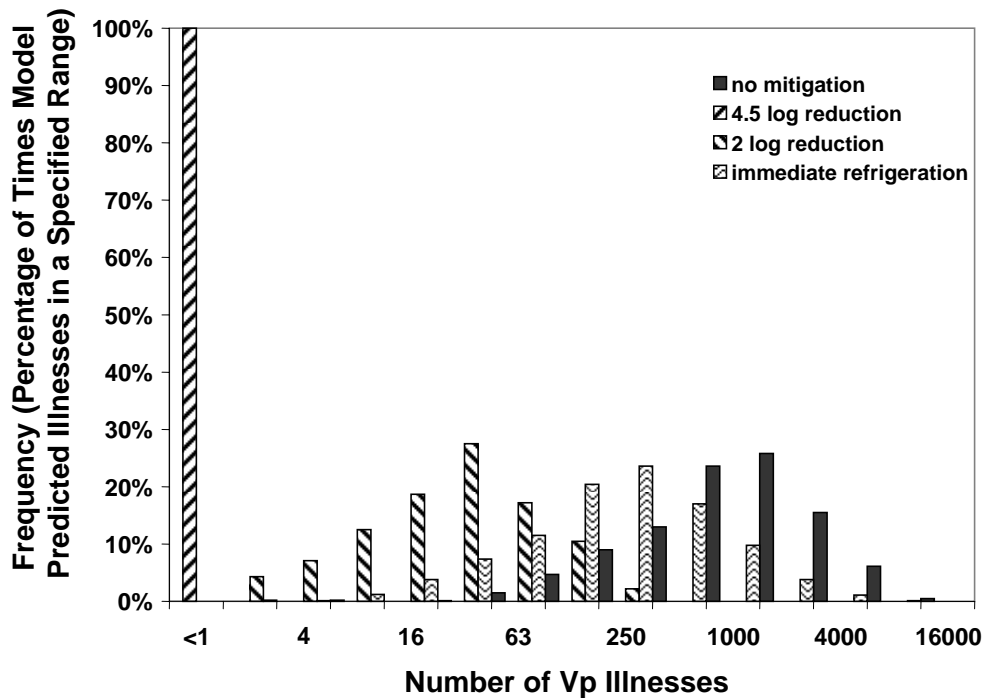


Figure VI-2. Effect of Potential Mitigations on the Distribution of Probable Number of Illnesses Associated with *Vibrio parahaemolyticus* in Oysters Harvested from the Gulf Coast (Louisiana) in the Summer

The effects of the mitigations on the mean risk per serving are shown in Figures VI-3 through VI-8 for the six region harvest areas. With the exception of immediate refrigeration, the effect of the potential mitigations on the number of illnesses is similar for the six regions and four seasons. The effectiveness of immediate refrigeration for the Pacific Northwest (Intertidal) is predicted to be much less than that in the Pacific Northwest (Dredge) and the other harvest regions. This is a consequence of the intertidal harvesting method as oysters are exposed to ambient air temperatures (e.g. on mud flats) for various time periods unrefrigerated. The 4 to 8 hours when the intertidal oysters are exposed to ambient air are included in the 1 to 11 hours harvest duration modeling. This period on the tidal flat allows for additional *V. parahaemolyticus* growth that cannot be effectively inhibited by refrigeration during the period of intertidal exposure. Immediate refrigeration is effective in the Gulf Coast but the effectiveness of the immediate refrigeration mitigation was found to be seasonal in the Mid-Atlantic, Northeast Atlantic

and Pacific Northwest regions but not in the Gulf Coast regions. This is an apparent consequence of considerably lower air temperatures (which may be at or below the growth temperature threshold for *V. parahaemolyticus*) during the winter season in those regions compared to the Gulf Coast regions.

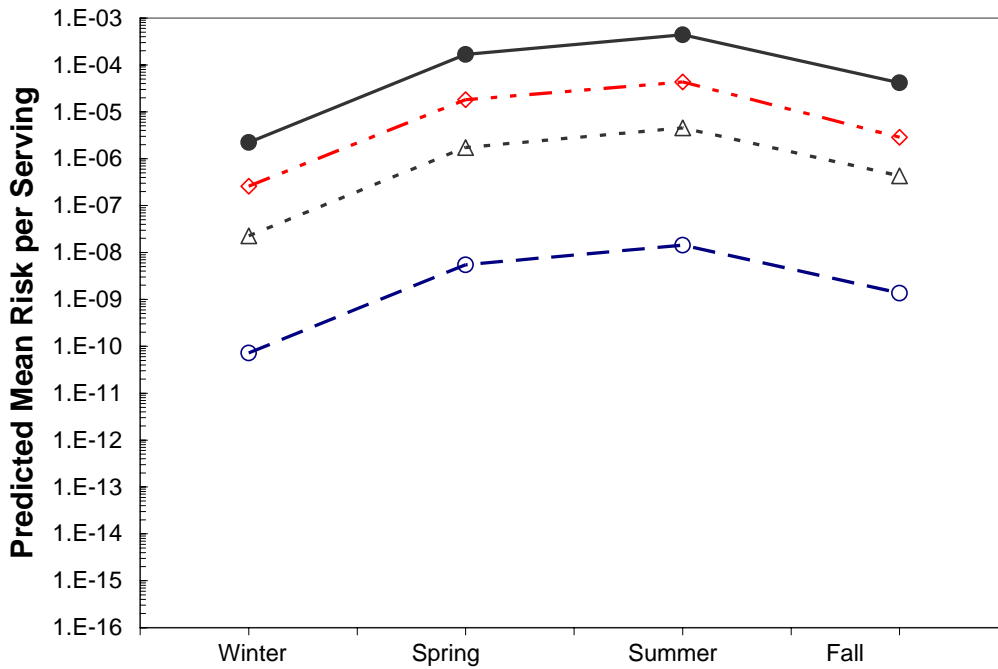


Figure VI-3. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Gulf Coast (Louisiana) Harvest

[No mitigation (●); immediate refrigeration upon harvest (◊); treatment resulting in a 2-log₁₀ reduction (Δ); treatment resulting in a 4.5-log₁₀ reduction (○).]

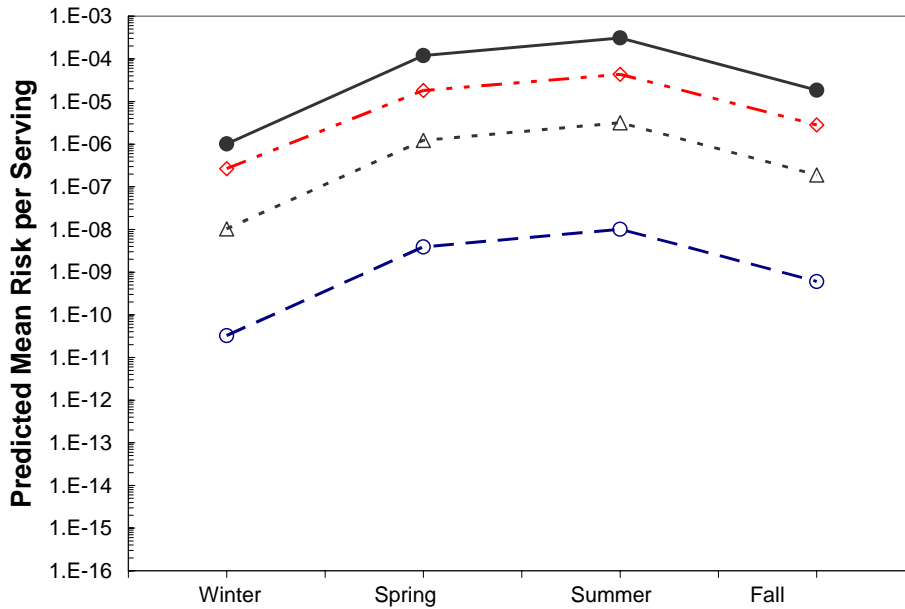


Figure VI-4. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Gulf Coast (Non-Louisiana) Harvest [No mitigation (●); immediate refrigeration upon harvest (◇); treatment resulting in a 2- \log_{10} reduction (△); treatment resulting in a 4.5- \log_{10} reduction (○).]

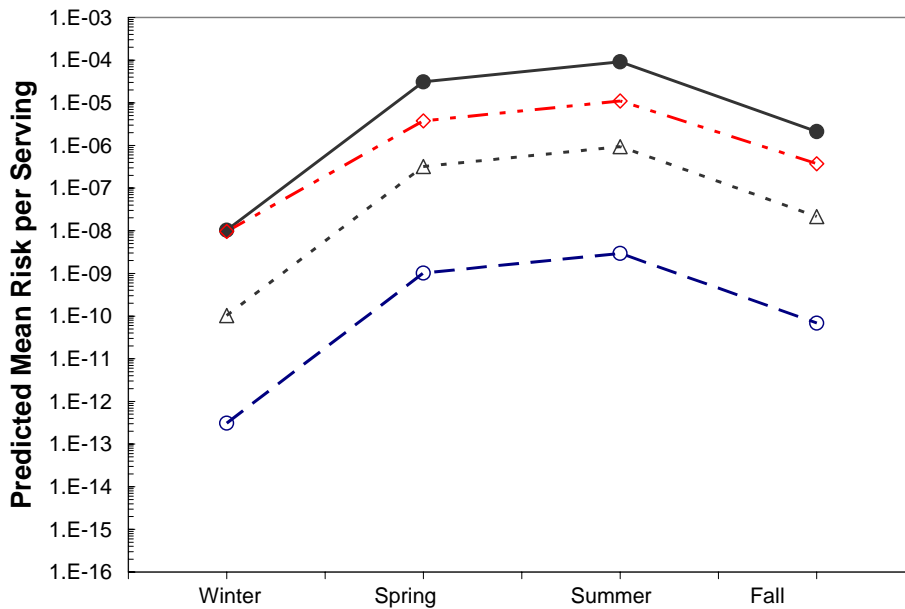


Figure VI-5. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Mid-Atlantic Harvest [No mitigation (●); immediate refrigeration upon harvest (◇); treatment resulting in a 2- \log_{10} reduction (△); treatment resulting in a 4.5- \log_{10} reduction (○).]

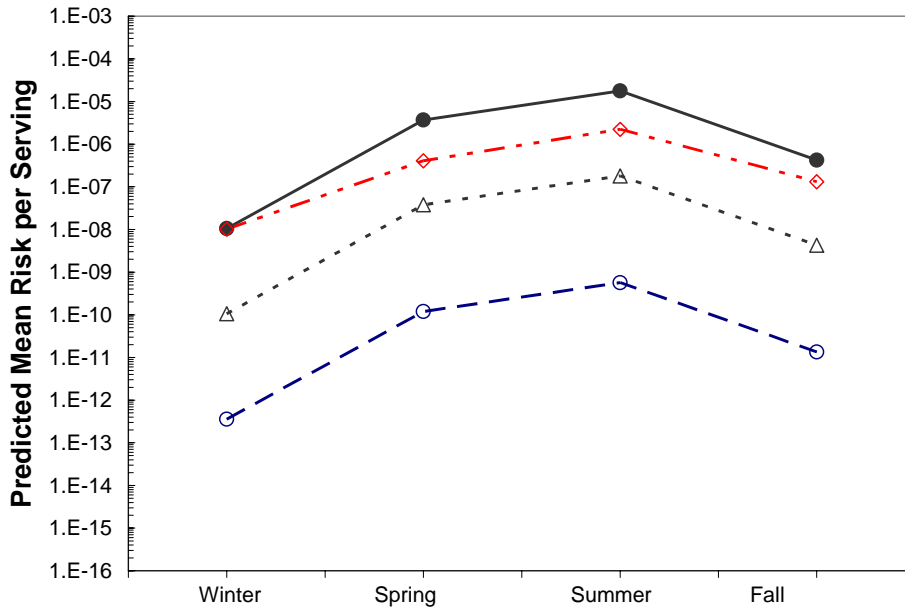


Figure VI-6. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Northeast Atlantic Harvest [No mitigation (●); immediate refrigeration upon harvest (◇); treatment resulting in 2- \log_{10} reduction (△); treatment resulting in a 4.5- \log_{10} reduction (○).]

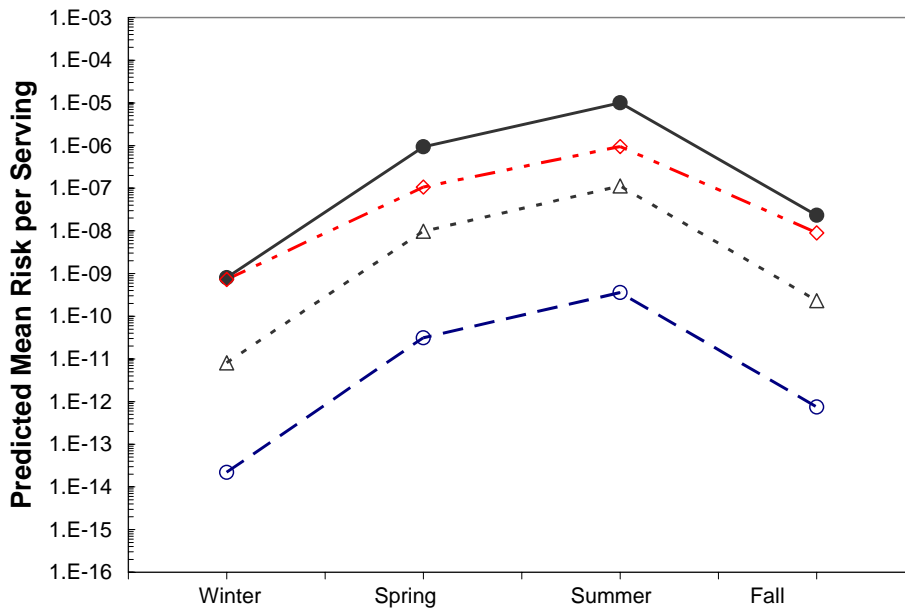


Figure VI-7. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Pacific Northwest (Dredged) Harvest [No mitigation (●); immediate refrigeration upon harvest (◇); treatment resulting in 2- \log_{10} reduction (△); treatment resulting in a 4.5- \log_{10} reduction (○).]

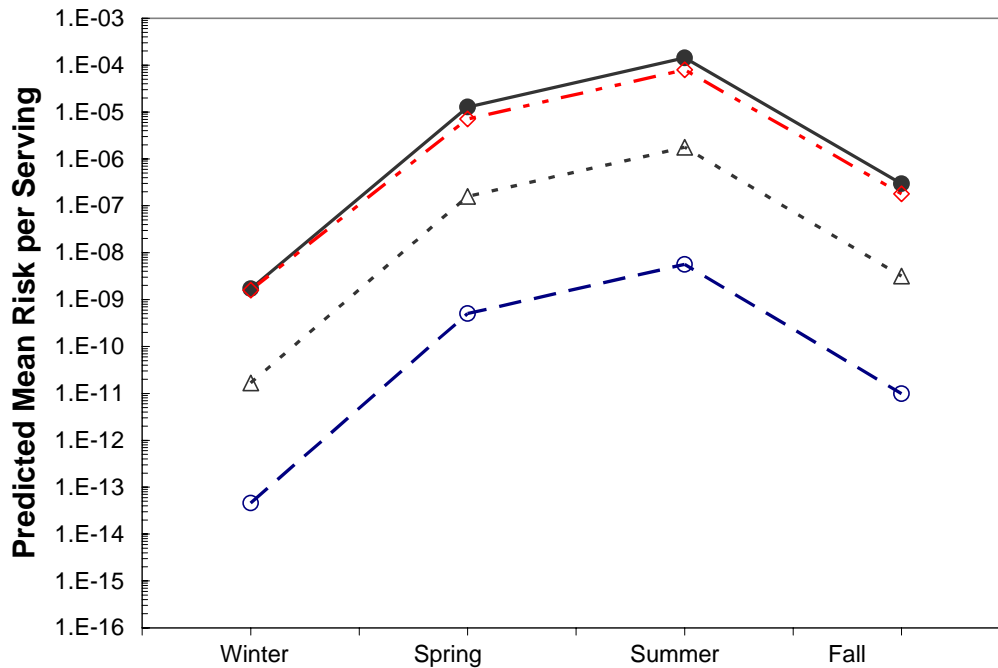


Figure VI-8. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Pacific Northwest (Intertidal) Harvest

[No mitigation (●); immediate refrigeration upon harvest (◇); treatment resulting in 2-log₁₀ reduction (△); treatment resulting in a 4.5-log₁₀ reduction (○).]

Reducing Time-to-Refrigeration

The effect of reducing the time that oysters are unrefrigerated was further investigated by comparing the impact on predicted illness for different times from harvest to when oysters are refrigerated. The predicted effect of “rapid” cooling (e.g., using ice or an ice slurry) was also compared to “conventional” cooling (e.g., immediate refrigeration after harvest). For conventional cooling, it is estimated to take up to 10 hours for oysters to cool to a temperature at which *V. parahaemolyticus* will no longer grow (Cook, 2002b). For rapid cooling, there is a much shorter time for oysters to reach a no-growth temperature for *V. parahaemolyticus*; it is about 1 hour (Schwarz, 2003b).

For the rapid cooling scenario, a one hour cooldown time to no-growth temperature was assumed after oysters are placed on ice or ice slurry. This estimate was based on studies by the Seafood Safety Laboratory, Texas A & M University at Galveston (Schwarz, 2003a). The average growth rate occurring during the one hour cooldown period was assumed to be equal to half the growth rate corresponding to the (variable) air temperature at the time of harvest. With the one hour cooldown time the mean times to “no-growth” temperature were approximately 2.0, 2.9, 3.7, and 4.3 hours over the set of 4 simulations.

For the conventional cooling scenario the same 1 to 4 hour range of maximum time unrefrigerated was combined with the assumed range of 1 to 10 hours to reach no-growth temperatures. This range was based on preliminary experiments (De Paola, 1999) and later confirmed by Cook (2002b) and Schwarz (2003b) for oysters in conventional (air-circulated) coolers. The amount of growth occurring during the cooldown period corresponded to that associated with the baseline model. Thus, for this scenario, the mean times to reach no-growth temperature were 5.5, 6.4, 7.2, and 7.8 hours over the set of 4 simulations corresponding to maximum times until first refrigeration of 1, 2, 3, and 4 hours, respectively.

Model simulations were run assuming maximum times of 1, 2, 3, and 4 hours for the time between harvest and first refrigeration. Specifically, the baseline distribution of duration of time from initial harvest until the initiation of oyster cooling was truncated at selected maximum times of 1, 2, 3, and 4 hours. All other variables (e.g., air and water temperatures) and uncertainties (e.g., dose-response) were taken to correspond to that specified in the baseline assessment.

For illustration, the results for the Gulf Coast (Louisiana and non-Louisiana) summer harvest are shown in Figure VI-9. As shown in the figure, the predicted reduction in *V. parahaemolyticus* illness from summer harvest of Gulf Coast oysters ranges from 46% to 97%, depending upon the specifics of the scenario. The results for all 24 region/season combinations are provided in Appendix 10.

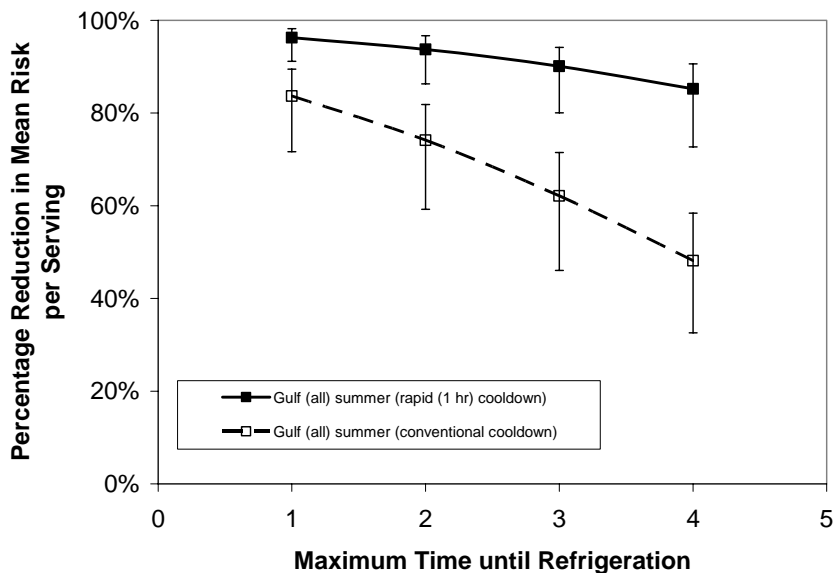


Figure VI-9. Predicted Effectiveness of Rapid versus Conventional Cooling on *Vibrio parahaemolyticus* Risk for Gulf Coast Summer Harvest

[The scenario represents a simultaneous consideration of both the Gulf Coast (Louisiana) and Gulf Coast (non-Louisiana) regions in the summer.]

Re-submersion of Intertidally Harvested Oysters

The impact of overnight submersion of oysters after intertidal harvesting on the predicted risk of illness was evaluated. The baseline model predicts the levels of *Vibrio parahaemolyticus* in intertidally-harvested oysters, i.e., oysters are placed into baskets and removed after the tide rises, a typical practice in the Pacific Northwest. Studies of intertidally harvested oysters have shown that *V. parahaemolyticus* levels increase 4 to 8 –fold in oysters during intertidal exposure (Nordstrom *et al.*, 2004; Herwig *et al.*, 2001). However, Nordstrom *et al.* (2004) also demonstrated that after overnight submersion for a single tidal cycle, *V. parahaemolyticus* levels were reduced to levels similar to those measured prior to the intertidal exposure.

The baseline risk assessment model estimates that in the summer the risk of illness increases from 1.1×10^{-5} for dredged to 1.5×10^{-4} for intertidal harvesting because of intertidal exposure and heating. Delaying harvest overnight until near the end of the next tidal cycle just before oysters are re-exposed again to ambient air reduces the risk to a level predicted for oysters harvested by dredge (1.0×10^{-5}) (see Appendix 10). The calculation for the percent reduction in risk obtained if the oysters are submerged overnight is based on the assumption that if *V. parahaemolyticus* levels after overnight submersion are similar to those in dredged oysters, then the risk decreases to that of dredged oysters. Results revealed that a 90% reduction in risk of illness could be obtained if intertidally harvested oysters were left submerged in the water overnight (Table VI-3). Further research is needed to determine whether this reduction could actually be achieved when oysters are stacked in baskets or by other means such as relaying or depuration.

Table VI-3. Effect of Overnight Submersion of Oysters during Intertidal Harvest on Predicted Risk in the Pacific Northwest Harvest Region

Type of Harvest	Season	Reduction in Illness (%)
Overnight Submersion of Intertidal Harvest ^a	Winter	51.5
	Spring	93.3
	Summer	93.0
	Fall	93.2

^aThis assumes levels of *V. parahaemolyticus* in oysters after submersion overnight are similar to dredged.

Sample-Based Control Plans

The level of total *V. parahaemolyticus* in oysters is useful as a convenient surrogate indicator of the risk of illness due to the level of pathogenic *V. parahaemolyticus* in oysters. The FDA guidance for *V. parahaemolyticus* in seafood recommends that levels not exceed 10,000 viable cells per gram (ISSC/FDA, 1997). The 1999 *V. parahaemolyticus* Interim Control Plan (ICP) for molluscan shellfish adopted by the

ISSC in 1999 and revised in 2001 included a microbiological criterion that if >10,000 cells/g are found in oysters, the area would need to be resampled for the presence of TDH⁺ strains. If any pathogenic (TDH⁺) *V. parahaemolyticus* were found in oysters, the harvest waters would be closed. In the 2001, revised plan, the number of total *V. parahaemolyticus*/g for resampling harvest waters was changed from 10,000 to 5,000.

The risk assessment cannot completely evaluate the effectiveness of such control plans because, as the model is constructed, there is no mechanism included to account for the possibility of persistence of either pathogenic or total *V. parahaemolyticus* in specific oyster harvesting areas and not others within the same region/season. The structure of the risk assessment does, however, allow consideration of the hypothetical impact on the incidence of disease if it were possible to exclude oysters from the raw market (or subjected to preventive controls) which have greater than any particular level of total *V. parahaemolyticus* at the time of harvest or at retail. The percentage of oyster harvest exceeding selected criteria levels for total *V. parahaemolyticus* can also be determined, giving an indication of the percentage of oysters that would no longer be available for raw consumption or for which preventative measures would need to be implemented to reduce *V. parahaemolyticus* growth under the assumption that the control plan could be implemented with 100% efficiency. For illustration, the results for the Gulf Coast (Louisiana) summer harvest are shown and the results for other region-season combinations can be found in Appendix 10.

Changes in the risk after removing varying percentages of the harvest greater than selected criteria levels were also determined in the simulations. Removal was simulated as occurring when a given criteria level was exceeded and the harvester/processor was compliant to that level. Varying levels of compliance (100%, 90%, 70%, 50%) were considered. For each criteria level and compliance probability, the proportion of harvest lost to the raw consumption market was estimated as the fraction of 10,000 simulated exposures for which initial *V. parahaemolyticus* levels exceeded the criteria level and the harvester/processor was compliant. The impact of deviation from compliance with these guidance levels was also evaluated, using the Gulf Coast region (Louisiana)/ Summer harvest as an example. As might be anticipated, the effectiveness of the guidance level to reduce illnesses is dependant on to the level of compliance (see Appendix 10).

At-Harvest Scenario. The at-harvest scenario included selected levels of 10 up to 100,000 total *V. parahaemolyticus*/g in order to estimate the relationship between illnesses potentially averted and harvest that would have to be diverted from the “raw market” (or subjected to preventive controls). The effect of uncertainties on this analysis was evaluated by considering the results of each uncertainty realization (sample) separately and then computing both a central estimate of probable effectiveness and a 90% uncertainty interval.

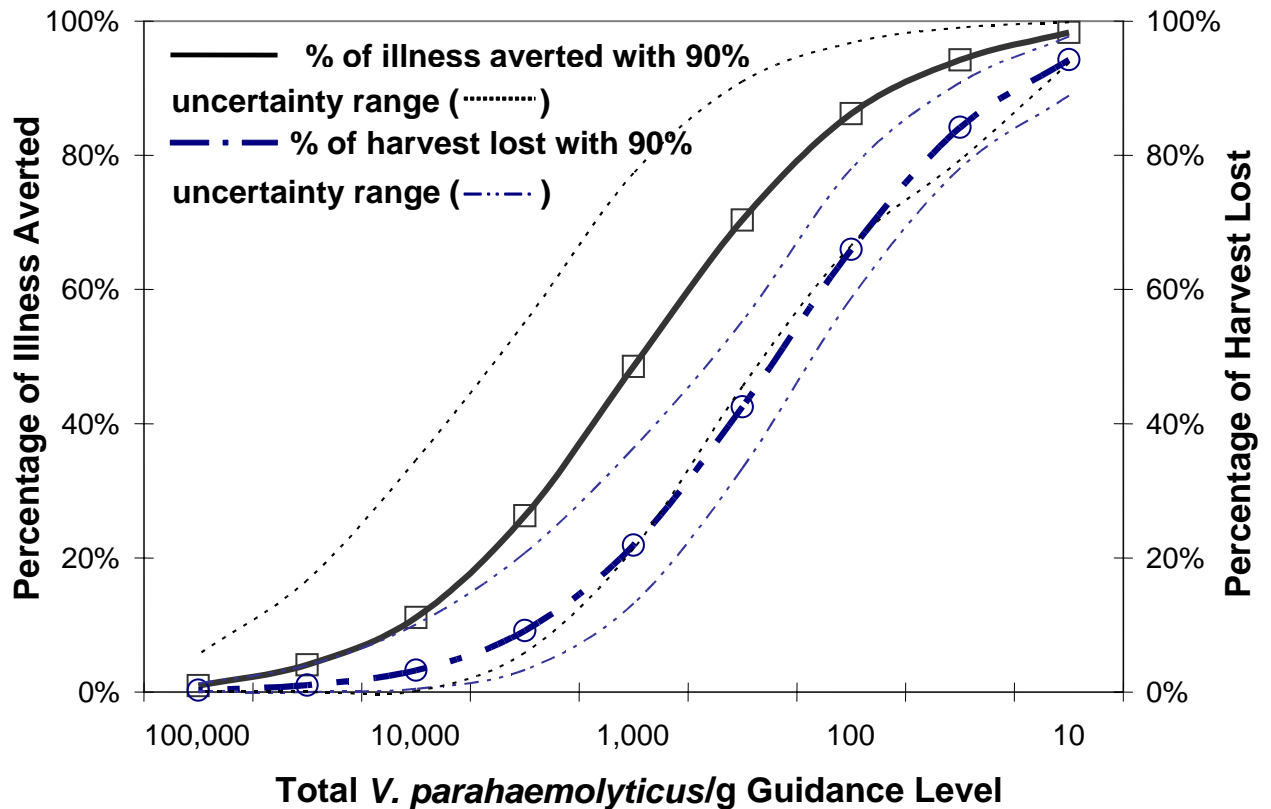


Figure VI-10. Predicted Effect of Control of Total *Vibrio parahaemolyticus* per Gram Oysters at Time of Harvest for the Gulf Coast (Louisiana) Summer Harvest
 [The term in Figure VI-10 “harvest lost” refers to the portion of the harvest that would have to be diverted from the “raw market.”]

Based on the means of the uncertainty distributions, the simulation results suggest that if all shellstock could be evaluated for total *V. parahaemolyticus* at time of harvest, excluding all oysters that had levels of 10,000 viable cells per g or more would reduce illness by 16% and 3% of the harvest would have to be diverted from the “raw market” or subjected to preventive controls. A 5,000 *V. parahaemolyticus* per g standard at time of harvest could (potentially) eliminate 28% of the illnesses associated with the consumption of oysters from this region/season with 6% of the harvest having to be diverted from the “raw market” or subjected to preventive controls. The relatively low (potential) reduction of illness is attributable to the large proportion of the harvest that would remain with a lower level of *V. parahaemolyticus* that would still grow to more significant levels after harvest. In comparison, the simulation results suggest that in the absence of subsequent post-harvest mitigations, “at-harvest” guidance levels of 5- \log_{10} (10^5 or 100,000), 3- \log_{10} (1,000 or 10^3) and 2- \log_{10} (100 or 10^2) total *V. parahaemolyticus* per g could (potentially) reduce the illness rate by 1.6%, 68% and 98% with corresponding impact of 0.25%, 21% and 66% of the harvest, respectively. There is, however, uncertainty associated with these predictions as indicated by the uncertainty bounds shown in Figure VI-10. It is important to note that these estimates are based on

the consideration of the baseline model only and do not take into account any other potential mitigations such as those evaluated earlier in this chapter.

At-Retail Scenario. The hypothetical impact on the incidence of disease if it were possible to exclude oysters (from the raw market) which have greater than any particular level of total *V. parahaemolyticus* at retail was also evaluated for different guidance levels following the same method described above for at-harvest control. The results are shown in Figure VI-11 for the Gulf Coast (Louisiana) summer harvest, with selected levels of 10 to 100,000 total *V. parahaemolyticus*/g included in order to estimate the relationship between illnesses potentially averted and harvest that would have to be diverted from the “raw market.”

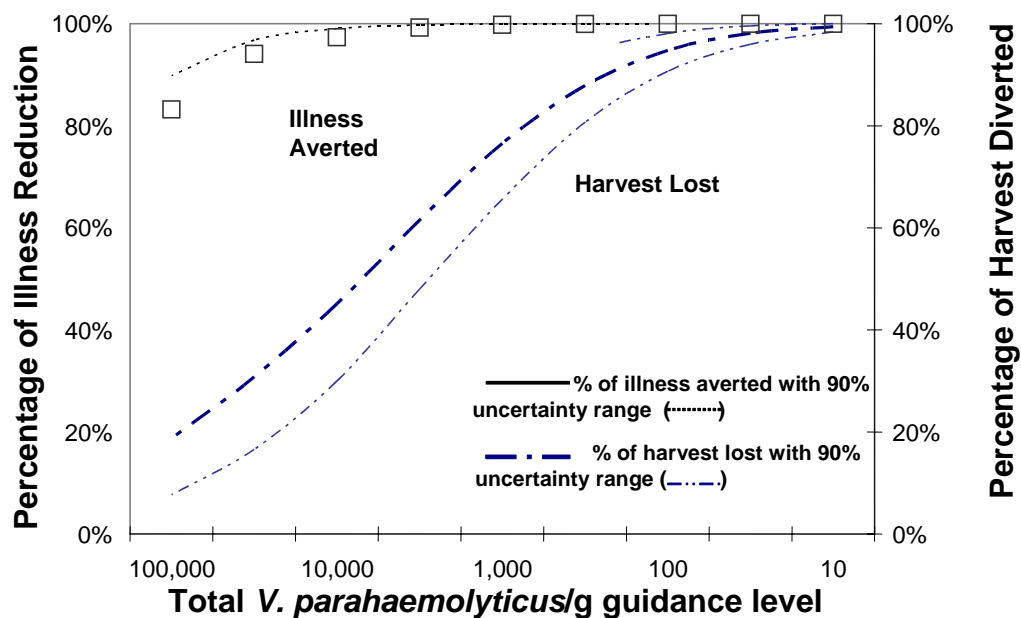


Figure VI-11. Predicted Effect of Control of Total *Vibrio parahaemolyticus* per Gram Oysters at Retail for the Gulf Coast (Louisiana) Summer Harvest

[The term in Figure VI-10 “harvest lost” refers to the portion of the harvest that would have to be diverted from the “raw market.”]

The effect of uncertainties on this analysis was evaluated as for the at-harvest control scenario. The simulation results suggest that at the same control levels, many more illnesses would be potentially eliminated, but with a much higher loss in harvest diverted from the raw market. For example, excluding all oysters that had levels of 10,000 viable cells per g at retail would reduce illness by 99% and 43% of the harvest would have to be diverted from the “raw market”, compared to 11% and 3%, respectively, for at-harvest control levels of 10,000 *V. parahaemolyticus* per gram. A 5,000 *V. parahaemolyticus* per g standard at retail could (potentially) eliminate almost 100% of the illnesses associated

VI. WHAT-IF SCENARIOS

with the consumption of oysters from this region/season with 70% of the harvest having to be diverted from the “raw market.” The greater effectiveness of guidance level applied at retail than at harvest with respect to illness aversion is because the former is applied after the effects of temperature abuse during harvesting operation.

VII. INTERPRETATION AND CONCLUSIONS

This risk assessment included an analysis of the available scientific information and data in the development of a model to predict the public health impact of pathogenic *V. parahaemolyticus* in raw oysters. The assessment focuses on comparing the relative risk of consuming raw oysters acquired from different geographic regions, seasons, and harvest practices. The scientific evaluations and the mathematical models developed during the risk assessment also facilitate a systematic evaluation of strategies to minimize the public health impact of pathogenic *V. parahaemolyticus*.

Regional and seasonal differences in climates and oyster harvesting practices occur within the United States. Therefore, the risk assessment was structured to assess regional, seasonal and harvesting practices influences on illness rates. Six separate geographic regions and harvesting practices combinations were considered: Northeast Atlantic, Mid-Atlantic, Pacific Northwest (Dredging), Pacific Northwest (Intertidal), Gulf Coast (Louisiana), Gulf Coast (non-Louisiana states). The predicted risk estimates must of course be evaluated in relation to the uncertainties as a result of limited scientific data and knowledge.

Although the risk assessment modeled sporadic *V. parahaemolyticus* illnesses, steps taken to reduce sporadic cases would be expected to reduce the size and frequency of outbreaks. The proportional reduction would depend on the virulence of the outbreak strain and on the survivability and growth of the strain following post-harvest treatments. Mitigation or control measures aimed at decreasing levels of *V. parahaemolyticus* in oysters will also likely decrease levels of other species in the *Vibrio* genus (or family), such as *Vibrio vulnificus*.

Below are the responses to the questions that the risk assessment team was charged with answering.

What is known about the dose-response relationship between consumption of *V. parahaemolyticus* and illnesses?

- Although an individual may become ill from consumption of low levels of *V. parahaemolyticus*, it is much more likely that he or she will become ill if the level is high. The probability of illness is relatively low (<0.001%) for consumption of 10,000 *V. parahaemolyticus* cells/serving (equivalent to about 50 cells/gram oysters). Consumption of about 100 million *V. parahaemolyticus* cells/serving (500 thousand cells/gram oysters) increases the probability of illness to about 50%.
- Anyone exposed to *V. parahaemolyticus* can become infected and develop gastroenteritis. However there is a greater probability of gastroenteritis developing into septicemia (and possibly death) among the subpopulation with concurrent underlying chronic medical conditions.
- The model predicts about 2,800 *V. parahaemolyticus* illnesses from oyster consumption each year. Of infected individuals, approximately 7 cases of gastroenteritis will progress to septicemia each year for the total population, of which

2 individuals would be from the healthy subpopulation and 5 would be from the immunocompromised subpopulation.

- This risk assessment assumed that pathogenic strains of *V. parahaemolyticus* are TDH⁺ and that all strains possessing this characteristic are equally virulent. Modifications can be made to the risk assessment if data become available for new virulence determinants. For example, data from outbreaks suggest that fewer microorganisms of *V. parahaemolyticus* O3:K6 are required to cause illness compared to other strains.

What is the frequency and extent of pathogenic strains of *V. parahaemolyticus* in shellfish waters and in shellfish?

- Pathogenic *V. parahaemolyticus* (i.e., TDH⁺ strains) usually occur at low levels in shellfish waters and oysters. This makes it difficult to monitor shellfish waters for pathogenic *V. parahaemolyticus* to prevent illnesses from this microorganism. As shown in Table VII-1, the predicted levels of pathogenic *V. parahaemolyticus* in oysters at the time of harvest are only a small fraction of the total *V. parahaemolyticus* levels. There are differences among regions. For example, the ratio of pathogenic to total *V. parahaemolyticus* is lower in the Gulf Coast (approximately 0.2%) compared to the Pacific Northwest (approximately 2.0%).

Table VII-1. Predicted Mean Levels of Total and Pathogenic *Vibrio parahaemolyticus* in Raw Oysters At-Harvest

Region	<i>Vibrio parahaemolyticus</i>	Mean Predicted Levels of <i>V. parahaemolyticus</i> per gram ^a			
		Summer	Fall	Winter	Spring
Gulf Coast ^b	Total	2,100	220	52	940
	Pathogenic	3.6	<1.0	<1.0	1.6
Mid-Atlantic	Total	780	51	3.5	200
	Pathogenic	1.3	<1.0	<1.0	<1.0
Northeast Atlantic	Total	230	33	3.7	42
	Pathogenic	<1.0	<1.0	<1.0	<1.0
Pacific Northwest (Dredged)	Total	5.0	<1.0	<1.0	<1.0
	Pathogenic	<1.0	<1.0	<1.0	<1.0
Pacific Northwest (Intertidal) ^c	Total	650	2.3	<1.0	61
	Pathogenic	15	<1.0	<1.0	1.4

^a Values rounded to 2 significant digits. See Appendix 7 for actual values of levels.

^b The at-harvest levels are similar for the Gulf Coast (Louisiana) and Gulf Coast (non-Louisiana) regions; this is a function of the model construction. Differences between these regions occur in the post-harvest module because time from harvest to refrigeration is typically shorter for Louisiana compared to non-Louisiana states (Florida, Mississippi, Texas, and Alabama).

^c Oysters harvested using intertidal methods are typically exposed to ambient air temperatures for longer times before refrigeration compared with dredged methods.

What environmental parameters (e.g., water temperature, salinity) can be used to predict the presence of *V. parahaemolyticus* in shellfish?

- The primary driving factor to predict the presence of *Vibrio parahaemolyticus* in oysters is water temperature. Salinity was a factor evaluated but not incorporated into the model. Salinity is not a strong determinant of *Vibrio parahaemolyticus* levels in the regions that account for essentially all the commercial harvest. Other factors such as oyster physiology and disease status may also be important but no quantifiable data were available to include these factors in the model.
- There are large differences in the predicted levels of *V. parahaemolyticus* in oysters at harvest among regions and seasons (see Table VII-1 above). For all regions, the highest levels of *V. parahaemolyticus* were predicted in the summer and spring and the lowest levels in the fall and winter. Overall, the highest levels of total and pathogenic *V. parahaemolyticus* were predicted for the Gulf Coast and the lowest levels in the Pacific Northwest (dredged).
- After harvest, air temperature is also an important determinant of the levels of *V. parahaemolyticus* in oysters. *Vibrio parahaemolyticus* can continue to grow and multiply in oysters until they are adequately chilled.
- Levels of *Vibrio parahaemolyticus* are lower in oysters after harvest in the cooler vs. warmer months (see Table VII-2 below). This means that reducing the time between harvest and cooling will be more important in the summer and spring than in the fall and winter.

Table VII-2. Predicted Mean Levels of Pathogenic *Vibrio parahaemolyticus* per Serving in Raw Oysters At-Harvest and At-Consumption

Region	Pathway Step	Mean Predicted Levels of <i>V. parahaemolyticus</i> per Serving ^a			
		Summer	Fall	Winter	Spring
Gulf Coast (Louisiana)	At-harvest	720	80	18	320
	At-consumption	21,000	2,000	98	7,900
Gulf Coast (Non-Louisiana) ^b	At-harvest	720	80	18	320
	At-consumption	15,000	880	47	5,600
Mid-Atlantic	At-harvest	260	18	1.2	66
	At-consumption	4,300	110	<1.0	1,500
Northeast Atlantic	At harvest	78	12	1.2	14
	At-consumption	860	17	<1.0	180
Pacific Northwest (Dredged)	At-harvest	24	<1.0	<1.0	4
	At consumption	460	1.2	<1.0	42
Pacific Northwest (Intertidal) ^c	At-harvest	3,000	10	<1.0	280
	At-consumption	7,500	17	<1.0	740

^a Values rounded to 2 significant digits. See Appendix 7 for actual values of levels.

^b Includes oysters harvested from Florida, Mississippi, Texas, and Alabama. The time from harvest to refrigeration in these states is typically shorter than for Louisiana.

^c Oysters harvested using intertidal methods are typically exposed to higher ambient air temperature for longer times before refrigeration compared with dredge methods.

How do levels of *V. parahaemolyticus* in shellfish at harvest compare to levels at consumption?

- Absent mitigation treatments, levels of *V. parahaemolyticus* are higher in oysters at consumption than at harvest (see Table VII-2 above). The difference between *V. parahaemolyticus* densities at-harvest versus at-consumption is largely attributable to the extent of growth that occurs before the oysters are cooled to no-growth temperatures.
- Levels of *V. parahaemolyticus* in oysters vary by region and season and are highest during the summer.
- During intertidal harvest, oysters are exposed to ambient air temperatures for longer times, allowing additional growth of *Vibrio parahaemolyticus* in oysters and leading to higher predicted risk of illness.
- Preventing growth of *V. parahaemolyticus* in oysters after harvest (particularly in the summer) will lower the levels of *V. parahaemolyticus* in oysters and as a consequence, lower the number of illnesses associated with the consumption of raw oysters.

What is the role of post-harvest handling on the level of *V. parahaemolyticus* in shellfish?

- Post-harvest measures aimed at reducing the *V. parahaemolyticus* levels in oysters reduced the model-predicted risk of illness associated with this pathogen.
- Reducing the time between harvest and chilling has a large impact on reducing levels of *Vibrio parahaemolyticus* in oysters and the number of illnesses. Predicted reductions were greater for shorter times to refrigeration using ice (oysters reach no-growth temperature in 1 hour) compared to cooling under conventional refrigeration (which may take up to 10 hours until oysters reach a no-growth temperature).

What reductions in risk can be anticipated with different potential intervention strategies?

- Overall. The most influential factor predicted to affect risk of illness was the levels of total *V. parahaemolyticus* in oysters at harvest. Intervention strategies should be aimed at reducing levels of *V. parahaemolyticus* and/or preventing its growth in oysters after harvest. These strategies, either at-harvest or post-harvest, must consider regional/seasonal differences. For example, the use of ice on harvest boats to cool oysters to the no-growth temperature of *V. parahaemolyticus* will have a larger impact on reducing illnesses in the summer than in the winter when air temperatures are cooler and *V. parahaemolyticus* levels are lower.
- Regional/seasonal Differences. Table VII-3 shows the relationship between the predicted number of illnesses and region/season combinations. The risk of *V. parahaemolyticus* illness is increased during the warmer months of the year, with the magnitude of this increase a function of the extent to which the growing waters (and ambient air temperatures) are at temperatures that support the growth of the pathogen (e.g., temperatures above 10 °C). For each region, the predicted numbers of illnesses are much higher for the summer compared to the winter months. Intervention

measures that depend on cooling oysters to no-growth temperatures for *V. parahaemolyticus* may be more important in warmer seasons and regions.

The risk of *V. parahaemolyticus* illness is substantial in the Gulf Coast region where water temperatures are warm over a large part of the year as compared to the Northeast Atlantic region where water temperatures support the growth of *V. parahaemolyticus* only during a relatively small portion of the year. A difference is seen among the regions due to different harvesting methods. Within the Gulf Coast, the predicted number of illnesses is much higher in Louisiana compared to other states in this region because the harvest boats in Louisiana are typically on the water longer, i.e., leading to a longer time from harvest to refrigeration. Harvest volume is also a determining factor; in the summer, Louisiana accounts for approximately 77% of the Gulf Coast harvest. This is also seen in the Pacific Northwest by comparing intertidal versus dredged harvesting. Intertidal harvesting accounts for 75% of the Pacific Northwest harvest and exposes oysters to higher temperatures longer, allowing greater growth of *V. parahaemolyticus*. Overnight submersion for a single tidal cycle, reduces levels of *V. parahaemolyticus* in oysters and the risk of illness.

Table VII-3. Predicted Mean Annual Number of Illnesses Associated with the Consumption of *Vibrio parahaemolyticus* in Raw Oysters

Region	Summer (July to September)	Fall (October to December)	Winter (January to March)	Spring (April to June)	Total
Gulf Coast (Louisiana)	1,406	132	7	505	2,050
Gulf Coast (Non-Louisiana) ^a	299	51	3	193	546
Mid-Atlantic	7	4	<1	4	15
Northeast Atlantic	14	2	<1	3	19
Pacific Northwest (Dredged) ^b	4	<1	<1	<1	4
Pacific Northwest (Intertidal) ^b	173	1	<1	18	192
TOTAL	1,903	190	10	723	2,826

^a Includes oysters harvested from Florida, Mississippi, Texas, and Alabama. The time from harvest to refrigeration in these states is typically shorter than for Louisiana.

^b Oysters harvested using intertidal methods are typically exposed to higher ambient air temperature for longer times before refrigeration compared with dredged methods.

- **Post-Harvest Treatments.** Measures aimed at reducing the levels of *V. parahaemolyticus* in oysters reduce the predicted risk of illness associated with this pathogen (Table VII-4). Post-harvest treatments that reduce levels of *V. parahaemolyticus* by 2 to 4.5-logs were found to be effective for all seasons and regions, with the most pronounced effects seen for regions and seasons with higher baseline risk. The model shows that any treatment that causes at least a 4.5-log decrease in the number of *V. parahaemolyticus* bacteria reduces the probability of

illness to such an extent that few illnesses would be identified by epidemiological surveillance. However, some outbreak strains (e.g., O3:K6) are more resistant to mitigations than endemic pathogenic *V. parahaemolyticus* strains, and the duration or extent of treatment may need to be more stringent to achieve an equivalent degree of reduction. Studies have shown that both *V. parahaemolyticus* and *V. vulnificus* respond similarly to control measures such as ultra high pressure, mild heat treatment, and freezing. Therefore, mitigations aimed at decreasing levels of *V. parahaemolyticus* will also likely decrease levels of *V. vulnificus*.

Table VII-4. Predicted Mean Number of Illnesses per Annum from Reduction of Levels of Pathogenic *Vibrio parahaemolyticus* in Oysters

Region	Predicted Mean Number of Illnesses per Annum			
	Baseline	Immediate Refrigeration ^a	2-log ₁₀ Reduction ^b	4.5-log ₁₀ Reduction ^c
Gulf Coast (Louisiana)	2,050	202	22	<1
Gulf Coast (Non-Louisiana)	546	80	6	<1
Mid-Atlantic	15	2	<1	<1
Northeast Atlantic	19	3	<1	<1
Pacific Northwest (Dredged)	4	<1	<1	<1
Pacific Northwest (Intertidal)	173	100	2	<1
TOTAL	2,826	391	30	<1

^a Represents refrigeration immediately after harvest; the effectiveness of which varies both regionally and seasonally and is typically approximately 1-log₁₀ reduction.

^b Represents any process which reduces levels of *V. parahaemolyticus* in oysters 2-log₁₀ reduction, e.g. such as may be expected for freezing (-30°C).

^c Represents any process which reduces levels of *V. parahaemolyticus* in oysters achieving a 4.5-log₁₀ reduction, e.g. such as mild heat treatment (5 min at 50°C), irradiation, or ultra high hydrostatic pressure.

The model also demonstrated that if oysters are not refrigerated soon after harvest, *V. parahaemolyticus* rapidly multiply resulting in higher levels. For example, the model indicates that for the Gulf Coast there is a significant reduction (~10-fold) in the probability of illness when the oysters are placed in a refrigerator immediately after harvest. Less pronounced reductions are predicted for the other regions. Predicted reduction in illness is less in colder seasons because oysters harvested in cooler weather are already at or below the temperature threshold for *V. parahaemolyticus* growth and as such refrigeration has little additional impact on levels of *V. parahaemolyticus*.

- **At-Harvest and At-Retail Controls.** Controlling the levels of *V. parahaemolyticus* in oysters at-harvest or at-retail (after refrigeration and storage) drastically reduces the number of predicted illnesses but would require diversion of oysters from the raw market or modification of handling practices to reduce post-harvest *Vibrio parahaemolyticus* growth. For the Gulf Coast (Louisiana) region in the summer, excluding all oysters with at least 10,000 *V. parahaemolyticus*/g at-harvest would reduce illness by approximately 16% with an impact of approximately 3% of the total harvest; and this same control level at-retail would reduce illness by about 99% with a 43% loss from the raw consumption market. The effectiveness of the control level either at-harvest or at-retail to reduce illnesses depends on the extent of compliance with that control level (see Table VII-5).

Table VII-5. Effect of Compliance with Guidance Levels for *Vibrio parahaemolyticus* In Raw Oysters At-Harvest and At-Retail for the Gulf Coast (Louisiana)/ Summer Harvest

Guidance Level ^a	Compliance Level (%)	At-Harvest		At-Retail	
		Harvest Diverted (%) ^b	Illnesses Averted (%) ^c	Harvest Diverted (%) ^b	Illnesses Averted (%) ^c
100	50	33	65	47	74
	100	66	98	94	100
1,000	50	11	37	37	69
	100	21	68	75	100
5,000	50	3	14	26	63
	100	6	28	53	100
10,000	50	1	8	22	60
	100	3	16	43	99

^a Guidance level is the level of total *V. parahaemolyticus* per gram of oyster. Assumes that the level of *V. parahaemolyticus* is known either at the time of harvest or at retail.

^b Refers to the amount of the total oyster harvest that would need to be diverted from the raw oyster market or subjected to preventive controls.

^c Refers to the number of illnesses that would be prevented in comparison to the baseline model predictions.

- In a sample-based control strategy, a reasonable surrogate for pathogenic *V. parahaemolyticus* may be total levels of this microorganism. Criteria for rejection of oysters based on the levels of this surrogate might have to vary by region. For example, an at-harvest control criterion based on total *V. parahaemolyticus* levels in the Pacific Northwest might need to be more stringent than in the Gulf Coast because the incidence of pathogenic strains appears to be higher in the Pacific Northwest. However, in an outbreak, the ratio of pathogenic to total *V. parahaemolyticus* may not be the same or consistent, and the model does not evaluate how well total *Vibrio parahaemolyticus* would serve as a surrogate for pathogenic *V. parahaemolyticus* in an outbreak situation.

VII. INTERPRETATION AND CONCLUSIONS

In conclusion, the risk assessment illustrates that the levels of *V. parahaemolyticus* at-harvest play an important role in causing human illness. However, other factors that either reduce or allow growth of *V. parahaemolyticus* in oysters are also important in determining the number of illnesses. For example, shortening the time-to-refrigeration of oysters in the summer controls growth of *V. parahaemolyticus* in oysters and subsequently reduces illnesses associated with this microorganism.

The results of this risk assessment are influenced by the data and assumptions that were used to develop the Exposure Assessment and Dose-Response models. The predicted risk of illness among consumers of raw oysters and the most significant factors which influence the incidence of illness could change as a result of future data obtained from continuing surveillance studies. It is anticipated that periodic updates to the model when new data and knowledge become available will reduce the degree of uncertainty associated with the factors that influence the risk. This risk assessment provides an understanding of the relative importance and interactions among the factors influencing risk. It will hopefully provide a useful tool to facilitate the formulation of effective guidance and requirements and the evaluation of risk mitigation strategies.

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APPENDICES