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

Center for Food Safety and Applied Nutrition
Food and Drug Administration
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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 heath 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	Additional information was obtained that further the following assumptions: • Growth rates of pathogenic and non-pathogenic *V. parahaemolyticus* are similar; • Time required for refrigerated oysters to cool down to temperatures that do not support the growth of *V. parahaemolyticus* is variable and may range from 1 to 10 hours.
Additional Data/ Information	• Prevalence of total and pathogenic *V. parahaemolyticus* at harvest for Pacific Northwest region (PNW) and Gulf Coast regions; • Relationship between water temperature and *V. parahaemolyticus* levels in oysters; • Time-to-refrigeration after harvest for the PNW region.
Modeling techniques	• Included intertidal harvesting in the PNW as an additional harvest region; • Evaluated mitigation effect of specific reduction levels of *V. parahaemolyticus* in addition to types of interventions; • Included regression-based sensitivity analysis; • Added two additional uncertainty parameters (total *V. parahaemolyticus* 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

	Mean Risk Per Serving[a]				
Region	**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

	Mean Annual Illnesses[a]				
Region	**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 *V. parahaemolyticus* consumed in oysters at one sitting.
Dose-response	The relationship of the levels of *V. parahaemolyticus* 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 then 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 *V. parahaemolyticus*	For the purpose of this risk assessment, pathogenic *V. parahaemolyticus* 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 *V. parahaemolyticus* 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 *V. parahaemolyticus* 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 *V. parahaemolyticus*	The summation of pathogenic (*tdh*+) and non-pathogenic (*tdh*-) *V. parahaemolyticus* 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 product / Pwater.

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 *Vibrio* Surveillance System
IAFP	International Association for Food Protection
ICP	ISSC Interim Control Plan for monitoring levels of pathogenic *V. parahaemolyticus* 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	digoxygenin
F	Fahrenheit
/g	per gram
g	grams
gyrB	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	*Vibrio parahaemolyticus*
Vp_{path}	pathogenic strains of *V. parahaemolyticus*

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.

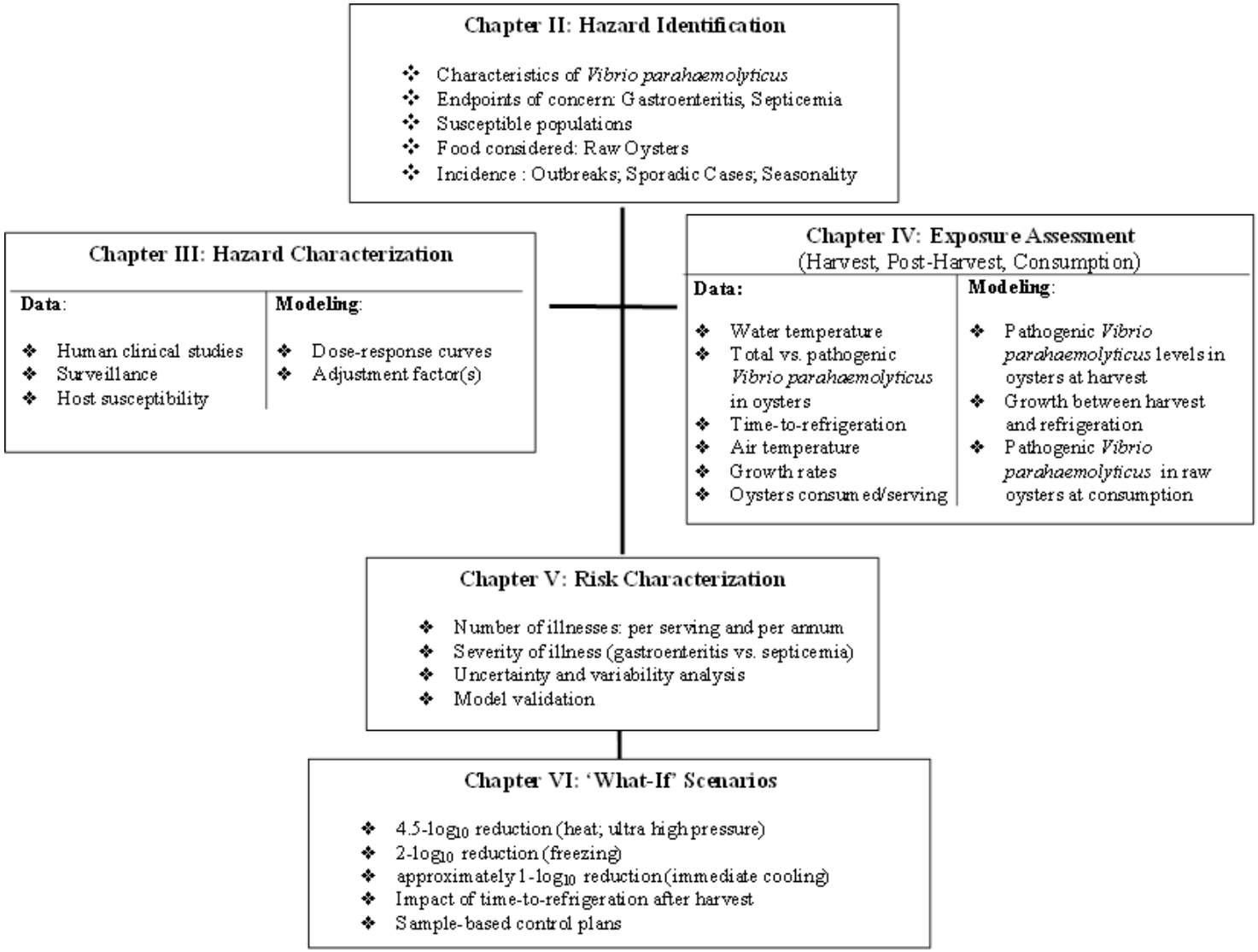

Figure I-1. Overview of *Vibrio parahaemolyticus* Risk Assessment Document

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 then 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])

[a] The Pacific Northwest includes California, Oregon, Washington State, and British Columbia.
[b] Number of cases that were culture-confirmed.
[c] Includes 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 parahameolyticus* 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 then 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 virulant 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 ID_{50} (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 10^6 cells. Diarrhea occurred in both individuals fed approximately 10^7 cells. The ingested doses were not directly determined, but were instead estimated assuming that *V. parahaemolyticus* cultures can reach maximum growth densities of approximately 10^{10} 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 10^9 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 x 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 x 10^5 viable cells, and 2 of 4 volunteers experienced abdominal discomfort and diarrhea at 3 x 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 *Vibrio parahaemolyticus*	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 *et al*, 1968	Yes	No	Yes	No
Kasai, 1971	Yes	Yes	No	No
Morris *et al*., 1990	No (*V. cholerae*)	Not applicable	Yes	No
Morris *et al*., 1995	No (*V. cholerae*)	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.

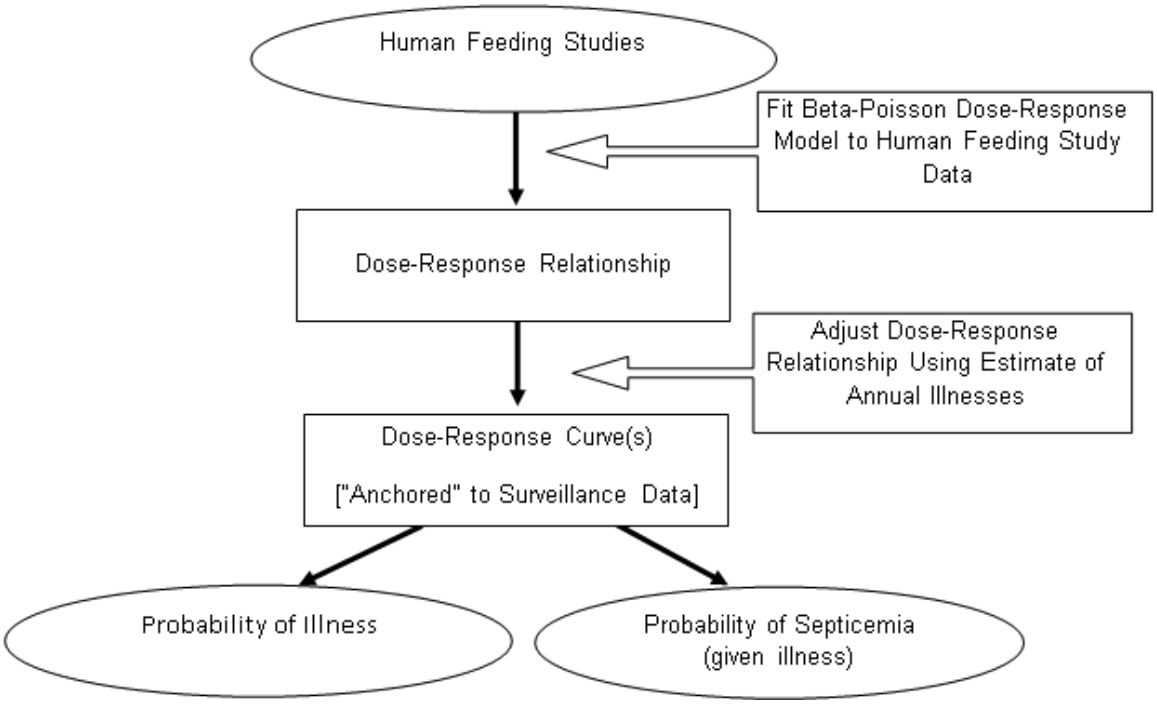

Figure III-1. Schematic Representation of the Development of the Vibrio parahaemolyticus Dose-Response Model

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 $2x10^2$ and $2x10^5$ cfu of *V. parahaemolyticus*. However, at higher doses (>$1x10^6$ *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	$\Pr(\text{ill} \mid \text{d}) = 1-(1+\text{d}/\beta)^{-\alpha}$
Probit	$\Pr(ill \mid d) = \Phi(\alpha+\beta*\log_{10}(d))$
Gompertz	$\Pr(ill \mid d) = 1-\exp[-\exp[\alpha+\beta*\log_{10}(d)]]$

[a] For 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 $\alpha << \beta$ (and $\beta>>1$). 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 ID_{50}) 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 ID_{50}, 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.

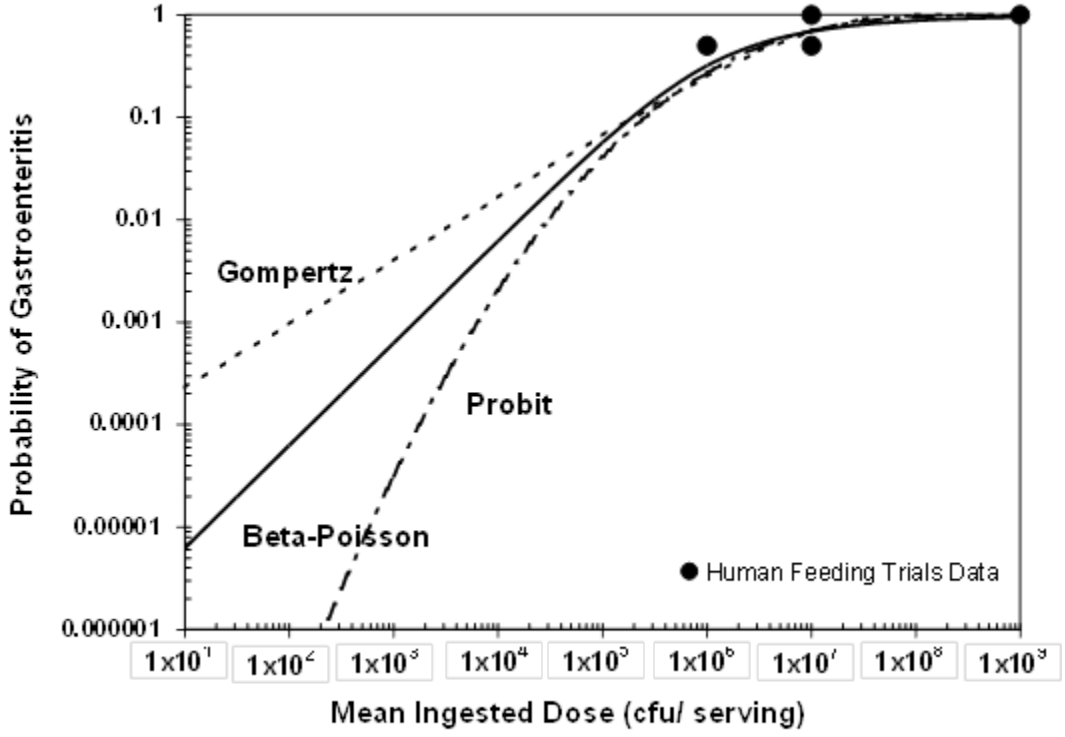

Figure III-2. Comparison of the Beta-Poisson, Gompertz, and Probit Dose-Response Models Fit to Data from Human Feeding Studies

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 ID_{50} 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:

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

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.

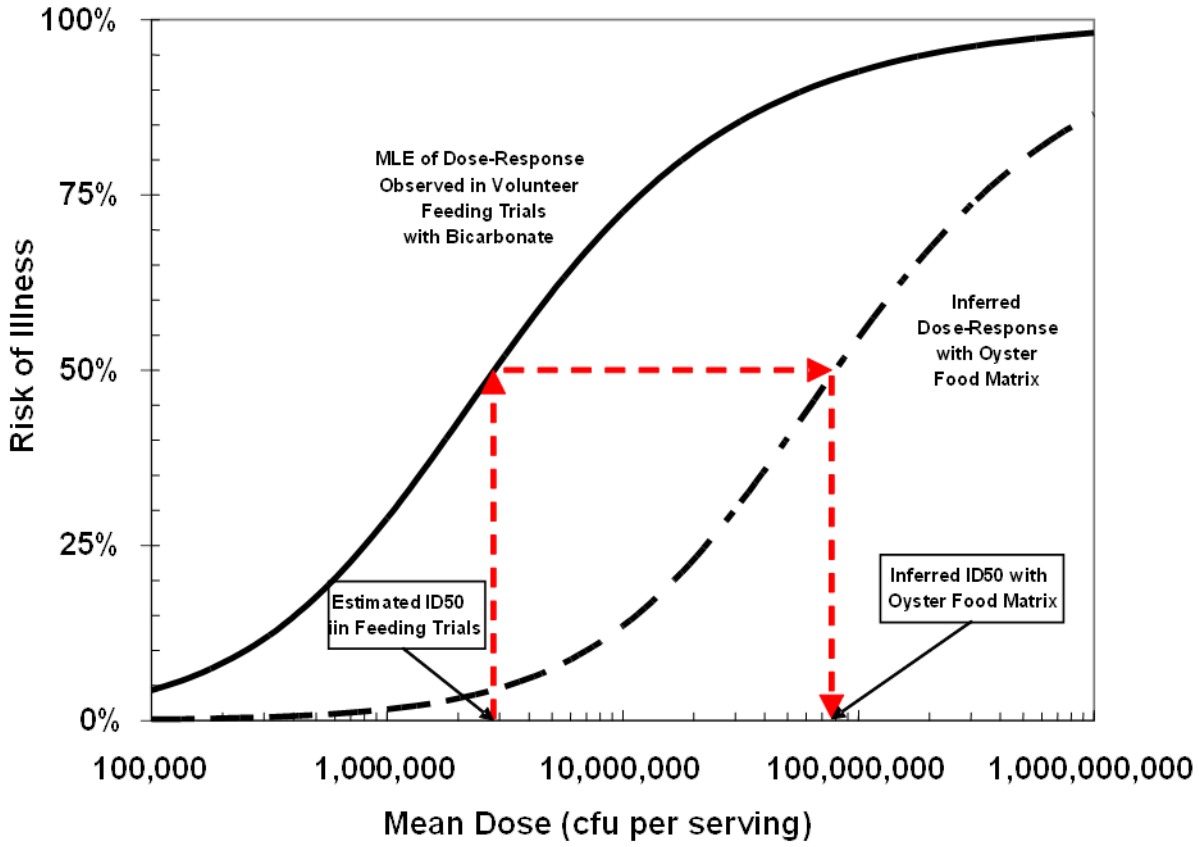

Figure III-3. The Beta-Poisson Dose-Response Model for *Vibrio parahaemolyticus* Fit to Human Feeding Trials and Adjusted Using Epidemiological Surveillance Data
[The solid line is the best estimate of the Beta-Poisson Model fit to pooled human feeding studies. The dashed line shows the shift adjustment so that the model predictions agree with epidemiological surveillance data. MLE denotes the maximum likelihood estimate. ID_{50} 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.

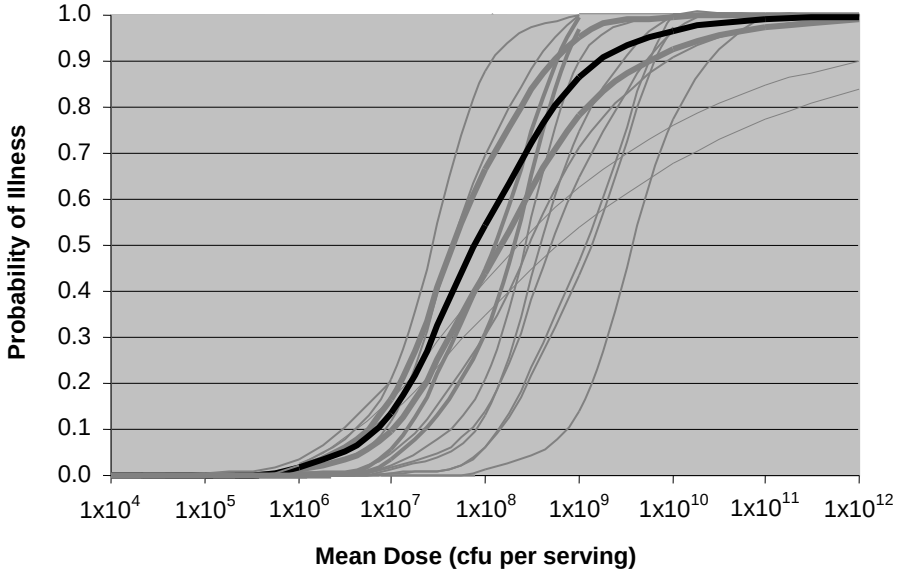

Figure III-4. *Vibrio parahaemolyticus* Dose-Response Curve and Uncertainty
[The dark line indicates the dose-response curve with the highest weighting (16.5%) and the 20 gray lines represent the dose-response curves with lower weightings (<1% to 13%).]

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 $1x10^9$ 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.

$$\Pr(\text{illness outcome} \mid \text{health status}) = \frac{\Pr(\text{health status} \mid \text{illness outcome}) * \Pr(\text{illness outcome})}{\Pr(\text{health status})}$$

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:

$$\Pr(\textit{septicemia} \mid \text{immunocompromised}) = \frac{\Pr(\text{immunocompromised} \mid \textit{septicemia}) * \Pr(\textit{septicemia})}{\Pr(\text{immunocompromised})}$$

$$= \frac{3/4 * 5/107}{23/79} = 0.12$$

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.

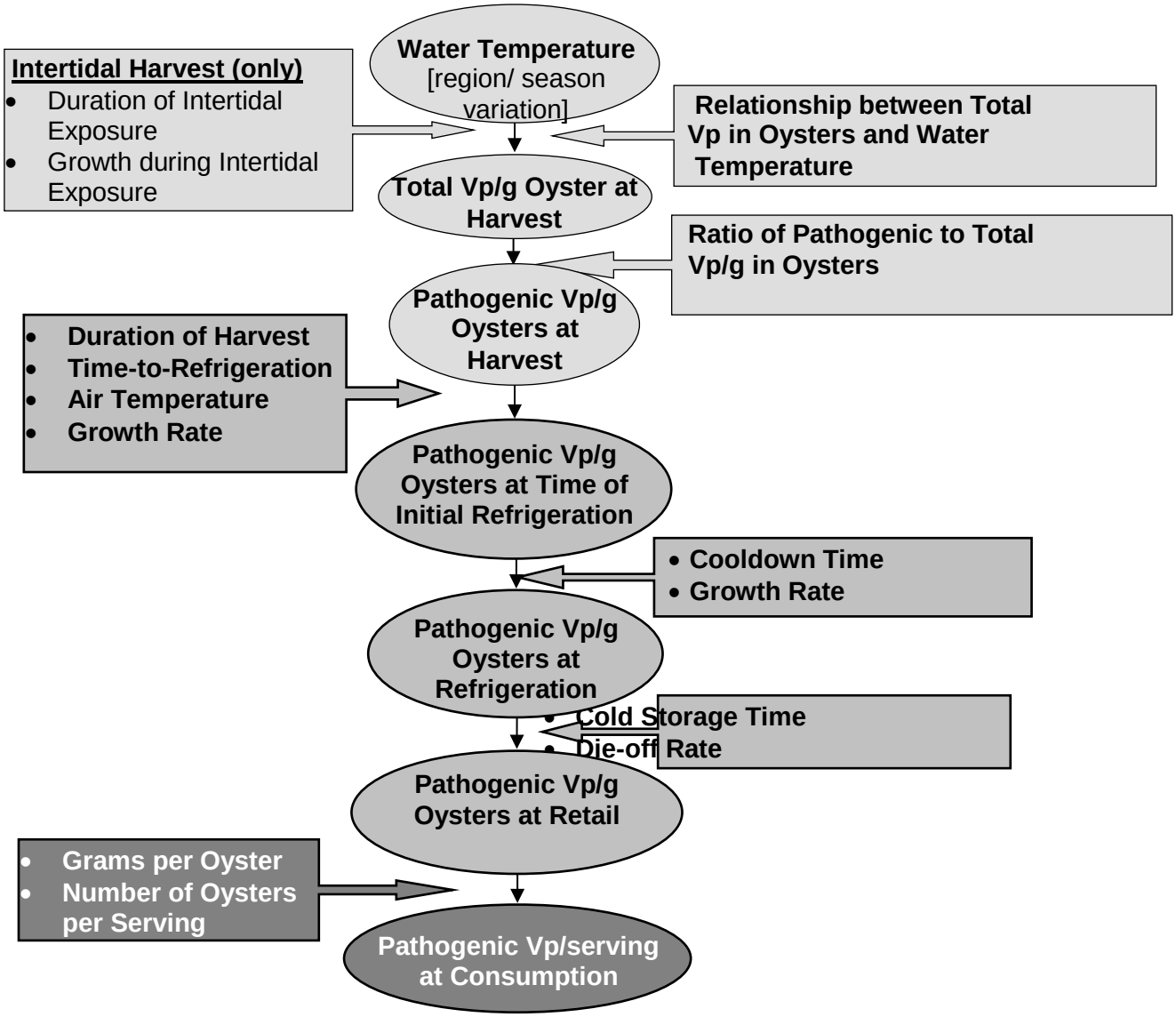

Figure IV-1. Schematic Representation of the Exposure Assessment Component of the *Vibrio parahaemolyticus* (Vp) Risk Assessment Model

[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.

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 to1999)	Yes	Yes (Pacific Northwest)
EPA STORET[c]	Yes (since 1964)	No	No
NERR[d]	No	Yes	No[e]

Study	Criteria		Used in Harvest Module?
	Long-Term Historical Data Base	Electronically Available Records	
	(since 1995)		
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.

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 ºC (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.

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.

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 *et al*., 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 *et al*.,1989	Yes	No	Not U.S.	No	No
Kiiyukia *et al*., 1989	Yes	Yes	Not U.S.	No	No
Ogawa *et al*.,1989	Yes	Yes	Not U.S.	Yes	No
Kaysner *et al.* , 1990a	Yes	No	No	No	No
Tepedino, 1982	Yes	No	No	No	No
Herwig and Cheney, 2001	Yes	Yes	No	No	No
Depaola *et al*., 2000	Yes	No	Yes	No	No
DePaola *et al*., 2002	Yes	No	No	No	No
Kaufman *et al*., 2003	Yes	Yes	No	No	No

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

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-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 *et al*., 2002	Yes	Yes	Yes
Kaufman *et al.*, 2003	Yes	Yes	Yes
DePaola *et al*., 2000	Yes	Yes	No[a]
FDA/ISSC, 2000; Cook *et al.*, 2002a	Yes	No[b]	No
FDA/ISSC, 2001; Cook *et al*., 2002b	Yes	Yes	No[c]
Thompson *et al*., 1976	Yes	No	No[d]
Kaysner *et al*., 1990	Yes	Yes	No[d]
DePaola *et al.*, 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.

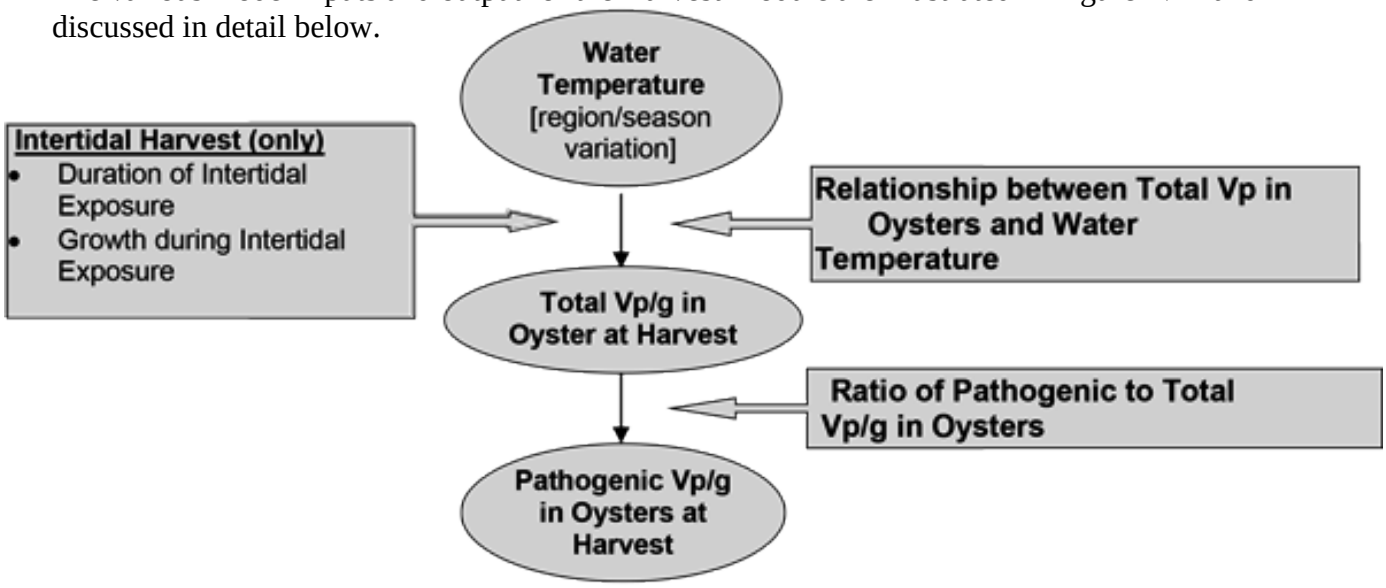

Figure IV-2. Schematic Depiction of the Harvest Module of the *Vibrio parahaemolyticus* (Vp) Exposure Assessment Model

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 ^{o}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 ^{o}C. Similarly, for the standard deviation of the within year temperature distributions, the central tendency across different years was an average of 3.5 ^{o}C with a variance of 0.27, which corresponds to a standard deviation of 0.52 ^{o}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 where 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 *et al*., 1990	Northeast Atlantic Mid-Atlantic Gulf Coast Pacific Northwest	61[a]	Membrane filtration	10 cfu/g
FDA/ISSC, 2001/ Cook *et al*., 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 5^{th} and 95^{th} 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 $\log_{10}$ total *V. parahaemolyticus* densities was approximately linear in the range of water temperatures sampled.

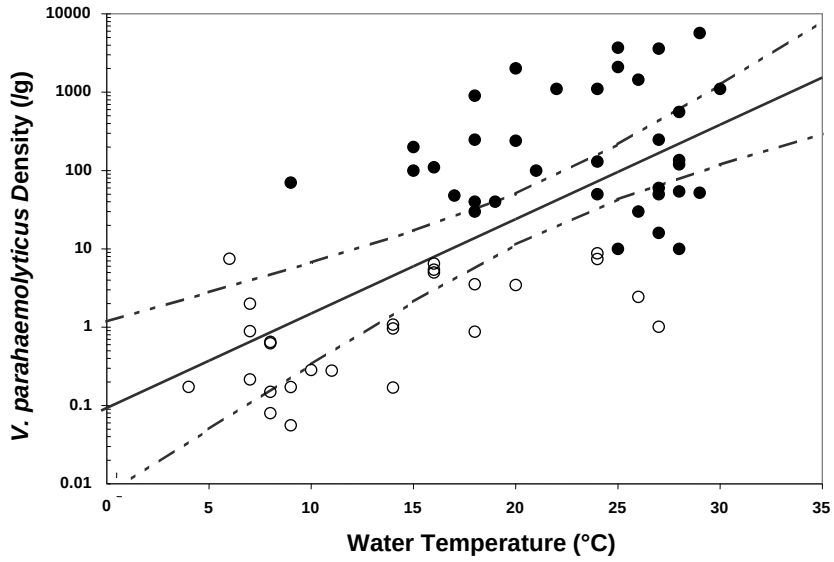

Figure IV-3. Tobit Regression Fit of *Vibrio parahaemolyticus* Densities in Oysters Versus Water Temperature Using the DePaola et al. (1990) Data Set

[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).]

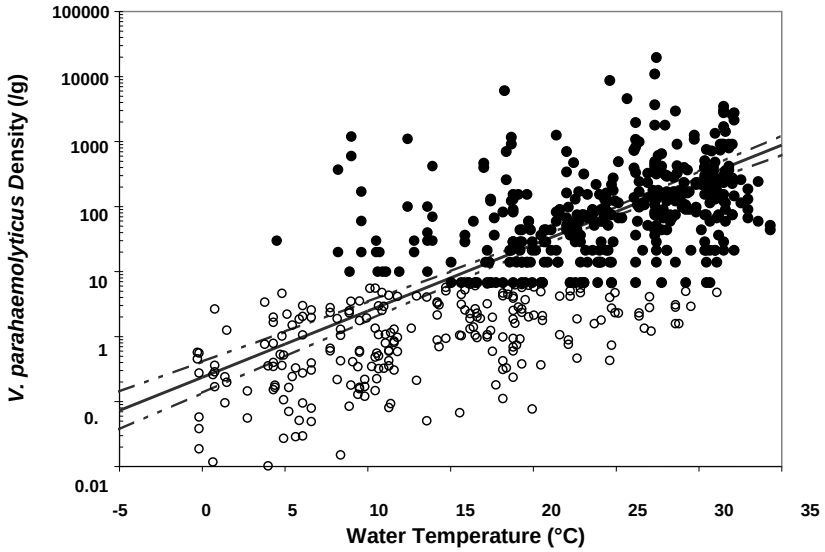

Figure IV-4. Tobit Regression Fit of the *Vibrio parahaemolyticus* Densities in Oysters Versus Water Temperature Using the FDA/ISSC (2001) Data Set

[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).]

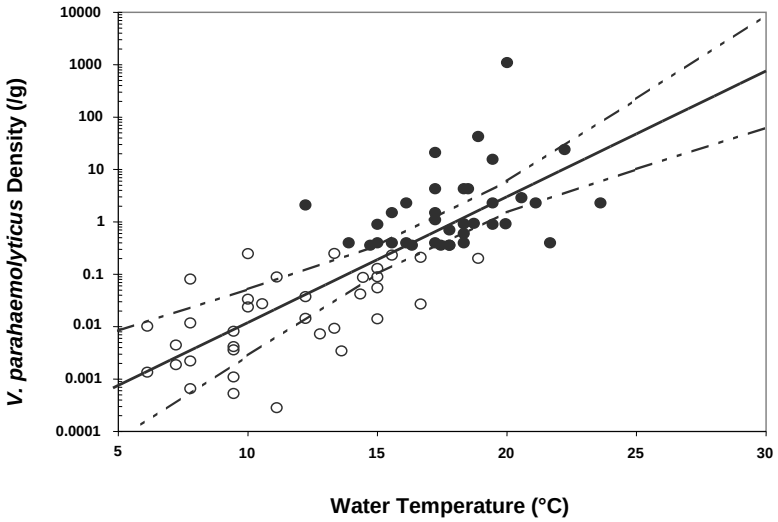

Figure IV-5. Tobit Regression Fit of the *Vibrio parahaemolyticus* Densities in Oysters Versus Water Temperature Using the State Department of Health (2000; 2001) Data Sets
[Solid line is the best estimate of the median *V. parahaemolyticus*/g. Dashed lines show the 5th and 95th % confidence limits. 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		*Vibrio parahaemolyticus* Isolates			Region
Number Tested	Number Pathogenic[a]	Number Tested[b]	Number Pathogenic[a]	Pathogenic (%)	(Study)
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 *et al*., 2003)
198	8	3,429 (DP)	9 TDH+	0.3	Gulf Coast, Mid-Atlantic, Northeast Atlantic (FDA/ISSC, 2000; Cook *et al.*, 2002a)
106	3	5,600 (MNP+DP)	16 TDH+	0.3	Texas (DePaola *et al.*, 2000)
156	34	6,018 (EB) 6,992 (DP)	46 31	0.76 0.44	Gulf Coast (DePaola *et al*., 2003a)[g]
65	13	1,103 [e] (DP)	27 [e]	2.3[f]	Pacific Northwest (DePaola *et al*., 2002)
23	1	308 (MPN)	10 TDH+	3.2	Pacific Northwest (Kaysner *et al*., 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 ^{o}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 *V. parahaemolyticus*/g [a]	Mean Pathogenic *V. parahaemolyticus*/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 ºC for 10 hours and a 790-fold increase after 24 hours. After refrigeration at 3 ºC 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.

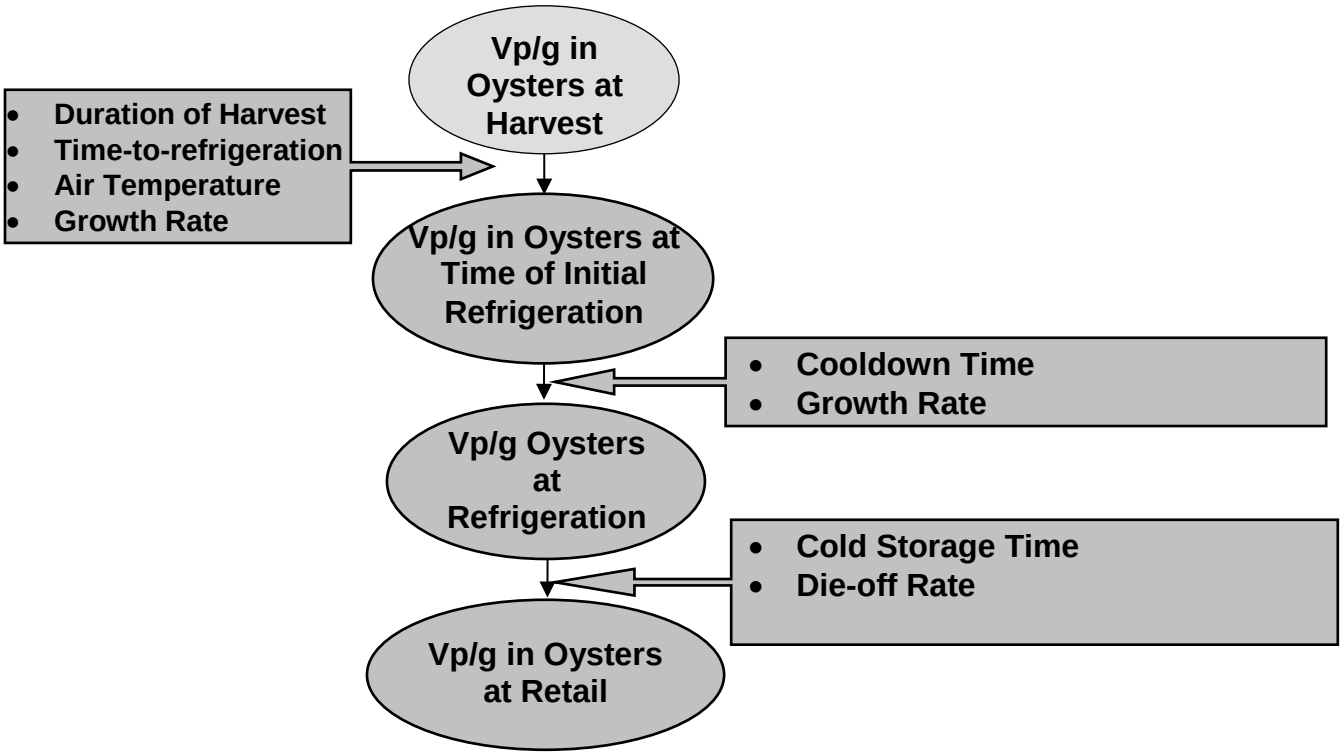

Figure IV-6. Schematic Depiction of the Post-Harvest Module of the *Vibrio parahaemolyticus* Exposure Assessment Model
[Vp/g is *Vibrio parahaemolyticus* per gram oyster. Levels of total and pathogenic *V. parahaemolyticus* were simulated by the model separately and in parallel.]

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 ^{o}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 ^{o}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 ^{o}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:

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

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 ^oC, 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 ^oC (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).

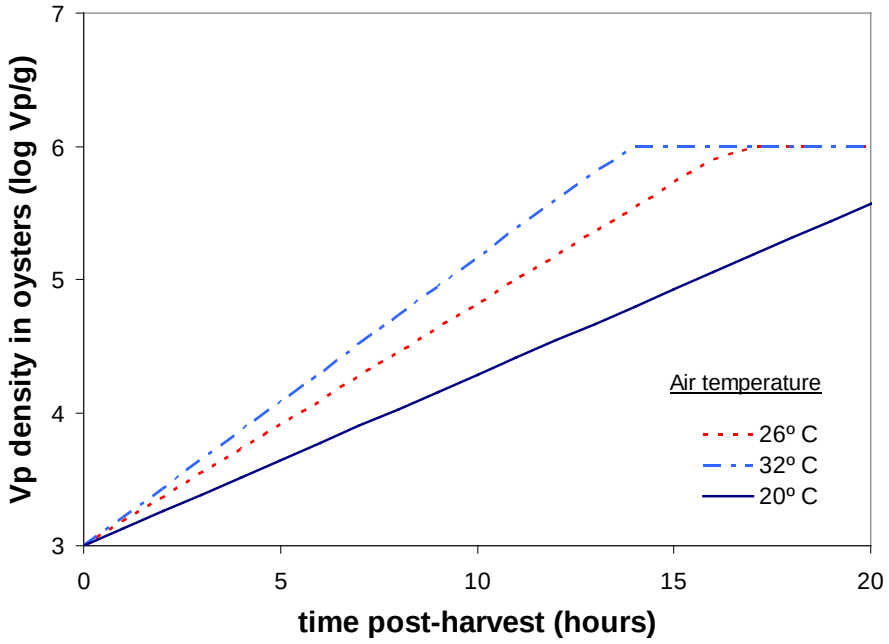

Figure IV-7. Predicted Mean Loglinear Growth of *Vibrio parahaemolyticus* in Oysters from an Initial Density of 1,000 (3-log$_{10}$) *Vibrio parahaemolyticus* per gram as a Function of Ambient Air Temperature

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 NDBC; 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).

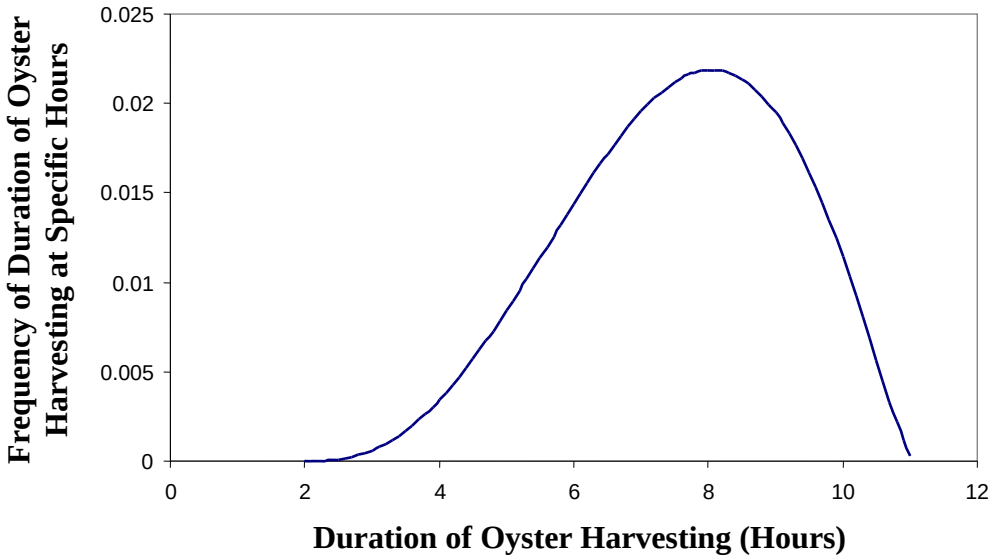

Figure IV-8. Example Beta-PERT Probability Density Distribution for Duration of Oyster Harvesting

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; μm=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-log_{10} per gram could not be exceeded. Specifically, the potential amount of additional growth is the sum of the growth over the T hours:

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

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

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

where N represents the density of *V. parahaemolyticus* at the time of first refrigeration and A is the maximum attainable density (6-log_{10} 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*μm in the absence of the truncation effect, where μm is the maximal growth rate determined by ambient air temperature at time of harvest. Thus, for an initial growth rate of 0.19-$\log_{10}$ per hour (i.e., at 26 ^{o}C), the average growth occurring during cooldown is approximately 0.6-$\log_{10}$ 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-$\log_{10}$ 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-$\log_{10}$ 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 *V. parahaemolyticus* [a]	Mean Pathogenic *V. parahaemolyticus* [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.

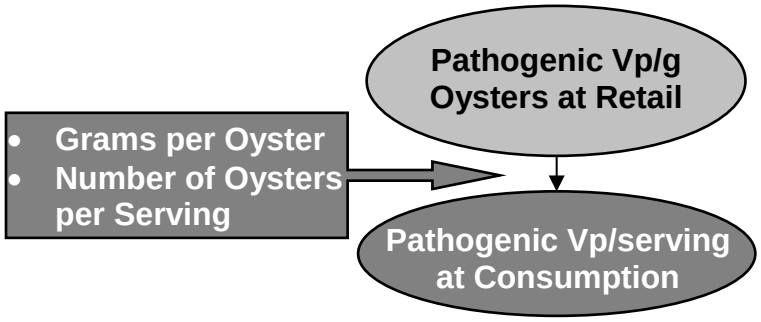

Figure IV-9. Schematic Depiction of the Consumption Module of the *Vibrio parahaemolyticus* Exposure Assessment Model

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.

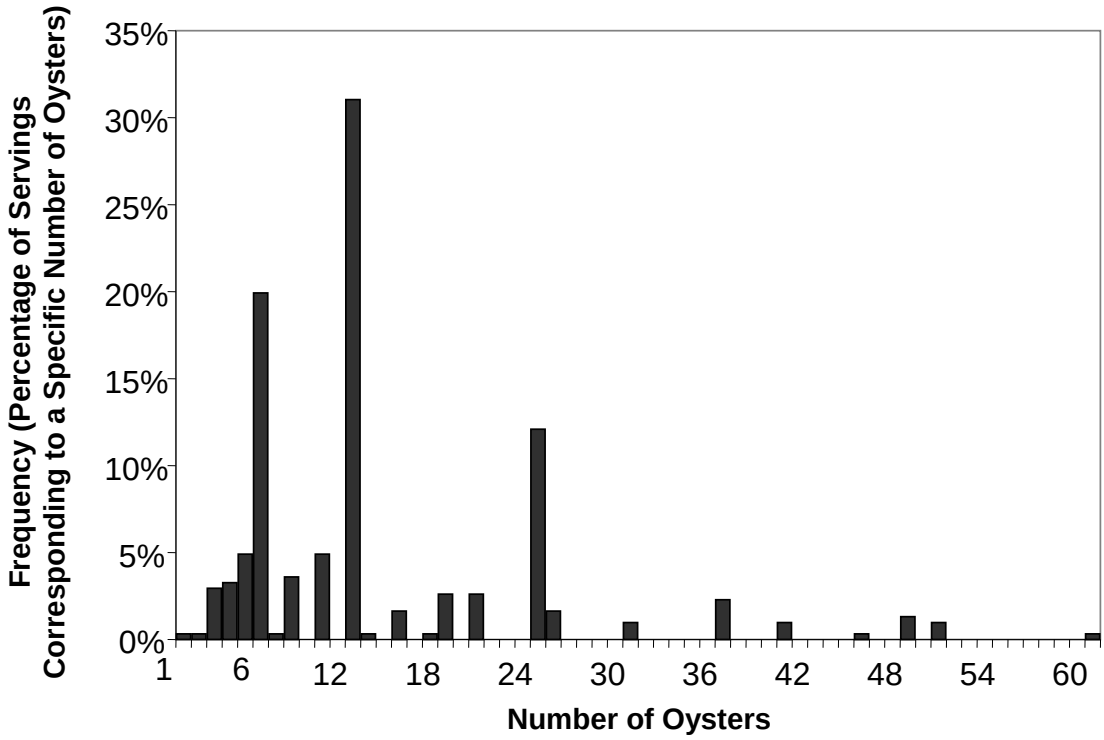

Figure IV-10. Self-reported Frequency of Number of Oysters Consumed per Serving (University of Florida Consumption Survey) (Degner and Petrone, 1994).

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]				
	Winter (Jan - March)	**Spring** (April - June)	**Summer** (July - Sept)	**Fall** (Oct - Dec)	**Total**
Gulf Coast					
Louisiana	2,751,000	2,630,000	2,854,000	2,769,000	11,004,000
Non-Lousiana	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 x 16,400,000 kg)/0.2 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]				
	Winter (Jan - March)	**Spring** (April - June)	**Summer** (July - Sept)	**Fall** (Oct - Dec)	**Total**
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 *V. parahaemolyticus* per Serving[a]	Mean Pathogenic *V. parahaemolyticus* 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)	$7.9\text{x}10^3$ ($2.3\text{x}10^3$, 1.8×10^4)
	Summer	1.2×10^7 (5.5×10^6, 2.2×10^7)	2.1×10^4 ($7.5\text{x}10^3$, 4.4×10^4)
	Fall	1.2×10^6 (2.6×10^5, 2.8×10^6)	$2.0\text{x}10^3$ (320, $5.1\text{x}10^3$)
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)	$5.6\text{x}10^3$ ($1.5\text{x}10^3$, 1.3×10^4)
	Summer	8.5×10^6 (3.6×10^6, 1.7×10^7)	1.5×10^4 ($4.9\text{x}10^3$, 3.2×10^4)
	Fall	5.0×10^5 (9.0×10^4, 1.3×10^6)	880 (110, $2.5\text{x}10^3$)
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)	$1.5\text{x}10^3$ (330, $3.5\text{x}10^3$)
	Summer	2.5×10^6 (5.4×10^5, 6.3×10^6)	$4.3\text{x}10^3$ (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, $2.6\text{x}10^3$)
	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	$1.9\text{x}10^3$ (2.3, $8.7\text{x}10^3$)	42 (0. 4, 160)
	Summer	$2.1\text{x}10^4$ ($1.3\text{x}10^3$, $8.7\text{x}10^4$)	460 (21, $2.1\text{x}10)^3$
	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, $3.7\text{x}10^4$)
	Summer	3.3×10^5 (2.4×10^4, 1.2×10^6)	$7.5\text{x}10^3$ (370, 3.0×10^4)
	Fall	800 (31, $3.5\text{x}10^3$)	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.

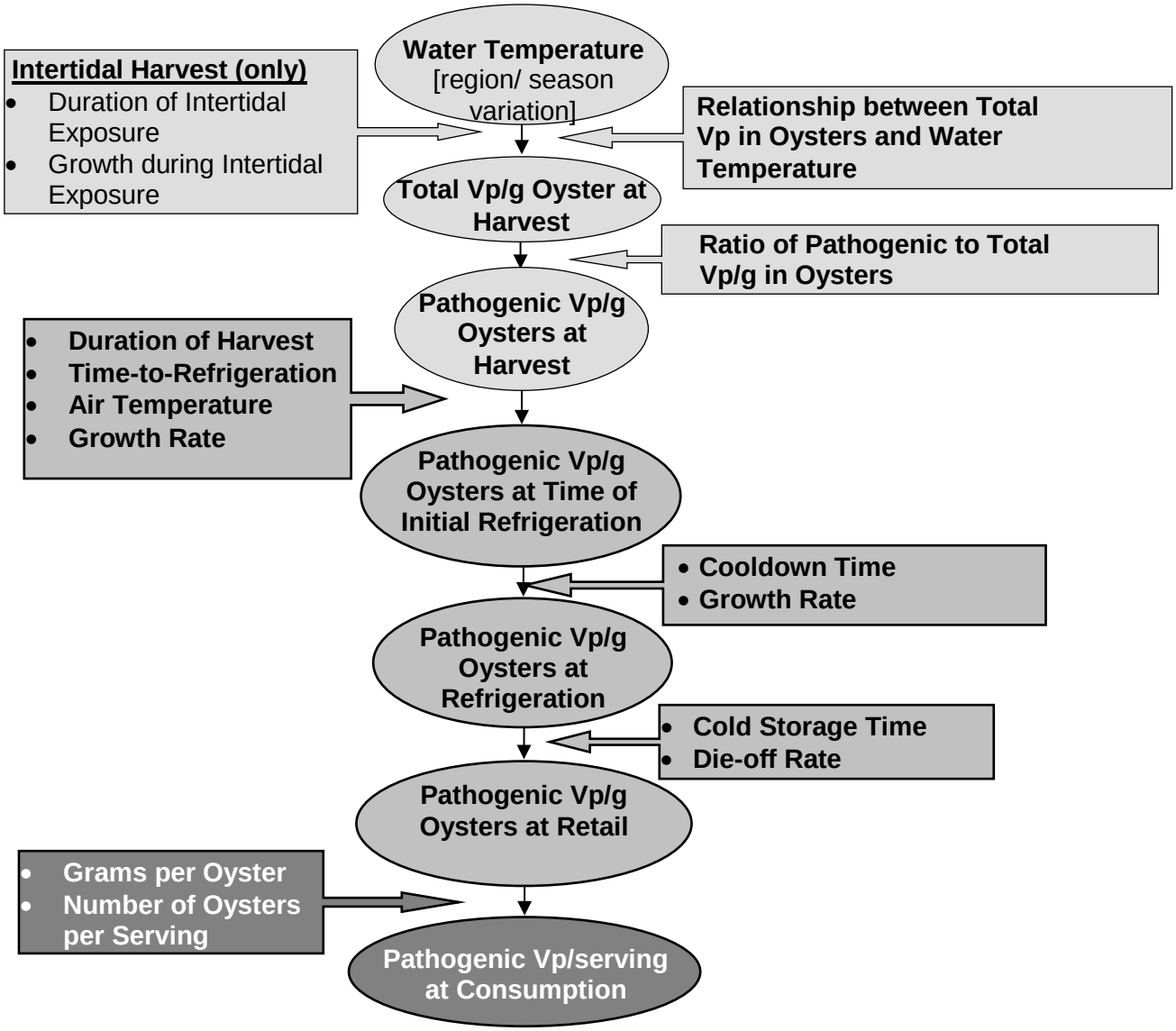

Figure IV-1. Schematic Representation of the Exposure Assessment Component of the *Vibrio parahaemolyticus* (Vp) Risk Assessment Model

[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.]

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 $1x10^{-8}$ 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)	4.4×10^{-4} (3.4×10^{-5}, 1.4×10^{-3})	4.3×10^{-5} (2.1×10^{-6}, 1.5×10^{-4})	2.1×10^{-6} (5.2×10^{-8}, 8.3×10^{-6})	1.7×10^{-4} (1.2×10^{-5}, 5.4×10^{-4})	6.6×10^{-4}
Gulf Coast (Non-Louisiana)[c]	3.1×10^{-4} (2.3×10^{-5}, 1.0×10^{-3})	1.9×10^{-5} (7.4×10^{-7}, 6.6×10^{-5})	1.1×10^{-6} (3.1×10^{-8}, 4.2×10^{-6})	1.2×10^{-4} (8.3×10^{-6}, 3.9×10^{-4})	4.5×10^{-4}
Mid-Atlantic	9.2×10^{-5} (4.9×10^{-6}, 3.3×10^{-4})	2.2×10^{-6} (4.9×10^{-8}, 1.0×10^{-5})	1.1×10^{-8} 4.9×10^{-10}, 3.8×10^{-8})	3.1×10^{-5} (1.8×10^{-6}, 1.1×10^{-4})	1.3×10^{-4}
Northeast Atlantic	1.8×10^{-5} (8.4×10^{-7}, 6.9×10^{-5})	4.0×10^{-7} (1.2×10^{-8}, 1.6×10^{-6})	1.1×10^{-8} (4.9×10^{-10}, 3.5×10^{-8})	3.6×10^{-6} (8.4×10^{-8}, 1.5×10^{-5})	2.2×10^{-5}
Pacific Northwest (Dredged)[d]	1.0×10^{-5} (1.6×10^{-7}, 4.2×10^{-5})	2.6×10^{-8} (6.9×10^{-10}, 9.5×10^{-8})	8.1×10^{-10} (3.2×10^{-11}, 3.2×10^{-9})	8.7×10^{-7} (4×10^{-9}, 3.1×10^{-6})	1.1×10^{-5}
Pacific Northwest (Intertidal)[d]	1.4×10^{-4} (3.2×10^{-6}, 6.2×10^{-4})	3.9×10^{-7} (3.1×10^{-9}, 1.6×10^{-6})	1.7×10^{-9} (5.5×10^{-11}, 6.5×10^{-9})	1.3×10^{-5} (2.3×10^{-8}, 5.8×10^{-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 in parentheses are the 5th and 95th percentiles of the uncertainty distribution. Values rounded to 2 significant digits.

[b] Note: 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.

[d] Oysters 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]				
	Summer (July to Sept)	**Fall** (October to December)	**Winter** (January to March)	**Spring** (April to June)	**Total**
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.

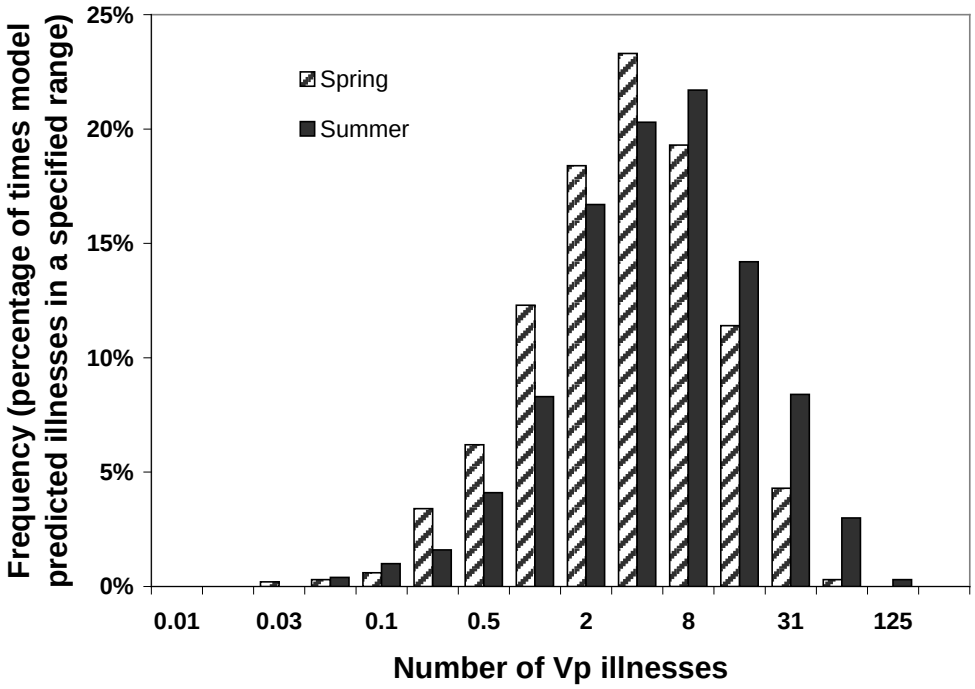

Figure V-2. Uncertainty Distribution of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Mid-Atlantic Harvests

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.

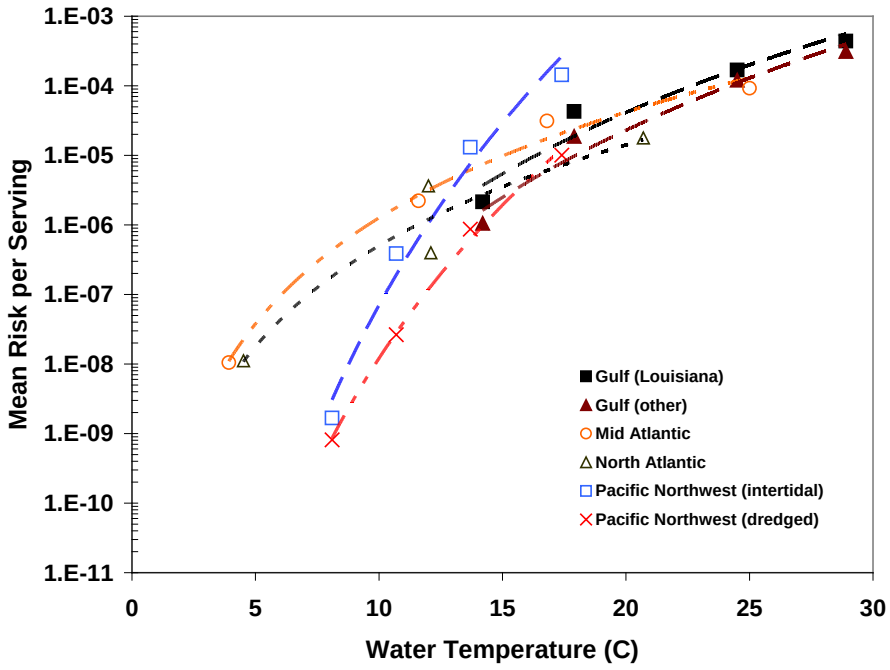

Figure V-3. Influence of Water Temperature on Variation of Mean Risk per Serving for Each Region

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

	Variability Factors in Order of Importance[a]					
Season	**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

[a]Log_{10} VP = log_{10} *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.

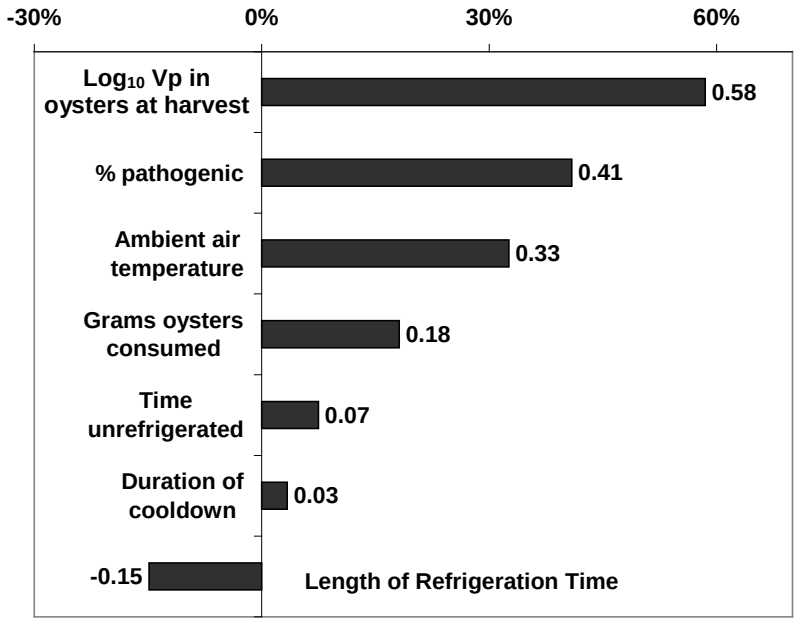

Figure V-4. Tornado Plot of Influential Variability Factors on *Vibrio parahaemolyticus* (Vp) Illness per Serving of Raw Oysters for the Gulf Coast (Louisiana) Winter Harvest

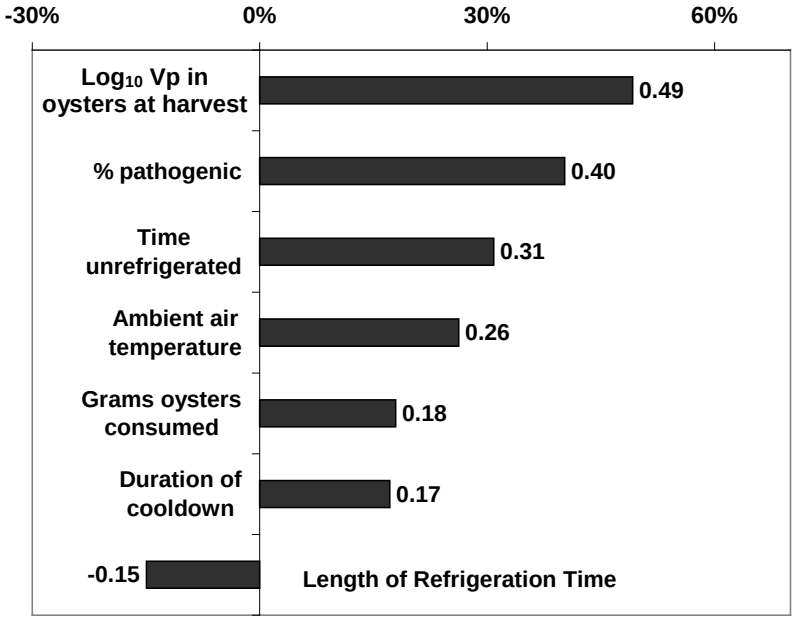

Figure V-5. Tornado Plot of Influential Variability Factors of *Vibrio parahaemolyticus* (Vp) Illness per Serving of Raw Oysters for the Gulf Coast (Louisiana) Summer Harvest

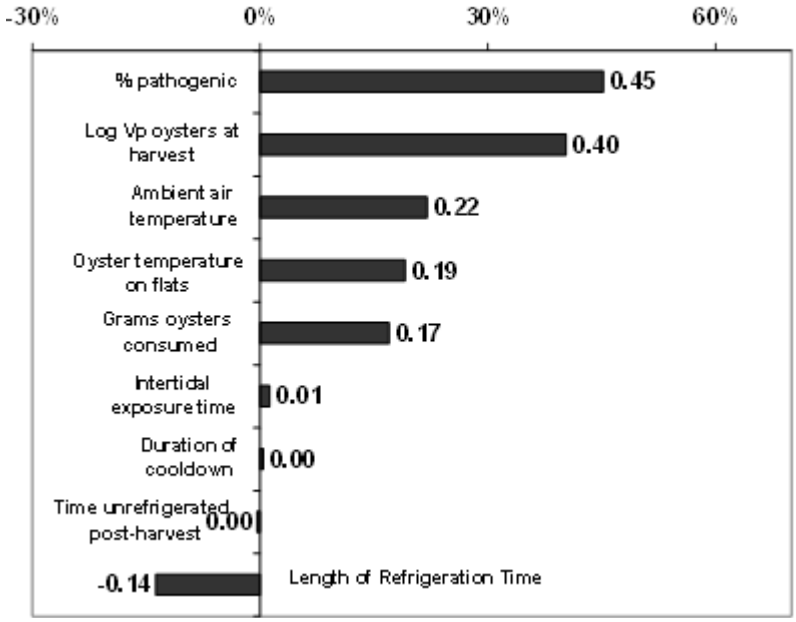

Figure V-6. Tornado Plot of Influential Variability Factors on *Vibrio parahaemolyticus* (Vp) Illness per Serving of Raw Oysters for the Pacific Northwest Coast (Intertidal) Spring Harvest

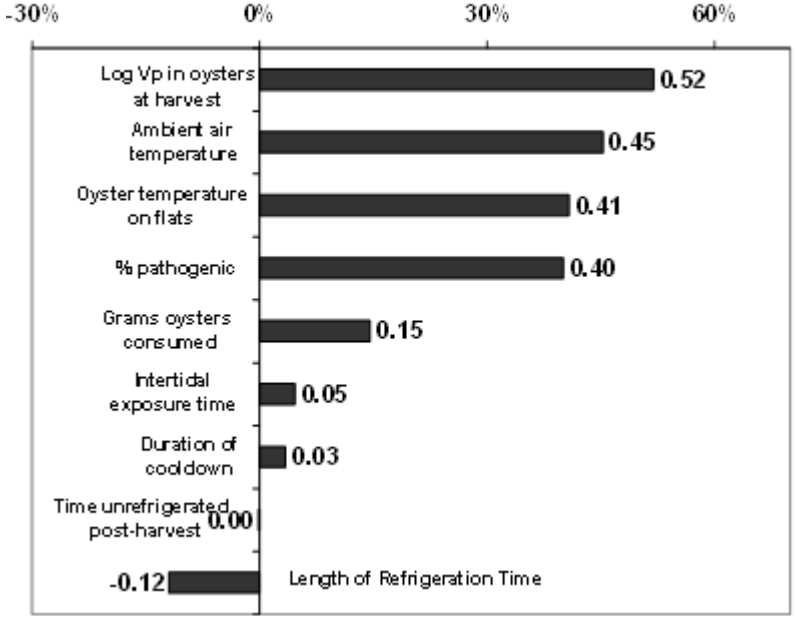

Figure V-7. Tornado Plot of Influential Variability Factors on *Vibrio parahaemolyticus* (Vp) Illness per Serving of Raw Oysters for the Pacific Northwest Coast (Intertidal) Winter Harvest

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.

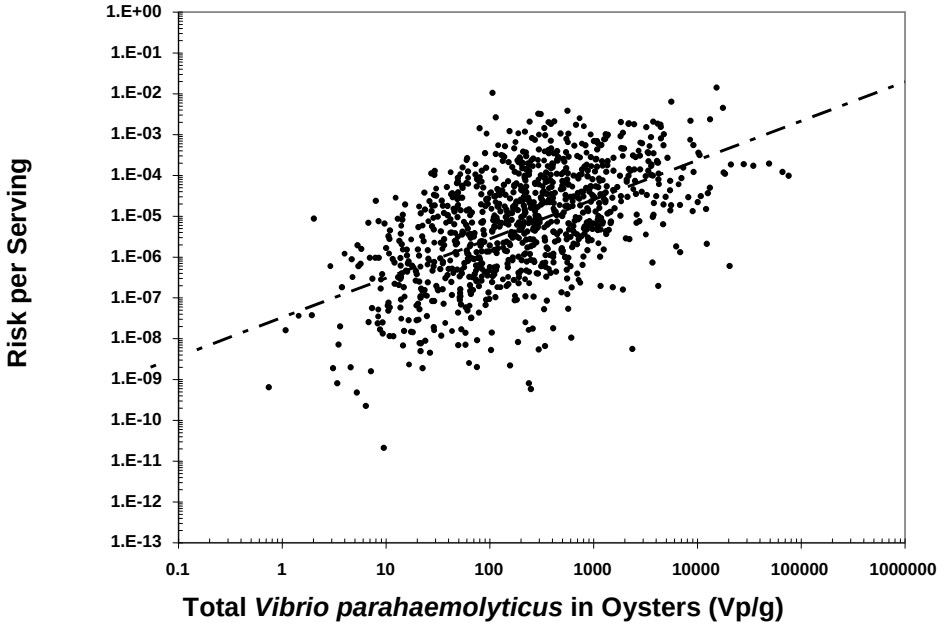

Figure V-8. Correlation of Risk per Serving and Total *Vibrio parahaemolyticus* in Oysters at Harvest for the Gulf Coast (Louisiana) Summer

[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.

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

where Var(risk) denotes the unconditional variance of the uncertainty distribution of mean risk per serving and Var(risk|no variation of the i^{th} 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	5.23×10^{-8}	75.4%
Percentage pathogenic	1.77×10^{-7}	16.5%
Growth rate in oysters	1.83×10^{-7}	13.8%
Relationship of total *V. parahaemolyticus* levels and water temperature	1.89×10^{-7}	11.1%
Year-to-year water temperature variation	2.08×10^{-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 2.12×10^{-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.

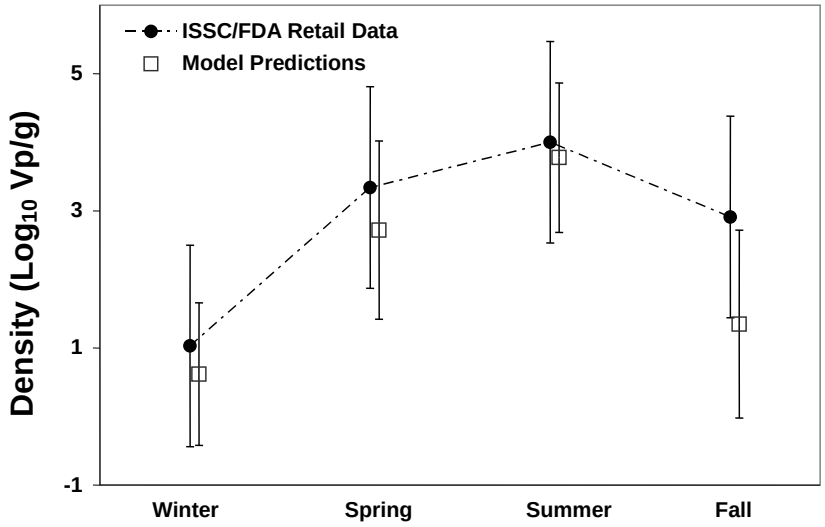

Figure V-9. Observed $\log_{10}$ Density of Total *Vibrio parahaemolyticus* at Retail (Cook et al., 2002a) Compared to Model Predictions for the Gulf Coast (Louisiana) Harvest

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

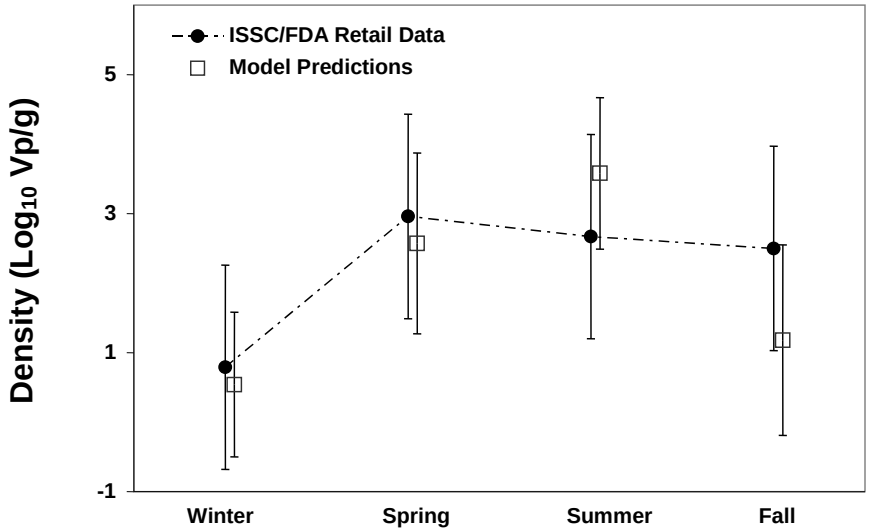

Figure V-10. Observed log10 Density of Total Vibrio parahaemolyticus at Retail (Cook et al., 2002a) Compared to Model Predictions for the Gulf Coast (non-Louisiana) Harvest

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

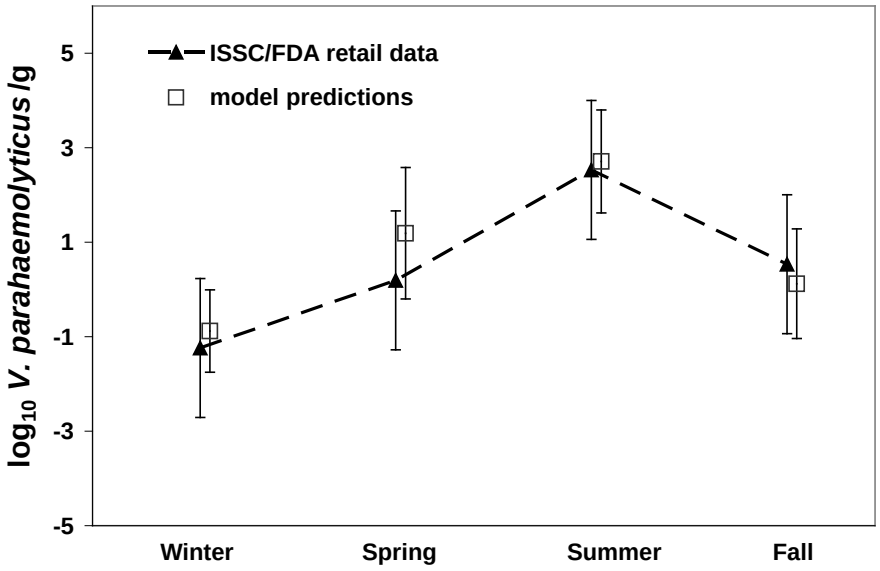

Figure V-11. Observed $\log_{10}$ Density of Total *Vibrio parahaemolyticus* at Retail (Cook *et al*., 2002a) Compared to Model Predictions for the Mid-Atlantic Coast Harvest

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

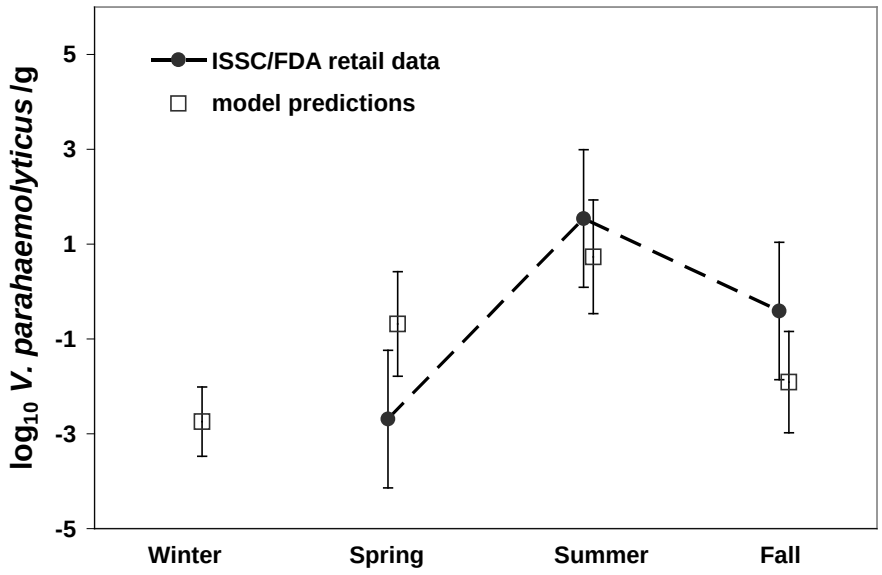

Figure V-12. Observed $\log_{10}$ Density of Total *Vibrio parahaemolyticus* at Retail (Cook et al., 2002a) Compared to Model Predictions for the Pacific Northwest (Dredged and Intertidal) Region

[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.

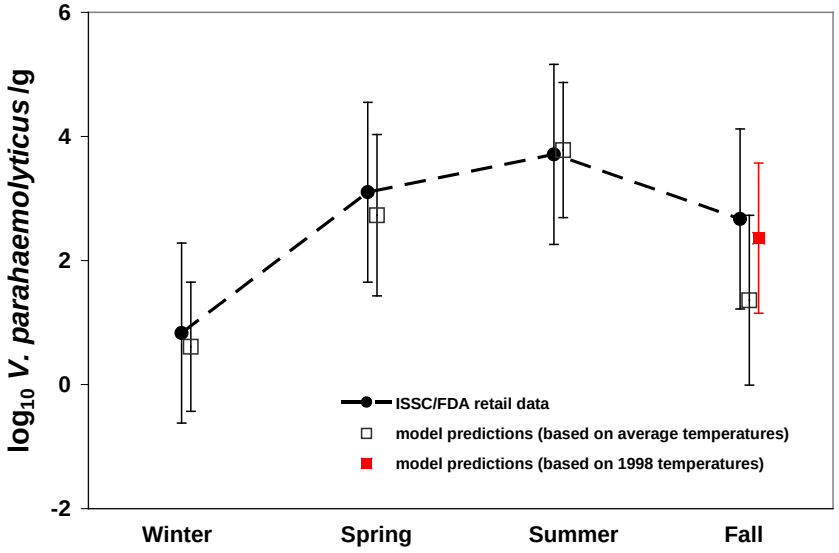

Figure V-13. Observed $\log_{10}$ Density of Total *Vibrio parahaemolyticus* at Retail (Cook *et al*., 2002a) Compared to Model Predictions for the Gulf Coast (Lousisana and non-Lousisana) Based on 1998 Fall Temperature

[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).

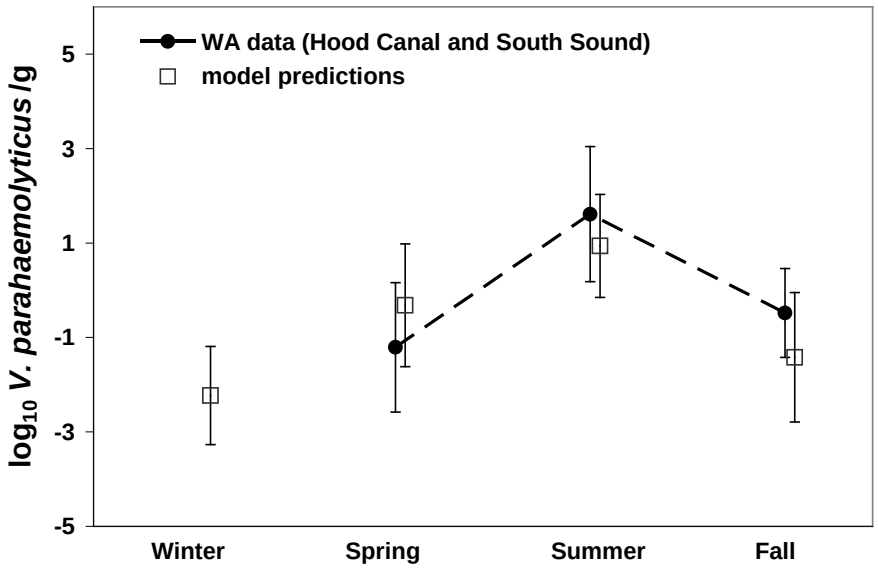

Figure V-14. Observed Log_{10} Density of Total *Vibrio parahaemolyticus* for the Pacific Northwest (Intertidal) Region (Washington State Department of Health, 2001) Compared to Model Predictions

[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-log_{10} 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 10^7 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-$\log_{10}$ 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 ^{o}C (59 °F) (Greenberg *et al*., 1982). Son and Fleet (1980) observed a 5-$\log_{10}$ reduction in lab-infected oysters (from $9x10^7$ to $8x10^2$ 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.

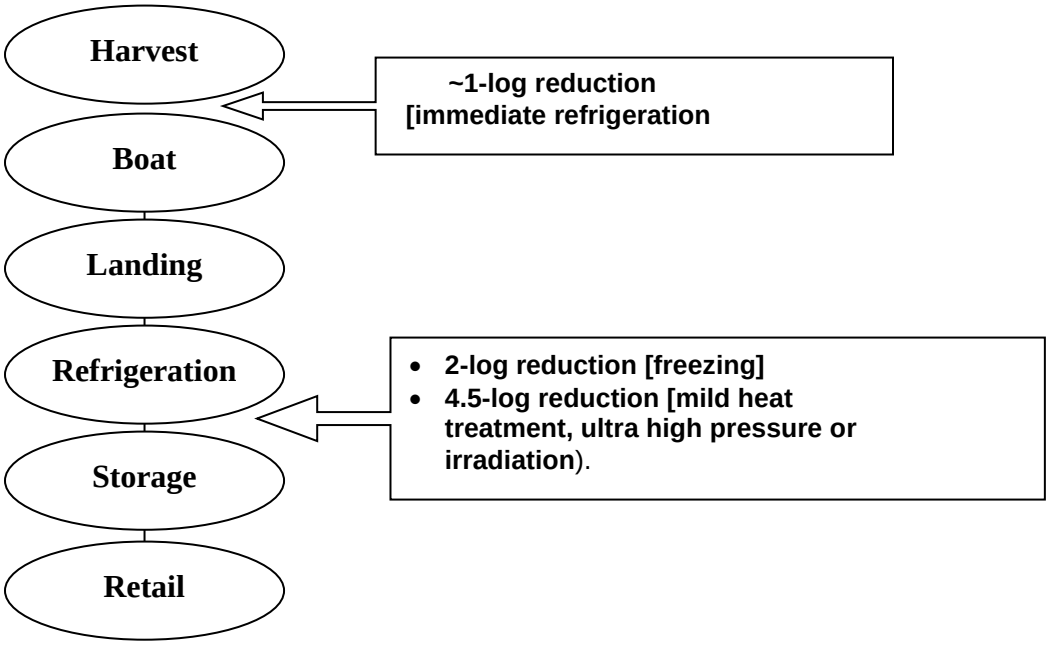

Figure VI-1. Schematic Representation from Harvest to Retail Showing Steps at which Evaluated Mitigations Occur

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

[a]Values 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.

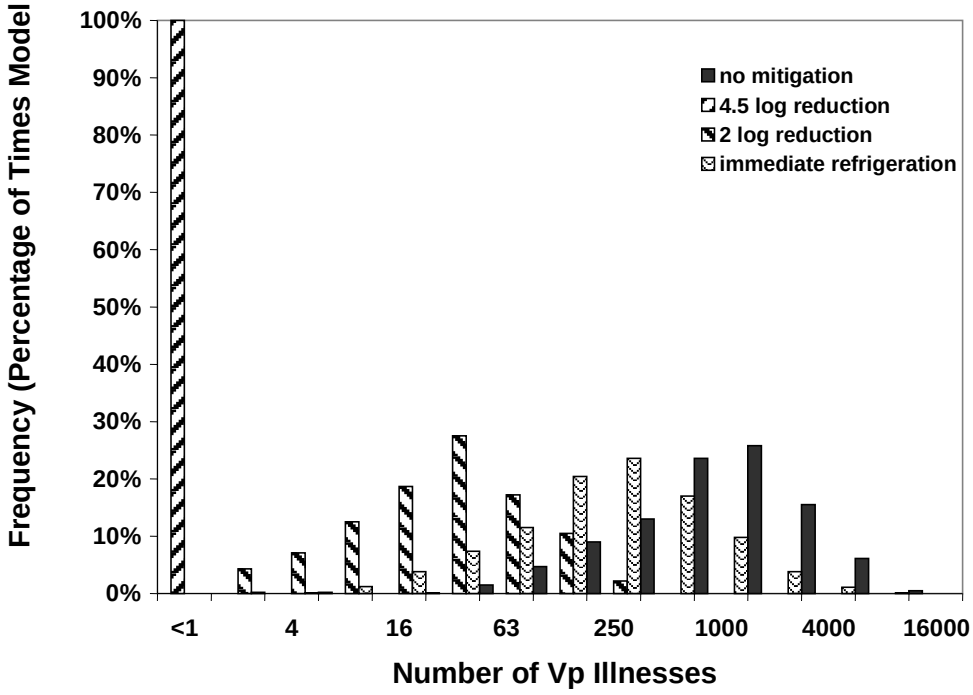

Figure VI-2. Effect of Potential Mitigations on the Distribution of Probable Number of Illnesses Associated with *Vibrio parahaemolyticus* in Oysters Harvested from the Gulf Coast (Louisiana) in the Summer

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.

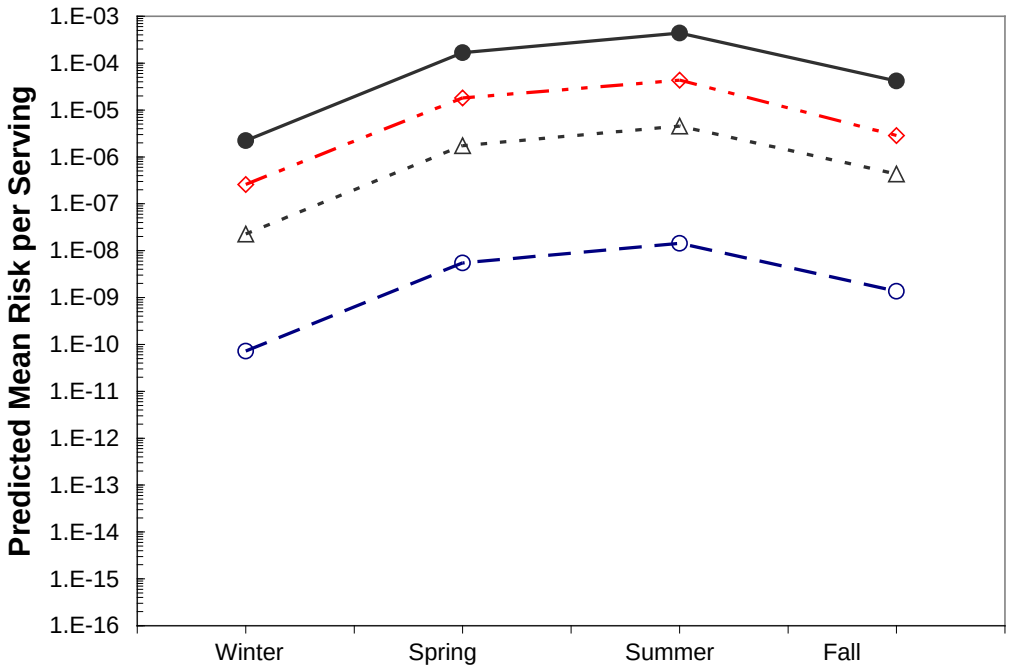

Figure VI-3. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Gulf Coast (Louisiana) Harvest

[No mitigation (●); immediate refrigeration upon harvest (◊); treatment resulting in a 2-$\log_{10}$ reduction (Δ); treatment resulting in a 4.5-$\log_{10}$ reduction (○).]

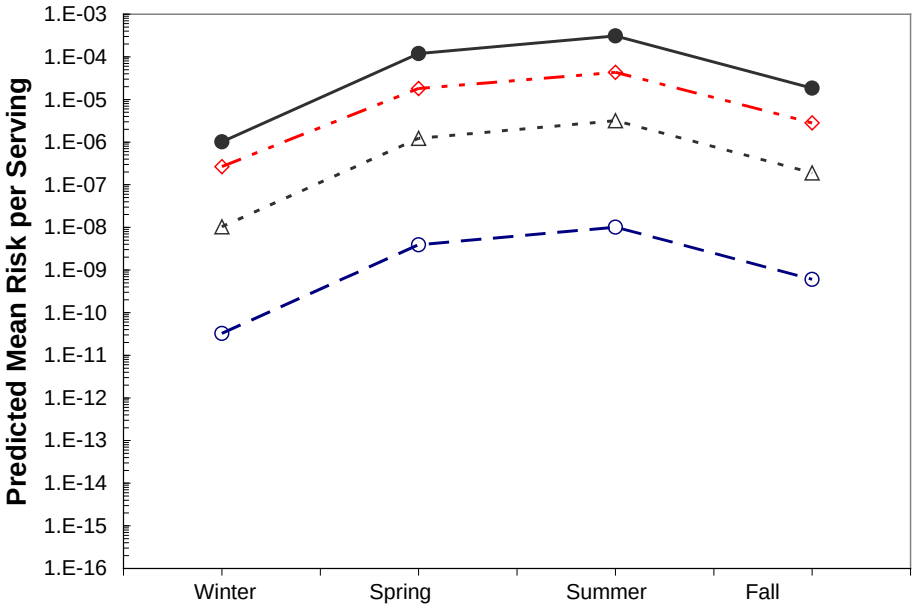

Figure VI-4. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Gulf Coast (Non-Louisiana) Harvest

[No mitigation (●); immediate refrigeration upon harvest (◊); treatment resulting in a 2-$\log_{10}$ reduction (Δ); treatment resulting in a 4.5-$\log_{10}$ reduction (○).]

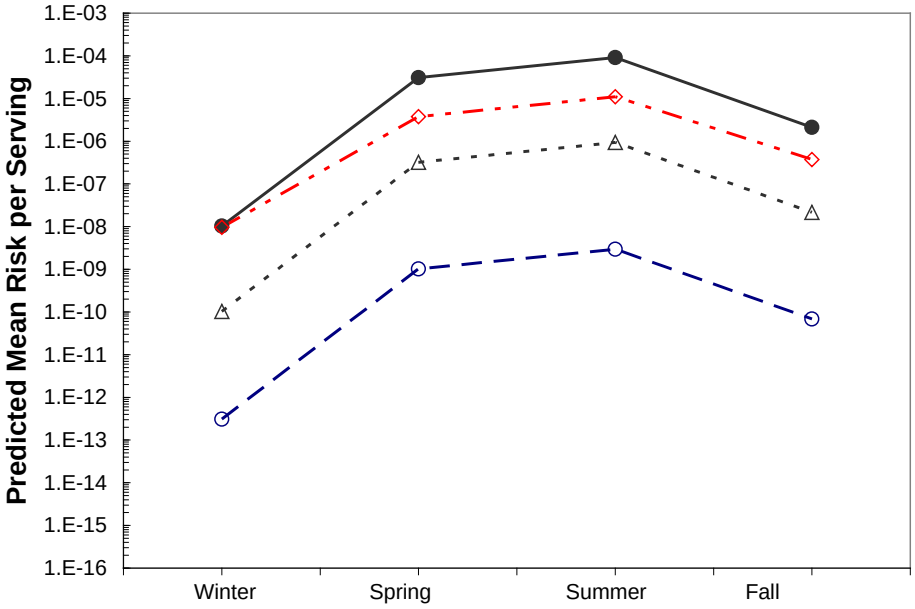

Figure VI-5. Effect of Potential Mitigations on Mean Risk of Vibrio parahaemolyticus Illnesses per Serving Associated with the Mid-Atlantic Harvest

[No mitigation (●); immediate refrigeration upon harvest (◊); treatment resulting in a 2-$\log_{10}$ reduction (Δ); treatment resulting in a 4.5-$\log_{10}$ reduction (○).]

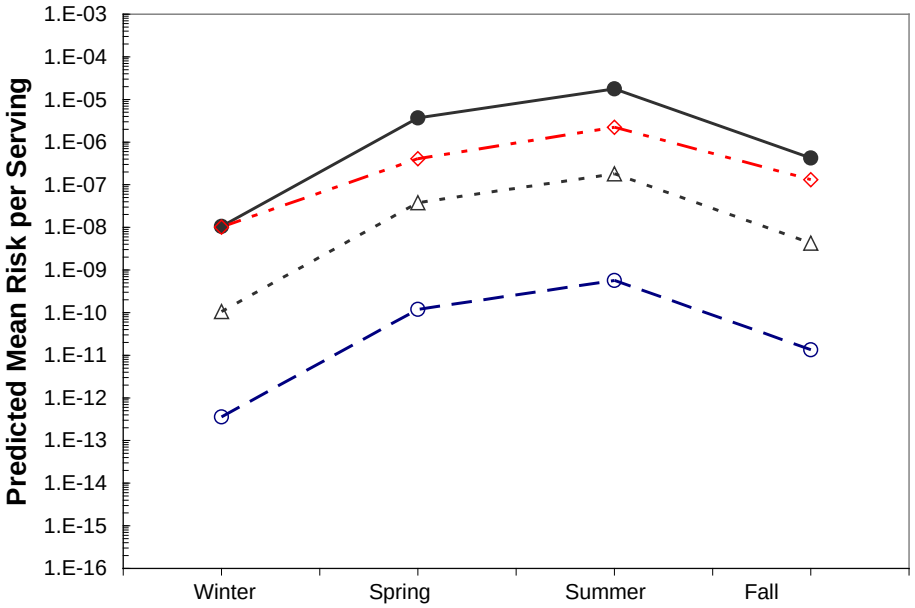

Figure VI-6. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Northeast Atlantic Harvest

[No mitigation (●); immediate refrigeration upon harvest (◊); treatment resulting in 2-$\log_{10}$ reduction (Δ); treatment resulting in a 4.5-$\log_{10}$ reduction (○).]

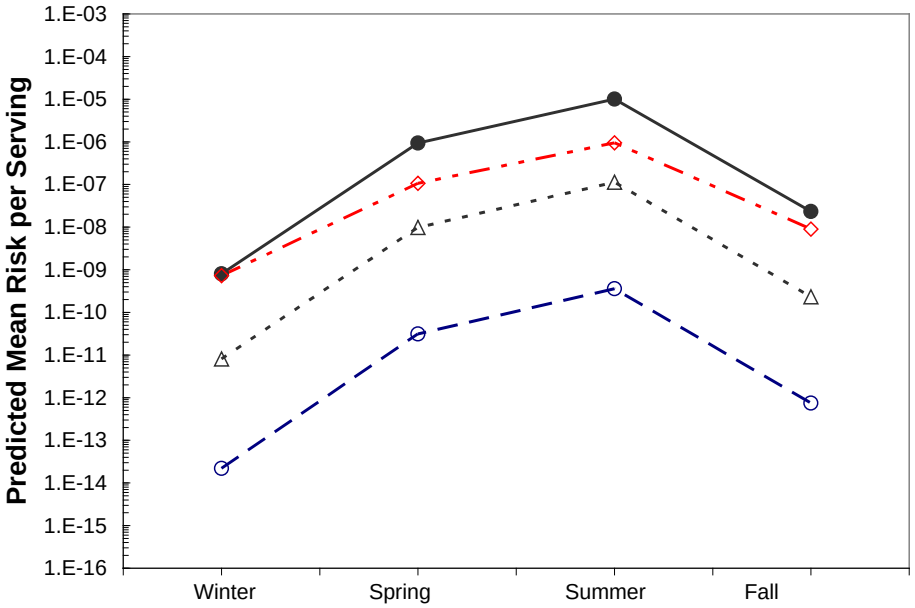

Figure VI-7. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Pacific Northwest (Dredged) Harvest

[No mitigation (●); immediate refrigeration upon harvest (◊); treatment resulting in 2-$\log_{10}$ reduction (Δ); treatment resulting in a 4.5-$\log_{10}$ reduction (○).]

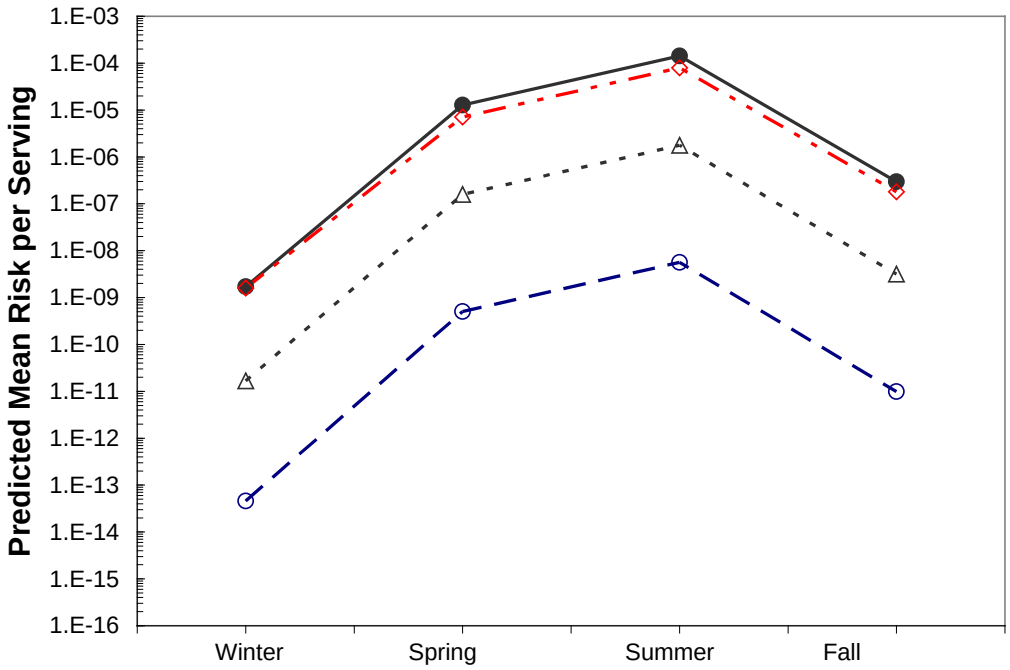

Figure VI-8. Effect of Potential Mitigations on Mean Risk of *Vibrio parahaemolyticus* Illnesses per Serving Associated with the Pacific Northwest (Intertidal) Harvest

[No mitigation (●); immediate refrigeration upon harvest (◊); treatment resulting in 2-$\log_{10}$ reduction (Δ); treatment resulting in a 4.5-$\log_{10}$ 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.

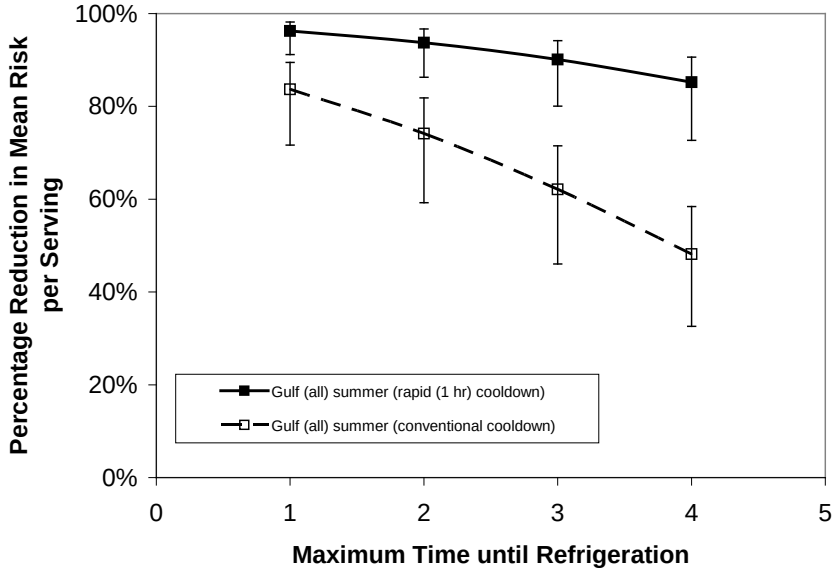

Figure VI-9. Predicted Effectiveness of Rapid versus Conventional Cooling on *Vibrio parahaemolyticus* Risk for Gulf Coast Summer Harvest

[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

[a]This 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.

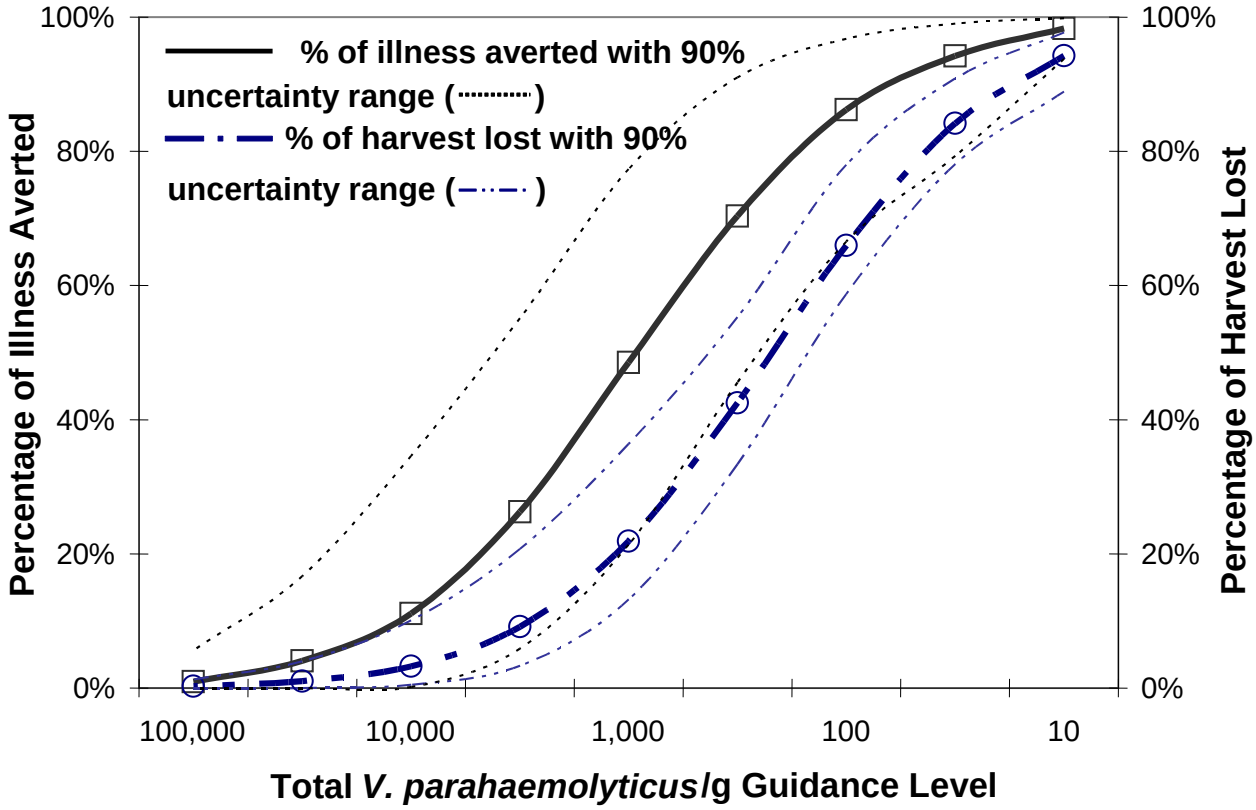

Figure VI-10. Predicted Effect of Control of Total *Vibrio parahaemolyticus* per Gram Oysters at Time of Harvest for the Gulf Coast (Louisiana) Summer Harvest
[The term in Figure VI-10 "harvest lost" refers to the portion of the harvest that would have to be diverted from the "raw market."]

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."

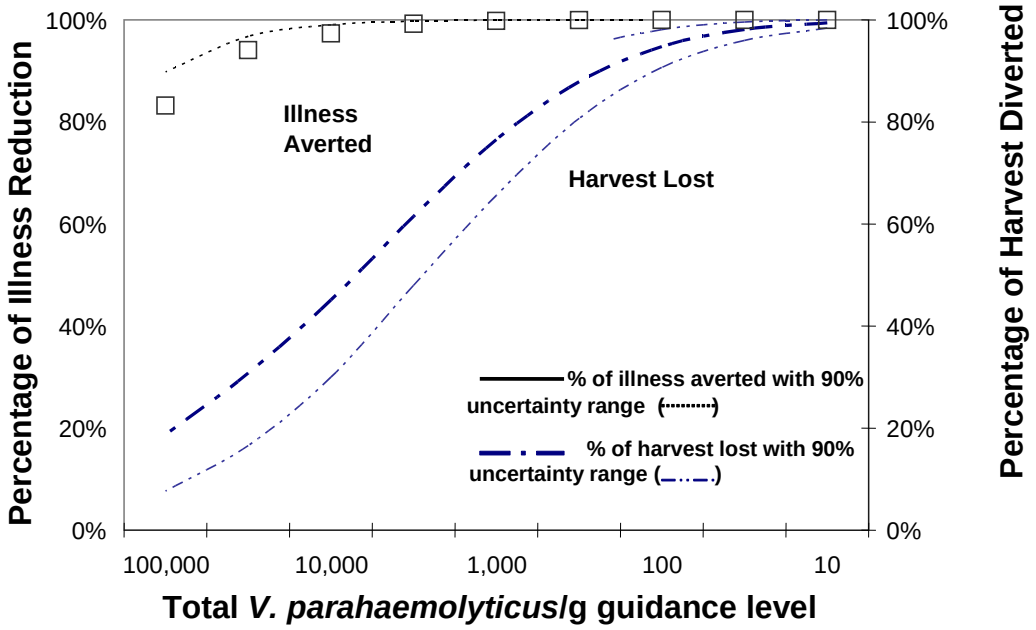

Figure VI-11. Predicted Effect of Control of Total *Vibrio parahaemolyticus* per Gram Oysters at Retail for the Gulf Coast (Louisiana) Summer Harvest

[The term in Figure VI-11 "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 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	*Vibrio parahaemolyticus*	Mean Predicted Levels of *V. parahaemolyticus* 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 *V. parahaemolyticus* 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 then 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-$\log_{10}$ reduction.
[b] Represents any process which reduces levels of *V. parahaemolyticus* in oysters 2-$\log_{10}$ 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-$\log_{10}$ 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.

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

Appendix 1: Chronology of Technical and Scientific Reviews of the FDA *Vibrio parahaemolyticus* Risk Assessment Document

FDA solicited the advice and opinions of scientific experts, State shellfish specialists, and the public throughout the conduct of this *Vibrio parahaemolyticus* risk assessment. A summary of the dates, type of review activity, and participants is provided here.

Chronology of Technical and Scientific Reviews of the FDA *Vibrio parahaemolyticus* Risk Assessment

Date	Activity	Reviewers
January 1999	*Vibrio parahaemolyticus* Risk Assessment (VPRA) team assembled	FDA
May 7, 1999	Federal Register Notice; request for comments and for scientific data and information	Public
May 27, 1999	Public meeting (Chicago, IL)	NACMCF; Public; VPRA team
August 13, 1999	Federal Register Notice of public meeting	Public
September 24, 1999	Public meeting; request for comments on the risk assessment approach and assumptions (Washington, DC)	NACMCF; Public; VPRA team
December 1999	Request for scientific review of draft risk assessment document	Interagency Risk Assessment Consortium (RAC) members
December 1999	Technical discussion of the draft risk assessment document	RAC members
December 1999	Intensive review of model	Dr. David Gaylor, FDA/NCTR (National Center for Toxicological Research)
March 31, 2000	Internal scientific review of draft document	FDA risk managers
May 29, 2000	Technical review of document	Special Government Employees (SGEs)
May 29, 2000	Review of model and mathematics	Government experts and SGEs
July 28, 2000	Internal scientific review of draft document	FDA risk managers
January 19, 2001	Publication of draft risk assessment document and request for comments	Public
March 2001	Public meeting; presentation of assumptions, approach, and results of	Public

Date	Activity	Reviewers
	the risk assessment and request for comment (66FR 13544)	
March 2001	1st extension of public comment period (66 FR13545)	Public
May 2001	2nd extension of public comment period (66 FR 28181)	Public
July 2001	Close of public comment period	
August 2001 to May 2002	Review public comments and plan changes needed to risk assessment	VPRA team members
May 2002	Internal scientific review of revised document and model	FDA risk managers and assessors
May 2002-November 2003	Additional modeling	VPRA team members
December 2003 – January 2004	Revision of risk assessment	VPRA team members
February 2004	Review of risk assessment	VPRA team members
February 2004	Peer review of model	Internal and external experts
April 2004	Review of risk assessment	FDA risk managers
May 2004	Editing of risk assessment document	VPRA team members
August 2004	Review of risk assessment	FDA risk managers
October 2004	Begin developing analysis to compare model with epidemiological data	CDC and VPRA team
January 2005	Begin preparation of report	VPRA team
May 2005	Review of risk assessment	FDA risk managers
June 2005	Began clearance/ approval process	FDA

Appendix 2: Response to Public Comments

Comments on the draft *Vibrio parahaemolyticus* Risk Assessment were solicited in the Federal Register notice of availability (Federal Register Docket No. 99N-1075) in the following areas:

(1) The assumptions made
(2) The modeling technique
(3) The data used, and
(4) The transparency of the draft risk assessment document.

FDA received comments from a total of eight institutions or individuals, within the U.S., and abroad: The Food Marketing Institute, The New York State Department of Environmental Conservation, Flow International Corporation, Ministry of Agriculture and Forestry (New Zealand), National Fisheries Institute, PCSGA, CSPI, and Aamir M. Fazil (Health Canada). FDA thanks all of the above-mentioned for taking the time and effort to provide us with their comments. We feel that these comments helped to a great extent to improve our risk assessment. The FDA VPRA team reviewed all the comments and we have addressed them to the best of our ability and the scientific data available. Below is a summary of the key comments and FDA's response to these comments.

COMMENTS ON THE ASSUMPTIONS

Comment 1. The assumption of "equal virulence for all pathogenic strains of *V. parahaemolyticus*" is debatable.

FDA Response 1. While it is almost certain that not all pathogenic *V. parahaemolyticus* are equally virulent, we are unaware of any definitive data indicating the magnitude of differences in virulence among pathogenic strains that would allow us to separate them into subcategories beyond that already done in the risk assessment. The availability of such data would likely have two impacts on the risk assessment. Better data on the relative virulence among TDH^+ strains would provide a better estimate of the variation among strains and thus decrease the uncertainty of the Hazard Characterization, but it would be unlikely that the additional data would greatly change the confidence intervals surrounding the Dose-Response relationship. What would have a great impact would be if there were additional or alternative virulence factors and if the prevalence of the more virulent strains varies seasonally or geographically. This would have the effect of further "concentrating" the risk within specific region/season combinations. The geographical variation of prevalence of TDH+ strains in the Pacific Northwest versus other areas of the country has already been incorporated into the assessment.

Comment 2. The assumption that all *V. parahaemolyticus* whether pathogenic or nonpathogenic have similar growth and survival rates is questionable.

FDA Response 2. Studies performed since the draft *V. parahaemolyticus* risk assessment was published comparing growth rate of pathogenic and non-pathogenic

strains in broth culture, demonstrated no significant difference in growth between the different strains (Cook, 2002a). More recent data on mitigation strategies, such as mild heat treatment and ultra high-pressure treatment have shown that O3:K6 strains are somewhat more resistant to these techniques than the other pathogenic strains (Cook, 2002c). The average D value for thermal treatment of non O3:K6 *V. parahaemolyticus* was 47.6 seconds (ranging from 25-89), whereas that of O3:K6 isolates was 137 seconds (ranging from 108-187). When ultra high pressure was used, the average D value for non O3:K6 strains at a pressure of 36,250 MPa was 24.6 seconds, and for O3:K6 strains, it was 51.9 seconds. These differences have been noted in the revised risk assessment.

Comment 3. Consumption patterns by immunocompromised and healthy populations should not be assumed to be the same.

FDA Response 3. There is little information currently available to estimate the impact of warning labels and other consumer advice on the behavior of individuals who may be more susceptible due to compromised immune function. In the absence of such information, we continue to feel that this is the most appropriate assumption regarding consumption patterns.

Comment 4. The assumption that lag time to growth of *V. parahaemolyticus* in oysters after harvest appears to be negligible is conservative and may result in an overestimate of the growth rate.

FDA Response 4. The specific behavior of the *V. parahaemolyticus* within the oyster at the time of harvest has not been studied extensively; however, there is a wealth of information available on the behavior of the microorganisms in relation to the lag phase. Lag in growth occurs when there is a change in an organism's environment or there is a substantial temperature change. At harvest, *V. parahaemolyticus* remains within the oyster, and is subjected to only modest temperature change over a substantial period of time. A lag phase is therefore not expected under these circumstances, and the original assumption remains the most biologically plausible.

Comment 5. The assumption that water activity of oysters does not vary substantially is conservative and may result in an overestimate of the growth rate.

FDA Response 5. A growth study of *V. parahaemolyticus* in oysters that was replicated during each month of the year indicated similar growth rates when salinity ranged from 8.5 to 25 ppt (Gooch *et al*., 2002). Reduced growth was observed in February when the salinity was 4 ppt but in a nationwide retail survey of shellstock oysters salinity below 8 ppt was rarely observed (FDA/ISSC, 2000; Cook *et al*., 2002a). This narrow range of encountered salinity does not support consideration of alternative assumptions related to the importance of water activity differences. Furthermore, the risk assessment examined in detail the influence of salinity and concluded that the effect of that variable was minor in relation to the primary determinant, water temperature.

Comment 6. The assumption that growth rate in oysters is a constant fraction of the growth rate in broth at all temperatures is conservative and may result in an overestimate of the growth rate.

FDA Response 6. It is possible that the assumption of a constant fraction of the growth rate in broth may not hold at temperatures much higher or lower than the experimental temperature (26 °C) that was used by Gooch *et al*. (2002). However, the ambient temperature of Gulf Coast oysters prior to refrigeration is very close to 26 °C (78.8 °F) from April through October, the period when most *V. parahaemolyticus* cases occur. For this reason, the assumption may not be overly critical to the risk assessment, but the risk assessment will be revised when more data are available. Furthermore, in spite of the possibility that the assumption may not hold for temperatures substantially different from 26 °C (78.8 °F), the exposure levels predicted by the VPRA for other regions and seasons (with cooler temperature) based on the assumption are in relatively good agreement with those observed during the retail study (FDA/ISSC, 2000; Cook *et al*., 2002a). See Figures V-9 to V-12 of technical document.

Comment 7. The assumption that the temperature of oyster meat equilibrates rapidly with that of the ambient air and air temperature as a surrogate for oyster meat temperature is conservative and may result in an overestimate of the growth rate.

FDA Response 7. Along the Gulf, water and air temperatures are nearly the same with water temperature slightly higher than air temperature on average. Consequently, for the Gulf the assumption of rapid equilibration of oyster temperature to air temperature is not conservative per se. When stored in a burlap sack, evaporative cooling has been observed to result in gradual equilibration of oysters to a temperature up to 4 °C cooler than air temperature (Cook, 2002b) but the prevalence of this storage practice (during harvesting) is unknown. For other regions and seasons, where water temperature is substantially lower than air temperature, the assumption of rapid equilibration to air temperature may be somewhat conservative. However, model predictions were compared to retail measurements of total *V. parahaemolyticus* and no substantial differences were noted between observed versus predicted levels. On the West Coast intertidal oysters are typically in a monolayer before harvest and would probably heat up quickly when exposed on a falling tide. During sunny days oyster temperatures were observed to be 5 to 10 °C (41 to 50 °F) warmer than air temperatures (DePaola *et al*., 2002; Herwig and Cheney, 2001). Furthermore, in spite of the possibility that the assumption may be conservative and may result in an overestimate of the growth rate, the exposure levels predicted by the VPRA based on the assumption are in relatively good agreement with those observed during the retail study (FDA/ISSC, 2000; Cook *et al*., 2002a). See Figures V-9 to V-12 of technical document.

Comment 8. The assumption that the *tdh* gene is the principal marker for pathogenic *V. parahaemolyticus* is conservative and does not take into account that in certain areas where tdh-positive isolates were being found, there were no illnesses reported.

FDA Response 8. The thermostable direct hemolysin (TDH) is a proven virulence factor (Nishibuchi *et al*., 1992) and occurs in over 90% of clinical strains in the U.S. (Daniels *et al*., 2000a; Okuda *et al*., 1997a) (Unpublished CDC data) and internationally (Miyamoto *et al*., 1969; Nishibuchi *et al*., 1985; Wong *et al*., 2000). Nearly all West Coast isolates that possess *trh* also possess *tdh* (DePaola *et al*., 2000). FDA data from the U.S. Gulf Coast and a nationwide retail survey indicated that over 95% of all environmental *V. parahaemolyticus* in the U.S. that possess *tdh* also have *trh*, but these isolates account for less than 50% of the recent clinical isolates (FDA/ISSC, 2000; Cook *et al*., 2002a). While it is clear that a small percentage of the *V. parahaemolyticus* isolated from clinical cases of illnesses are strains with *trh* but not *tdh*, it is uncertain that these strains caused the illnesses. Even if they did, it also is uncertain whether a combination of these genes increases *V. parahaemolyticus* virulence.

COMMENTS ON THE MODELING TECHNIQUES

Comment 9. It is troubling that the quantitative risk assessment and modeling is based on only one study, that of DePaola *et al*., 1990.

FDA Response 9. In order to address this particular concern, additional studies bearing on the estimated relationship between *V. parahaemolyticus* densities and water temperature have been evaluated and incorporated into the model. One of these studies, the ISSC/FDA *V. parahaemolyticus* harvest study, was ongoing at the time the risk assessment was initiated. In the ISSC/FDA study, samples were collected nationwide with the exception of the Pacific Northwest. Unpublished data on *V. parahaemolyticus* densities in the Northwest from 1997 through 2001 were also provided to the *V. parahaemolyticus* Team by Washington State authorities (WA State Department of Health, 2002a). These data were also analyzed to better quantify the apparent differences in the *V. parahaemolyticus* harvest densities in the Pacific Northwest compared to other regions of the country, particularly the Gulf Coast. The Washington State data were previously excluded from consideration due to the apparent effects of intertidal exposure on the *V. parahaemolyticus* densities in collected samples. Therefore, a subset of the Washington State samples corresponding to predominantly dredged areas were evaluated with respect to predicting *V. parahaemolyticus* levels in submerged oysters, prior to intertidal exposure effects (DePaola *et al*., 2002).

Comment 10. On page 32 of the draft assessment, the Pacific Coast Shellfish Growers are credited with stating that shellfish go into refrigeration post-harvest within a maximum of four hours. To clarify: while many growers on the West Coast can and do meet this standard, this should not be construed as the norm. There are situations in large bays with extended boat travel requirements (Willapa Bay, for example) or remote harvest locations where time from harvest to refrigeration may be significantly longer than this. More accurately, the assessment should reflect the growers on the West Coast meet the time/temperature requirements of the National Shellfish Sanitation Program.

FDA Response 10. Based on this information, we have remodeled the Pacific Northwest using a minimum time of 2 hours, a maximum of 11 hours and a mean of 8 hours for time-to-refrigeration that is still well within the NSSP requirements (see Table III-7 in the technical document).

Comment 11. A very significant portion of the shellfish cultured on the West Coast is harvested at low tide. However, intertidal exposure of oysters to ambient air temperatures is not reflected in the draft risk assessment.

FDA Response 11. The effect of intertidal harvest is included in our remodeling efforts for the West Coast. A collaborative study with FDA, Washington State and ISSC in August of 2001 generated data indicating significant increases in *V. parahaemolyticus* levels during intertidal exposure (DePaola *et al*., 2002). These data along with data from an ISSC funded study at University of Washington (Herwig and Cheney, 2001) were used to model the effects of intertidal exposure on *V. parahaemolyticus* levels. Washington State data indicate that *V. parahaemolyticus* levels at harvest in Willapa Bay, where most oysters are not exposed at low tide, are generally below detectable levels.

Comment 12. A decrease in the growth rate of *V. parahaemolyticus* during a cool-down (initial refrigeration or icing) of molluscan shellfish was modeled. This should be verified by collaborative scientific studies based on measurements of the actual growth rate of a *tdh+ V. parahaemolyticus* population in naturally contaminated (preferable) or inoculated oysters.

FDA Response 12. A direct measurement of the growth rate of pathogenic *V. parahaemolyticus* during the cooldown process was not undertaken. There is likely to be considerable variation in the temperature and storage conditions of oysters under commercial conditions. Consequently a direct measurement of the growth rate under one or several sets of specific and controlled refrigeration conditions does not fully determine variation in growth likely to occur under commercial conditions. Validation of assumptions underlying the predictions of growth during cooldown were addressed by measuring oyster temperature during cooldown and, in a separate experiment, measuring the growth rate of *tdh*+ and *tdh*- strains in broth culture at 25° C (77° F) (Cook, 2000a).

Comment 13. Remodel β-Poisson dose-response curve using β parameters to obtain a β-distribution, so that each individual eating occasion will have an individual likelihood of illness based on dose-response selecting from the β-distribution.

FDA Response 13. Although the simulation of the Beta-Poisson dose-response within the current assessment might be modified to incorporate the implied variation of risk according to the "exact" Beta-Poisson model, evaluation of the impact that this modification would have on the assessment suggests that it would be minimal. The principle outputs of the assessment are the uncertainty distributions of total number of illnesses across different region and season combinations. As a consequence of the Central Limit Theorem and the relatively large number of servings involved, the mean risk per serving (rather than variation of risk) is what is of particular relevance.

Variability is important to the extent that it impacts mean risk per serving and the additional variation in risk implied by the exact Beta-Poisson model would need to be heavily asymmetric or skewed about the median in order to impact the mean risk per serving (and consequently total number of illnesses) compared to that of the approximate model. We expect that such skewness is unlikely to be substantial and would therefore have little impact on the uncertainty distributions of total number of illnesses relative to identified uncertainties (e.g., other dose-response models). This expectation was evaluated by conducting simulations.

In conducting these simulations the implications of the exact versus approximate models was made assuming that parameter estimates obtained by fit of the approximate model applied to both. This is not strictly correct, as discussed by Furumoto and Mickey (1967), but parameter estimates corresponding to the exact Beta-Poisson model per se could not be readily identified. The results of the simulations indicated that, at the (relatively high) levels of exposure estimated to occur, there is no appreciable difference between using the approximate rather than the exact Beta-Poisson model. The document has been revised to more clearly indicate that the approximate Beta-Poisson model was utilized and that this implies less variation (in individual risk) than that of the exact model.

COMMENTS ON INTERVENTION STRATEGIES

Comment 14. The draft Risk Assessment identified several possible interventions that might be used to control or reduce the level of *V. parahaemolyticus* in shellfish, including reducing time-to-refrigeration, mild heat treatment, freezing, hydrostatic pressure, depuration, irradiation, and relaying. However, only three of these mitigation strategies were actually evaluated in the Risk Assessment, and none of the three interventions on which the draft Risk Assessment focuses are appropriate for use by retailers to enhance the safety of raw molluscan shellfish. The Risk Assessment, in citing a variety of studies, dismisses depuration as ineffective at reducing *V. parahaemolyticus* in oysters. The West Coast industry believes refrigerated wet storage should be investigated as a means of reducing *V. parahaemolyticus* post harvest and instead of being dismissed, become a priority for research.

FDA Response 14. The 2004 risk assessment focuses on the degree of reduction in the levels of *V. parahaemolyticus* in oysters. The results demonstrate that **any** mitigation strategy that reduces the level of *V. parahaemolyticus* in oysters also reduces illness (Chapter VI: What-If Scenarios). The predicted reduction in illness depends on the level of *V. parahaemolyticus* reduced in oysters. In general, as *V. parahaemolyticus* levels are reduced, there is a subsequent reduction in the predicted number of illnesses. Different intervention/ mitigation strategies produce different levels of reduction. We have provided some more commonly used mitigation strategies as examples of the different effects on the levels of *V. parahaemolyticus*. However, by no means do we imply that these are the only strategies that are effective.

Comment 15. It is premature to consider intervention strategies as part of the risk assessment modeling at this time.

FDA Response 15. We do not agree that it was premature to consider intervention strategies as part of the Risk Assessment. Evaluation of mitigation strategies is an important component of process pathway risk assessments. The second objective of the risk assessment is to evaluate the likely public health impact of different control measures, including the efficacy of current and alternative microbiological standards.

COMMENTS ON DATA USED

Comment 16. The prevalence of *tdh*+ *V. parahaemolyticus* strains in the Pacific Northwest was based on a total of only 25 composite oyster samples from 2 studies. This sample size is small, therefore at least 2 more years of data on the percent of pathogenic *V. parahaemolyticus* (*tdh*+) and specific serotypes of *tdh*+ isolates should be collected in a national collaborative study like the FDA-ISSC survey (FDA/ISSC, 2000) of shellfish from each of the five geographic regions used in the risk assessment models.

FDA Response 16. We have used data from more recent studies in the Pacific Northwest and in the Gulf Coast in the current version of the model (DePaola *et al*., 2002; Kaufman *et al*., 2003). There were approximately 60 samples analyzed in each study for the prevalence of both total *V. parahaemolyticus* and *tdh*+ strains. These data are substantially more detailed than in previous studies (where isolates were typically pooled over multiple samples). The data was found to be adequate to statistically estimate both the mean relative prevalence of *tdh*+ and the variation of the relative prevalence of *tdh*+ from one sample to the next, for both the Pacific Northwest and the Gulf Coast.

Comment 17. The data in Table III-4 summarize the minimum, maximum and mean lengths of oyster harvesting in different regions during different seasons. It is unclear whether FDA assumed that the harvesting duration was a distribution of the harvest times from both the pre- and post-NSSP time-to-refrigeration requirements. Only the data from the post requirement period are relevant since these requirements are now mandatory.

FDA Response 17. Our assumptions concerning the length of harvesting times are more clearly described in the current version of the risk assessment document. The distributions do reflect the (self-reported) changes in harvesting evident in the dealer survey data after the post- NSSP refrigeration requirements took affect, but only with respect to those regions and seasons for which the mean water temperature is high enough for the requirements to be applicable. For the colder region/season combinations, not substantially effected by the post-NSSP time-to-refrigeration requirements, the dealer survey data corresponding to pre-NSSP time-to-refrigeration requirements were assumed to apply. Regarding the West Coast, it was our impression from information obtained previously that the maximum length of harvest time was 4 hours. As mentioned above, based on comments received in response to the risk assessment, we have since revised the assumptions to reflect the NSSP requirements appropriate to the West Coast.

Comment 18. The risk assessment did not appear to consider the possible immunological effects of oyster consumers' exposure to low levels of new or virulent strains over time and whether that might subsequently reduce the number and severity of illnesses over time.

FDA Response 18. We have found no evidence that eating raw oysters increases immunity to *V. parahaemolyticus* illnesses. FDA encourages the submission of data to support this assertion. The risk assessment is consistent with the CDC's definition of the risk group for gastroenteritis caused by *V. parahaemolyticus*, i.e., all persons (see Disease Information via www.cdc.gov).

Comment 19. If consumer advisories about the risks associated with the consumption of raw molluscan shellfish are at all effective, then the population of consumers of raw molluscan shellfish should not be growing at the same rate as the general population.

FDA Response 19. Consumption of raw oysters was estimated based on oyster landings data, expert opinion on the percentage of the total landings consumed raw and estimates of the mean serving size obtained from a telephone survey conducted in Florida. A point estimate of consumption was obtained using average landings data from 1990 through 1998. Over this period of time, yearly oyster landings have fluctuated somewhat with a modest increasing trend. We have used the point estimate of past consumption as an estimate of current (and near-future) consumption. We do not have information necessary to investigate the potential effectiveness of education on the change in the number of consumers of raw oysters.

Comment 20. It is not clear how or if the effects of differing levels of virulence in particular strains of *V. parahaemolyticus*, may have been incorporated into the risk assessment.

FDA Response 20. A basic assumption of the risk assessment is that only *tdh*+ strains are virulent and that all strains possessing this characteristic are equally virulent. Although experimental studies suggest that additional pathogenic factors may modulate the virulence of *tdh*+ strains, these have not been incorporated into the present assessment. However, even with the assumption that all pathogenic strains are equally virulent there is structural (model) and parameter uncertainty associated with the estimated dose-response. These uncertainties are substantial and are a consequence of the limited data available with human subjects. Although differing levels of virulence associated with additional pathogenic factors potentially increase variability and the uncertainties associated with the output distributions for probable number of illnesses, the effect may be relatively small given the dose-response uncertainties already identified and incorporated into the assessment.

Comment 21. The risk assessment was not able to estimate an infective dose that might cause illness in the consumers of raw oysters.

FDA Response 21. As stated above, the dose-response model reflects the uncertainty and variability associated with an infective dose. Typically, data were used to estimate distributions rather than point estimates, and consequently, our results are in the form of distributions reflecting both uncertainty and variability. The available feeding studies in human subjects were evaluated to estimate the dose-response associated with pathogenic *V. parahaemolyticus* administered with antacid to healthy subjects. Epidemiological rates of illness in the U.S. population, probable rates of underreporting and model-based estimates of exposure were then considered to determine the likely effect of the food matrix and host factors on the dose-response. For the dose-response models that were considered there is no infectious dose level per se above which the rate of illness is 100% and below which the rate of illness is 0%. A step function dose-response implied by a single infectious dose level was considered implausible and was not evaluated. Moreover, it has been assumed that some *V. parahaemolyticus* serotypes such as O3:K6 require a lower dose to cause illness than other strains (Daniels *et al*., 2000b). Nevertheless, infectious dose was estimated in the sense that for each dose-response model considered an estimate of the dose associated with 50% probably of illness was obtained as well as the doses associated with other probabilities of illness. The uncertainties associated with these estimates were also determined. In a report by FAO/WHO (2003), mechanistic considerations of the probable independent action of bacterial pathogens imply dose-response relationships that are linear at low dose (i.e., no threshold levels).

Comment 22. On page 20 of the draft risk assessment, there is an apparent contradiction in the risk assessment, which estimates that the average percentage of *V. parahaemolyticus* that is pathogenic relative to total *V. parahaemolyticus* on the West Coast is ~3% and that the average percentage of pathogenic *V. parahaemolyticus* in the Gulf Coast and other areas of the country is 0.2 to 0.3%. This supposed high presence of virulent *V. parahaemolyticus* would seem to suggest the West Coast should have the highest incidence of illness, yet this appears to be contradicted on page 62 where the report finds that, based on the Beta-Poisson model, the largest numbers of projected illnesses were attributable to Gulf Coast product.

FDA Response 22. Although there is a higher percentage of pathogenic *V. parahaemolyticus* on the West Coast, there is a lower incidence of total *V. parahaemolyticus* in comparison to the Gulf Coast. The difference in total *V. parahaemolyticus* levels is a consequence of lower water temperatures and higher salinities on the West Coast. The low incidence of illness estimated in the original draft risk assessment was probably due to the shorter harvest time assumed in the model previously, as well as the failure to take the effects of intertidal harvesting into consideration. In the current risk assessment version, we have extended the harvest time to up to 11 hours and have included modeling of intertidal harvesting, which has resulted in an increase in incidence of illness closer to that reported to the Washington State Department of Health. However, risk is still lower than on the Gulf Coast because lower water temperatures (and total *V. parahaemolyticus* levels) compensate for the higher percentage of pathogenic *V. parahaemolyticus*. If all other factors such as prevalence of total *V. parahaemolyticus*, water and air temperature, harvest and post harvest practices,

etc. were equivalent among the regions, then more illnesses would be expected to occur with the Pacific Northwest harvest than that of the Gulf Coast.

COMMENTS ON TRANSPARENCY

Comment 23. The use of complex mathematical models prevents all but the most knowledgeable risk assessors from completely understanding the degree to which uncertainties in the assessment affect the outcome.

FDA Response 23. We agree and that is why FDA issued an "Interpretive Summary" of the risk assessment in conjunction with the technical document. This interpretive summary includes the essential elements of the risk assessment in a manner that can be understood by non-scientists. It states simply why the risk assessment was conducted, what was required of the risk assessment team and what was done to address these requirements, what the results were, and what these results signify. We have also attempted to explain the uncertainties as clearly and simply as possible. In addition, we provide more in the way of technical discussions related to the modeling and statistics in four appendices (3-6) in order to make the calculations more transparent. Some, but not all, technical discussion, figures and tables were moved from the document to the appendices to make the main text more readable.

Comment 24. The draft document, on page 38, appears to erroneously associate 23 cases of *Vibrio parahaemolyticus* related illnesses to the consumption of raw molluscan shellfish harvested in New York State in 1998.

FDA Response 24. We have corrected the numbers in the document.

Appendix 3: The *Vibrio parahaemolyticus* Risk Assessment Simulation Model

Overview

A Monte Carlo simulation model was developed for the *V. parahaemolyticus* risk assessment to capture the variability and uncertainty of the description of the processes associated with *V. parahaemolyticus* densities in oysters, the effects of oyster harvesting, consumer consumption, and human response to the pathogen. This model is made up of biogenic and abiogenic factors. Abiogenic factors include environmental air, water temperatures, and storage times; and biogenic factors include predicted harvest behavior and amounts of oysters consumed. Within the model these factors are combined with growth rate, death rate, and dose-response models. The result is a probabilistic simulation predicting a distribution of baseline risk for each region/season and distributions of risks associated with mitigations.

The model simulations were implemented in @Risk (Palisade). All of the calculations were performed by the Monte Carlo method of resampling from specified input distributions and appropriately combining the sampled values to generate the corresponding output distributions. For each region and season, a total of 10,000 servings were simulated for combinations of 1,000 samples of the uncertainty parameters. Due to the relatively large number of servings consumed within each of the region and season combinations, the number of illnesses implied by the model was determined by the average risk per serving multiplied by the number of servings consumed. The appropriateness of this calculation follows from the Central Limit Theorem. The sum of a large sequence of independent Bernoulli random variables, representing simulated illness outcomes, will converge to the product of the number of variables in the sequence times the average risk of illness (i.e., as the number of variables in the sequence increases). This it true even when a sequence of random variables are not identically distributed, as is the case here due to differing levels of exposure and hence risk per serving.

Iteration of the Model: Variability

For each iteration of the model the prediction of the density of pathogenic *V. parahaemolyticus* per gram of oyster tissue was determined at harvest by applying the estimated distribution of the percentage of *V. parahaemolyticus* that are pathogenic to the predicted distribution of total *V. parahaemolyticus* at the time of harvest and then evaluating changes in the density of pathogenic *V. parahaemolyticus* through the post-harvest module. Levels of total *V. parahaemolyticus* were also evaluated from time of harvest through the post-harvest module. This approach was adopted because total *V. parahaemolyticus* levels were necessary to implement the bound on the level at 6 $\log_{10}$, so that the comparable pathogenic levels would not be exceeded. Also, results from the FDA/ISSC retail study (FDA/ISSC, 2001), the only post-harvest/retail study available, provide levels of total *V. parahaemolyticus*, but not pathogenic *V. parahaemolyticus* for all regions. We used this study to validate the exposure assessment of our model by

comparing levels of total *V. parahaemolyticus* found at retail with the model predicted levels.

Exposure distributions of predicted numbers of pathogenic *V. parahaemolyticus* ingested per serving were obtained by combining distributions describing the probabilistic variation of number and meat weight of oysters in a serving and the expected variation of the density of pathogenic *V. parahaemolyticus* per gram at the end of the Post-harvest process. Individual iterations of the model predicting the number of pathogenic *V. parahaemolyticus* consumed by an individual were used to calculate a risk of illness. @Risk keeps track of the value of each calculation of risk for the 10,000 iterations. When one simulation is completed summary statistics are available for the 10,000 calculations of risk under both baseline and mitigation scenarios.

The number of iterations was set high enough to allow for a range of all the variables to be run through the model. At this number of iterations the summary statistics (e.g., mean values) calculated for the risk of illness were found to converge during the simulation; meaning that, by the $10{,}000^{th}$ iteration, these values were nearly constant. The Monte Carlo simulation error associated with this aspect of the simulation was determined to correspond to an average coefficient of variation of 0.2% up to ~5%. The precision was lowest when the mean dose (and risk) was low and it approached 0.2% for those regions and seasons that collectively account for >95% of the estimated annual illness burden.

The estimates of mean risk determined by the average of simulated illness outcomes for selected high risk region/seasons confirmed the appropriateness of just using the mean risk rather than directly simulating illness outcomes (as Bernoulli random variables). Thus, predicted numbers of illnesses were obtained by determining the mean risk and then calculating the associated number of illnesses as the product of this estimate and the number of servings (based on the NMFS landings statistics).

Simulations with New Parameters: Uncertainty

These simulations of 10,000 sampled exposures (and risks) were repeated 1,000 times with selected uncertainty parameters in order to evaluate their influence the model's output (risk). Parameters evaluated on this level of the simulation included the effect of likely year-to-year variation in the distributions of water temperatures, and the uncertainties associated with parameters such as the percentage of total *V. parahaemolyticus* which are pathogenic, the dose-response and the relative growth rate of *V. parahaemolyticus* in oysters versus broth cultures. A sample size of 1,000 was selected based on practical time constraints. The software selected for Monte Carlo simulations (@Risk) does not directly facilitate a fully nested two-dimensional (variability and uncertainty) simulation approach. Consequently, the uncertainty dimension of the simulation was conducted by performing simple random sampling of uncertainty parameters in Microsoft Excel *per se* and then calling a sequence of 1,000 @Risk simulations with the uncertainty parameters fixed at the values corresponding to each of the uncertainty samples obtained.

Simple random sampling is considerably less precise than Latin hypercube (or other types of stratified) sampling. The relative precision or Monte Carlo error of the mean simulation output with respect to uncertainty at the selected sample size of 1,000 was estimated to correspond to a coefficient of variation of ~3-4% of the nominal mean for each region and season combination. It was determined that the most significant source of this variation was due to the Monte Carlo error of the simple random sampling of the dose-response uncertainty. As a consequence of this degree of simulation error, calibration or "anchoring" of the model to CDC estimates of annual illness burden was accomplished by using "rejection"-sampling to obtain a single fixed representative sample of specified precision from the distribution of the dose-response uncertainty. A criteria of <0.1% relative difference between the sample versus the actual (population) mean was used. Thus, a fixed sample of 1,000 dose-response parameters satisfying this criteria was obtained by iteratively taking samples (of size 1,000) from the uncertainty distribution via simple random sampling and rejecting all but the 1st (collection of 1,000) satisfying the chosen criteria. After having obtained a representative sample of 1,000 dose-response parameters, the adjustment factor associated with food-matrix and pathogen-host effects was estimated by anchoring the model to be consistent with CDC estimates of annual illness burden. This was accomplished by running the model with different adjustment factors and then interpolating between the results to obtain a suitable estimate.

Although the model implementation fixes the samples of the uncertainty parameters rather than randomizing them on each model invocation, the effect of the Monte Carlo error is minimal with respect to both the identification of influential variables and the evaluation of effectiveness of mitigations.

Description of Calculations for Each Step of the Model (@Risk implementation)

A copy (CD-ROM) of the model is available. Fax request for the model to the CFSAN Outreach and Information Center at 1-877-366-3322. Additional information can be found on the spreadsheets:

- Spreadsheet 1. Values used to generate correlated uncertainty distributions used in the assessment, including water temperature data.
- Spreadsheet 2. Simulation results of two-dimensional uncertainty and variability simulations for all regions and seasons (Figure A3-1).

The basic @RISK model showing each step as described below is shown in Figure A3-1. Figures A3-2 to A3-6 show how various parameters are used to derive levels of *V. parahaemolyticus* at different stages in the pathway. For example, Figure A3-5 shows how the mitigation strategies are incorporated into the model and Figure A3-6 shows how the model is adjusted to include intertidal parameters for the Pacific Northwest region as described in the Exposure Assessment and Appendix 5.

	A	B	C	D	E	F	G
1							
2	Appendix 3. Spreadsheet 2. Model for the FDA *Vibrio parahaemolytic*						
3							
4	Gulf Coast Louisianna				Light green cells contain the 'no mitigation' calculation		
5	Winter						
6	1				No mitigation		
7		Water parameters					
8		mean μ			17.322351		
9		mean σ			3.3939456		
10		Water temperature			16.362461		degrees C
11		Temperature-> Vp statistics					
12		intercept			-0.4683393		
13		slope			0.0937913		
14		sigma			0.7242419		
15							
16							
17					**total Vp**	**pathogenic Vp**	
18						0.000756876	% pathogenic at harvest
19		Log Vp level density at harvest			1.4901003	-1.63087492	
20		Log Vp level in environ. Trunc			1.490100	-1.630875	log counts/gram
21		Time on flat					hours
22		estimated growth rate					
23		outgrowth on flats					log counts/gram
24		Log Vp level on flats					log counts/gram
25		Harvesting parameters					
26		min time on water			7		
27		likely time on water			12		
28		max time on water			13		
29		Time on the water			11.263151		hours
30		Time unrefrigerated			7.650752		hours
31		Air temperature parameters					
32		μ			-1.07		
33		σ			3.3		
34		Ambient air temp			18.004714		degree C
35							
36		sqrt(max growth rate)			0.1107692	0.110769217	
37							
38		Estimate growth rate in oysters			0.068122	0.068121998	log counts/hr
39							
40		outgrowth1			0.5211845	0.521184514	
41		Predicted counts at 1st refrigeration			2.011285	-1.109690	log counts/gram
42		Duration of cooldown			8		hours
43		outgrowth2			0.306549	0.306548993	
44		Predicted counts after cooldown			2.317834	-0.803141	
45		Length of refrigeration time			2.4		days
46							
47		Predicted level after die off			2.162352	-0.958623	log counts/gram
48							
49		Grams oysters consumed			211.5197		grams
50							
51		Vp exposure per meal			30739.916	23.26630638	mean count per serving
52		Pathogenic Vp consumed				21	counts
53						1.322219295	log mean counts
54		probability of illness				4.16301E-07	per this serving
55							

Figure A3-1. Spreadsheet Showing Each Step of the @RISK *Vibrio parahaemolyticus* Risk Assessment Model

1. Input of the water parameters: For each uncertainty simulation, new values are inserted into cells E8 and E9 which are the mean and standard deviation of the region/season temperature distribution.

2. Simulation of water temperature during harvest: Based on the values in cells E8 and E9 a water temperature is probabilistically selected based on a Normal distribution.

3. Total *V. parahaemolyticus* at harvest: The total Vp/g density at harvest is determined by using the (regression-based) prediction parameters from cells E12, E13, and E14 to input into cell E19 where the density is calculated (Figure A3-2). If the density is higher than 10^6, then the density is truncated in cell E20.

4. Percent pathogenic *V. parahaemolyticus*: Uncertainty values are set in Cells O35 and O36 for the Beta distribution values that are then calculated in Cell M33 (Figure A3-2). The percent pathogenic is then copied to Cell F18 to make the calculation easier to follow.

5. Pathogenic *V. parahaemolyticus* /g at harvest: The percent pathogenic *V. parahaemolyticus* (cell F18) is multiplied by the total Vp/g density E19 in Cell F19. The value is truncated at an amount proportional to the 10^6 value for the total Vp/g density. The proportionality constant is the % pathogenic for this particular iteration.

6. Time-to-refrigeration: Time-to-refrigeration is the duration of time between when the oysters are harvested and initiation of refrigeration, which typically involves the delivery of the oysters to a land based refrigeration site. The time the oyster harvesters were out on the water (cell E29) is estimated using a Beta-Pert distribution with parameters taken from cells E26 (minimum time), E27 (most likely time), and E28 (maximum time). Within the estimated time period, the time the oysters were out of the water is selected from a uniform distribution with one hour as the minimum time and the maximum being the time on the water value (cell E30).

7. Air temperature: The air temperature (cell E34) is calculated based upon the water temperature plus a probabilistically selected value from a normal distribution using the parameters from cells E32 and E33.

8. Pathogenic *V. parahaemolyticus*/g at 1^{st} refrigeration:
    a. For oysters that are harvested by dredging (i.e., all oysters except those harvested intertidally in the Pacific Northwest), the increase in the *V. parahaemolyticus* densities (cfu/g) from the time the oysters come out of the water to the time they are first placed in refrigeration is estimated as a function of time (E30) and temperature (E34). Additional growth parameters are provided in cells J7 to J14. Cell E38 calculates the square root of the growth rate which is used to predict the out growth reported in

Cell F40. As the growth rate estimate is for *V. parahaemolyticus* growth in culture with the absence of competitors, a factor (M27) based on experimental observation is used to adjust the growth rate to that of *V. parahaemolyticus* in an oyster where competition with other microorganisms is present. The factor is an uncertainty factor and is changed for each uncertainty simulation. Predicted growth (presented as $\log_{10}$) (cell F40) is added to the initial predicted *V. parahaemolyticus* density (presented as $\log_{10}$) (cell F20) to obtain predicted counts at first refrigeration (F41) (Figure A3-3).

b. For oysters that are harvested intertidally, additional factors, such as the time the oysters are on tidal flats before being harvest (E21) and the temperature increase the oysters experience from being in the sun are taken into consideration (Figure A3-6). The time the oysters are on the flats are modeled as a time between 4 and 8 hours and the increase in temperature experienced by the oysters is modeled to be between 0 and 10 °C. Based on the time on the flats and the increased temperature, an estimate of growth is computed to add to the initial *V. parahaemolyticus* density.

9. Cooldown time: A cooldown time in hours is randomly selected from a uniform distribution between 1 and 10 hours in cell E42.

10. Pathogenic *V. parahaemolyticus*/g at cooldown: The *V. parahaemolyticus* continue to grow while the oyster is cooling. The growth rate (cell F43) is a fraction of the initial growth rate estimated as a function of the length of the cooling time. The growth is added to the initial *V. parahaemolyticus* density of the oyster (cell F44).

11. Storage time: The time that oysters are stored is generated in cell E45 based on a Beta-Pert distribution with the minimum time being 1 day, the most likely time being 6 days and the longest time being 21 days.

12. Pathogenic *V. parahaemolyticus*/g at consumption: Pathogenic *V. parahaemolyticus*/g at consumption (cell F52) is determined by multiplying the number of days under refrigeration (cell E45) by the cell death rate under cold storage conditions (cell F47) and then subtracting this amount from the level at cooldown.

13. Number of oysters per serving: The number of oysters in a serving is selected from an array of probable serving sizes (M29) that are weighted to match the estimated numbers of oysters eaten based on an identified consumer survey.

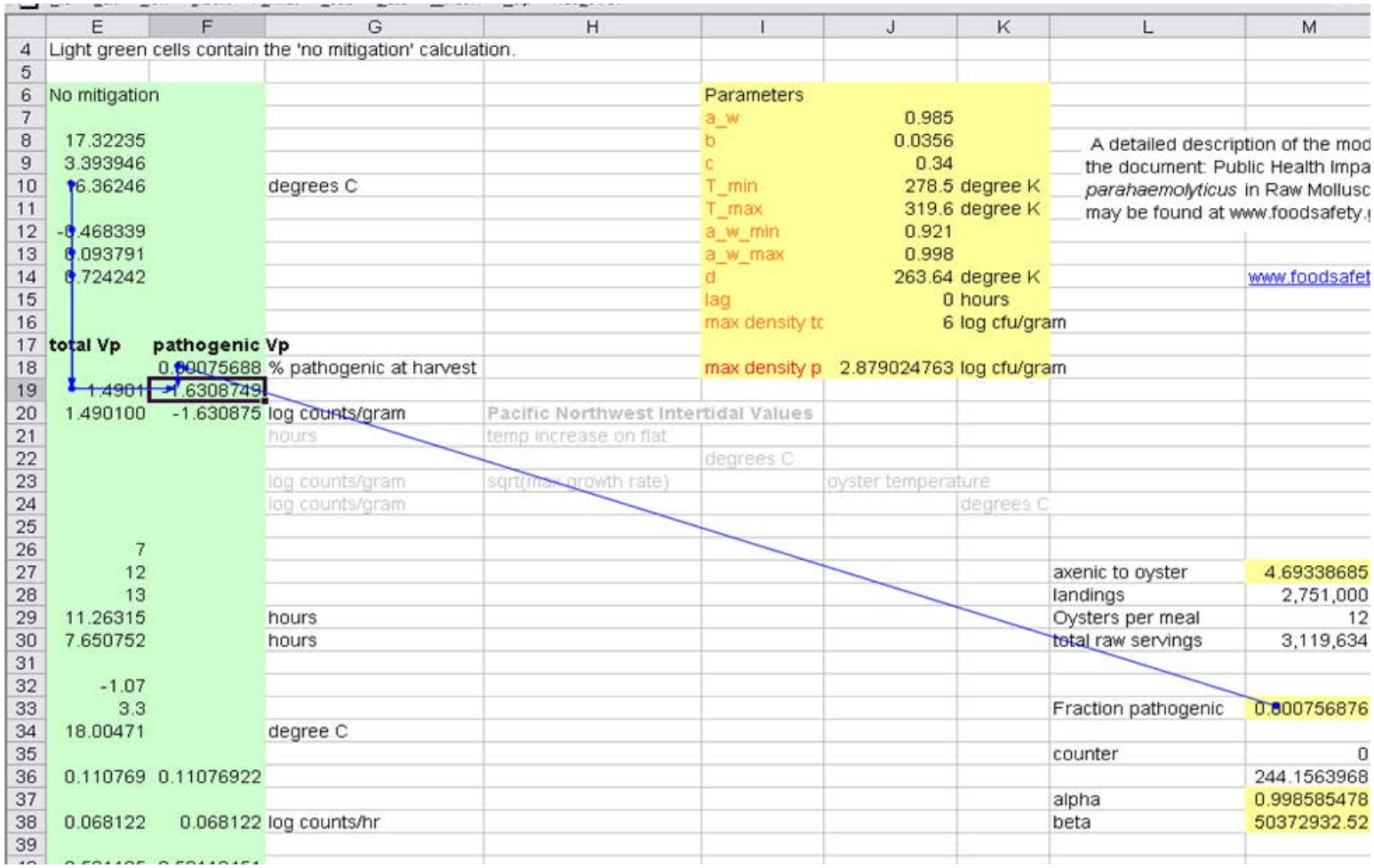
14. Meat weight per oyster serving: The weight of the oyster is probabilistically selected from a lognormal distribution fit to available data on oyster weights. The sampled (or simulated) value is multiplied by the number of oysters consumed

corrected for the fraction of whole oyster that is not consumed (mantle fluid) and multiplied by the mean and standard deviation from the lognormal distribution fit. Finally the simulated value (cell E49) is truncated if the total weight of consumed oyster exceeds 2 kg and rounded to 10 grams if the total weight consumed is less than 10 grams.

15. Ingested dose: The ingested dose (cell F51) is determined by multiplying the mass of the oysters consumed (cell E49) by the density of the *V. parahaemolyticus* present (cell F47). This amount is converted to a whole number by using a probabilistic Poisson estimation of the number of *V. parahaemolyticus* (cell F52).

16. Dose-Response: The dose response parameters are changed for each simulation and are copied to Cells M37 and M38.

17. Risk of Illness: The calculation of the risk of illness is made in Cell F54. The inputs to the calculation are the dose-response parameters and the ingested dose of pathogenic *V. parahaemolyticus* (Figure A3-4).



	E	F	G	H	I	J	K	L	M
4	Light green cells contain the 'no mitigation' calculation.								
5									
6	No mitigation				Parameters				
7					a_w	0.985			
8	17.32235				b	0.0356		A detailed description of the mod	
9	3.393946				c	0.34		the document: Public Health Impa	
10	6.36246		degrees C		T_min	278.5	degree K	*parahaemolyticus* in Raw Mollusc	
11					T_max	319.6	degree K	may be found at www.foodsafety.g	
12	-0.468339				a_w_min	0.921			
13	0.093791				a_w_max	0.998			
14	0.724242				d	263.64	degree K		www.foodsafet
15					lag	0	hours		
16					max density tc	6	log cfu/gram		
17	**total Vp**	**pathogenic Vp**							
18		0.00075688	% pathogenic at harvest		max density p	2.879024763	log cfu/gram		
19	1.4901	-1.6308749							
20	1.490100	-1.630875	log counts/gram	Pacific Northwest Intertidal Values					
21			hours	temp increase on flat					
22					degrees C				
23			log counts/gram	sqrt(max growth rate)		oyster temperature			
24			log counts/gram				degrees C		
25									
26	7								
27	12							axenic to oyster	4.69338685
28	13							landings	2,751,000
29	11.26315		hours					Oysters per meal	12
30	7.650752		hours					total raw servings	3,119,634
31									
32	-1.07								
33	3.3							Fraction pathogenic	0.00756876
34	18.00471		degree C						
35								counter	0
36	0.110769	0.11076922							244.1563968
37								alpha	0.998585478
38	0.068122	0.068122	log counts/hr					beta	50372932.52
39									

Figure A3-2. Screen Shot of @RISK Spreadsheet Showing How the Levels of Pathogenic Vibrio parahaemolyticus at Harvest were Determined

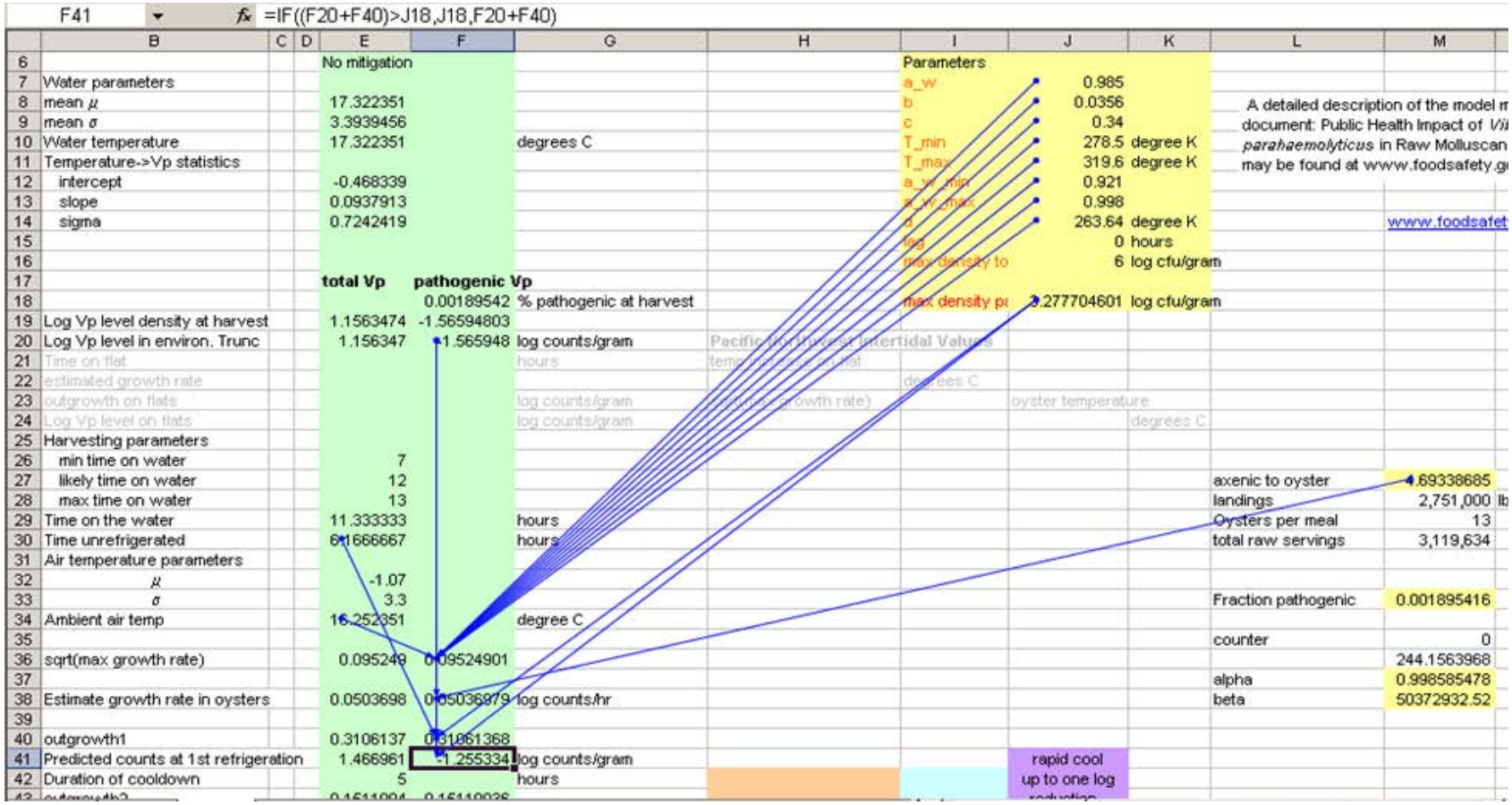


Figure A3-3. Screen Shot of @RISK Spreadsheet Showing How Vibrio parahaemolyticus Levels at First Refrigeration were Derived

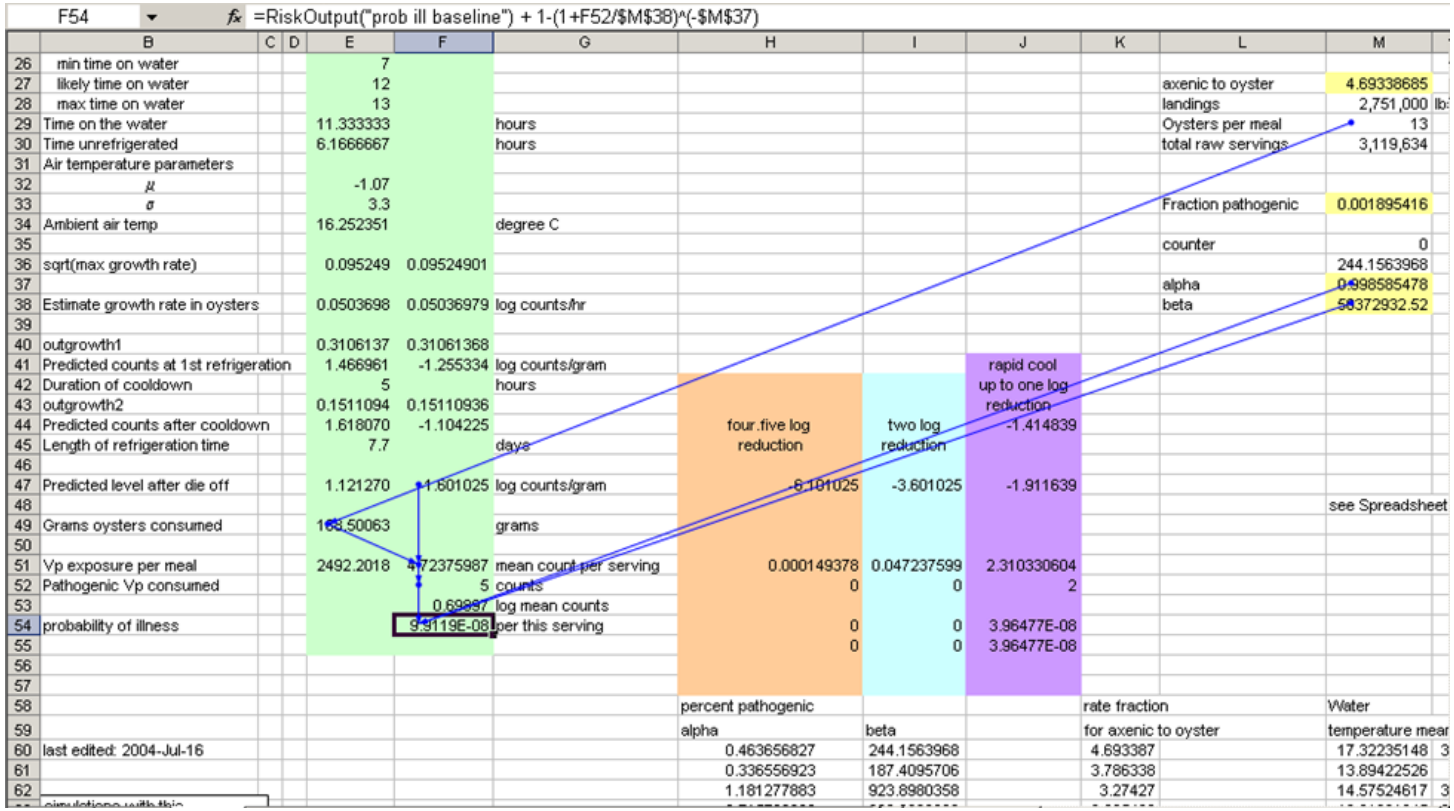
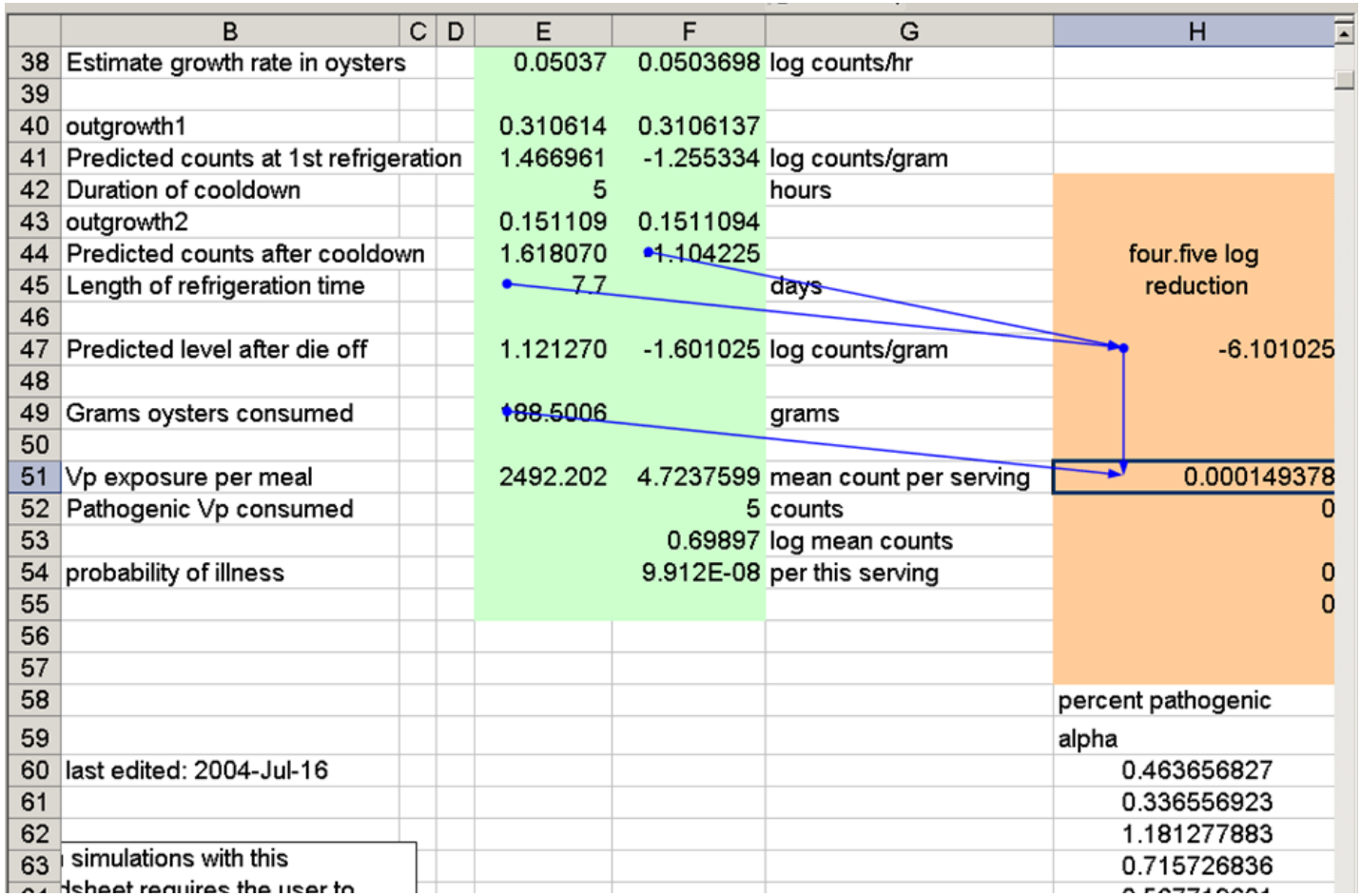
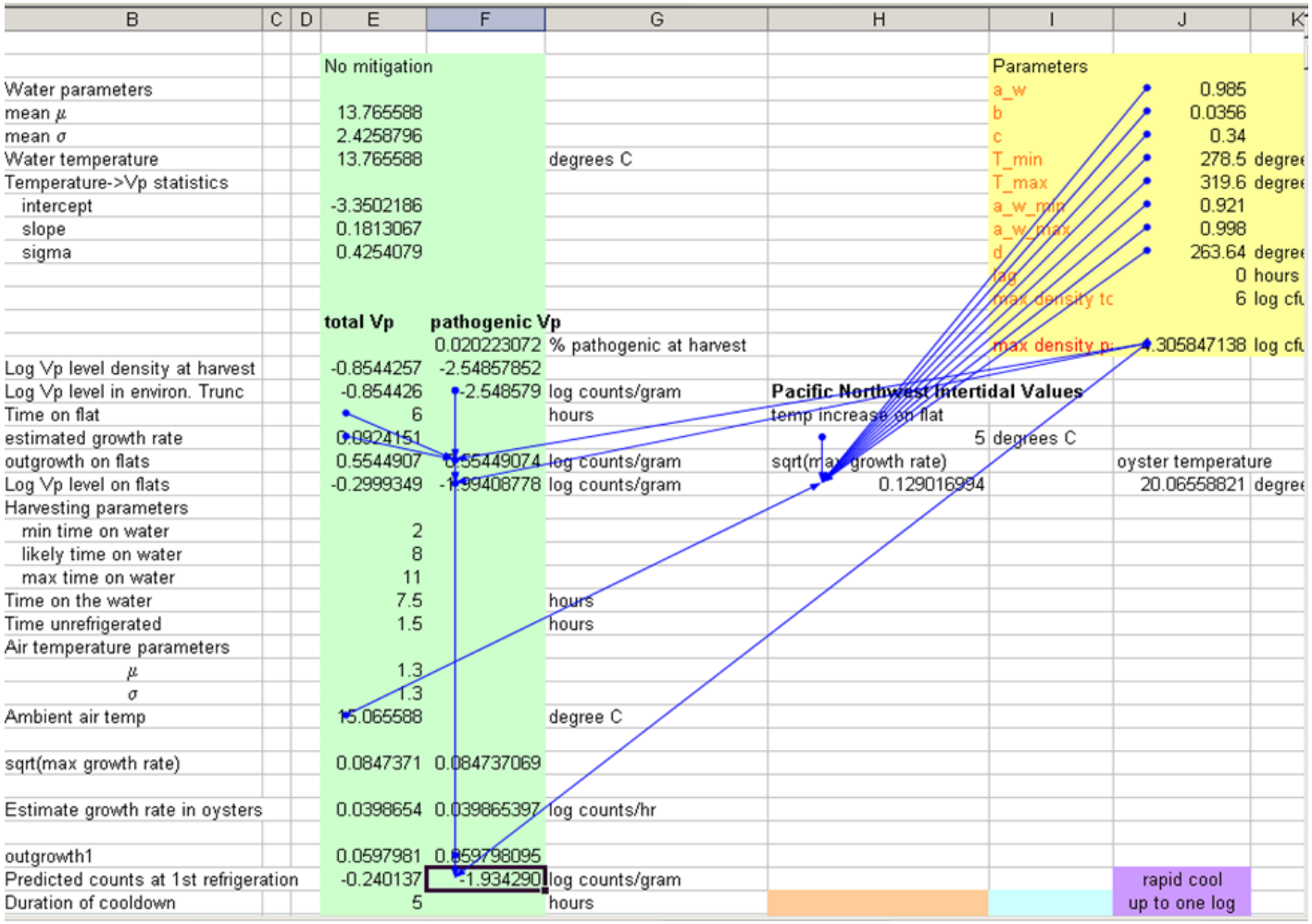


Figure A3-4. Screen Shot of @RISK Spreadsheet Showing How Probability of Illness was Derived using Oyster Servings, Levels of Pathogenic Vibrio parahaemolyticus Consumed and the Dose-Response Parameters



	B	C	D	E	F	G	H
38	Estimate growth rate in oysters			0.05037	0.0503698	log counts/hr	
39							
40	outgrowth1			0.310614	0.3106137		
41	Predicted counts at 1st refrigeration			1.466961	-1.255334	log counts/gram	
42	Duration of cooldown			5		hours	
43	outgrowth2			0.151109	0.1511094		
44	Predicted counts after cooldown			1.618070	-1.104225		four.five log
45	Length of refrigeration time			7.7		days	reduction
46							
47	Predicted level after die off			1.121270	-1.601025	log counts/gram	-6.101025
48							
49	Grams oysters consumed			188.5006		grams	
50							
51	Vp exposure per meal			2492.202	4.7237599	mean count per serving	0.000149378
52	Pathogenic Vp consumed				5	counts	0
53					0.69897	log mean counts	
54	probability of illness				9.912E-08	per this serving	0
55							0
56							
57							
58							percent pathogenic
59							alpha
60	last edited: 2004-Jul-16						0.463656827
61							0.336556923
62							1.181277883
63	simulations with this						0.715726836
	dsheet requires the user to						

Figure A3-5. Screen Shot of @RISK Spreadsheet Providing an Example of How the Effect of Mitigation on Levels of Pathogenic Vibrio parahaemolyticus Per Serving was calculated



			No mitigation				Parameters		
Water parameters							a_w	0.985	
mean μ			13.765588				b	0.0356	
mean σ			2.4258796				c	0.34	
Water temperature			13.765588		degrees C		T_min	278.5	degre
Temperature->Vp statistics							T_max	319.6	degre
intercept			-3.3502186				a_w_min	0.921	
slope			0.1813067				a_w_max	0.998	
sigma			0.4254079				d	263.64	degre
							lag	0	hours
							max density tc	6	log cfu
			total Vp	**pathogenic Vp**					
				0.020223072	% pathogenic at harvest		max density p:	4.305847138	log cfu
Log Vp level density at harvest			-0.8544257	-2.54857852					
Log Vp level in environ. Trunc			-0.854426	-2.548579	log counts/gram	**Pacific Northwest Intertidal Values**			
Time on flat			6		hours	temp increase on flat			
estimated growth rate			0.0924151			5	degrees C		
outgrowth on flats			0.5544907	0.55449074	log counts/gram	sqrt(max growth rate)		oyster temperature	
Log Vp level on flats			-0.2999349	-1.99408778	log counts/gram	0.129016994		20.06558821	degre
Harvesting parameters									
min time on water			2						
likely time on water			8						
max time on water			11						
Time on the water			7.5		hours				
Time unrefrigerated			1.5		hours				
Air temperature parameters									
μ			1.3						
σ			1.3						
Ambient air temp			15.065588		degree C				
sqrt(max growth rate)			0.0847371	0.084737069					
Estimate growth rate in oysters			0.0398654	0.039865397	log counts/hr				
outgrowth1			0.0597981	0.059798095					
Predicted counts at 1st refrigeration			-0.240137	-1.934290	log counts/gram			rapid cool	
Duration of cooldown			5		hours			up to one log	

Figure A3-6. Screen Shot of @RISK Spreadsheet Showing the Effect of Intertidal Harvesting on Levels of Vibrio parahaemolyticus in Oysters in the Pacific Northwest

Advantages and Limitations of the Model

The modeling approach adopted in the present assessment is similar in structure to that of other risk assessments, but has several unique aspects. Foremost, risk has been analyzed in terms of region and season to take proper account of differing harvest practices and climates. Second, the model that was developed is scalable in that it may be applied to finer levels of spatial and/or temporal resolution as data become available. Thirdly, the modeling approach has separated variability from uncertainty by identifying four key variables as uncertain, selecting values for these variables according to specific distributions, and then simulating the effects of variability parameters for all randomly selected values of the uncertainty parameters. In this manner, parameters that represent variability in the model are not mixed with parameters that are uncertain. However, parameters like water temperature can represent uncertainty as well as variability. This separation has allowed for an estimate of the reduction in the overall uncertainty of the analysis that would be gained if the uncertainty of individual variables were reduced. Other microbial risk assessments have separated variability from uncertainty; however, this risk assessment has investigated the gain in information that results from reduction in uncertainty of individual variables. Each of these points is discussed in turn.

The model developed here analyzes risk within categories defined by the four seasons and six primary harvesting regions (Northeast Atlantic, Mid-Atlantic, Gulf of Mexico [divided into 2 regions], and Pacific Northwest [divided into dredged harvesting and intertidal harvesting]) due to differing harvest practices and climates. The analysis could have subdivided further; however, the limitations of acquiring data with respect to a finer level of detail are such that the analysis was conducted at the specified regional level. Analyzing the regions separately allows for an assessment of mitigations that may be tailored to specific regions and seasons. The results may then be used in a subsequent cost benefit analysis. The principle limitation of this approach, which effectively segments the risk assessment into spatial and temporal groupings, is that the results are generally conditional to the *a priori* definitions of region and season. In particular, the selective application of sensitivity and importance analyses to predefined regions/seasons one at a time (i.e., to determine influential parameters and uncertainties) yields results that pertain to specific regions and seasons. Consequently, overarching and comparatively more influential effects may be obscured. In the present assessment, air/water temperatures are highly influential variables across region/season groupings and this is partially obscured by the necessity of presenting sensitivity analysis results for selected region/season combinations. The fact that air/water temperatures may be relatively homogeneous within some of the defined region/seasons in the present assessment, and hence relatively inconsequential in such a context, does not obviate the fact that there are wide (and important) variations in these parameters across regions and seasons.

The structure of the model is amenable to further subdivision of locality and season because it is scalable. Specifically, the model is structured to simulate the density of *V. parahaemolyticus* in oysters at selected steps from harvest to consumption as a function of environmental and industry-specific parameters (e.g., air/water temperatures, harvest

practices) corresponding to locality and season. Given the existence of appropriate data, the model can be used to simulate this process for any appropriately defined harvest location and time frame. The selected level of spatial/temporal categorization (regional and seasonal) was determined by consideration of data availability; most specifically, the quantity and quality of data that could be obtained pertaining to air/water temperatures, harvest practices, *V. parahaemolyticus* prevalence, and shellfish landing information. Given that more detailed data on air/water temperatures is available from satellite observations a finer level of categorization (e.g., by state and/or by month) may be possible and/or other methodological approaches (e.g., harmonic regression) may be applicable to incorporate the effects of climate into further assessments without "segmenting" by region/season categories. However, the utility of such a level of detail in modeling of air/water temperature effects is mitigated by the fact that additional uncertainties may arise if the model is applied on a finer level of detail (e.g. harvesting areas) for which more refined data on industry-specific harvesting practices are missing or incomplete. The effects of such incomplete (or inaccurate) data on the results of the model have not been evaluated at this time, but an analysis of this type may be appropriate in the future if the model is to be applied on the State or shellfish harvesting area level using more refined temperature data available from satellite observations or other sources.

Variability and uncertainty have been separated in the analysis because this separation provides a more informative characterization. We distinguish between model inputs that are less well characterized because of lack of knowledge (uncertainty) and model inputs that are inherently heterogeneous (variable). A model input which is designated as heterogeneous is a parameter that is considered to be naturally variable, even when there is no uncertainty present. For example, the day-to-day water temperatures within each of the different regions and seasons are considered inherently variable and have been modeled as normal distributions with given means and standard deviations. At the same time, the relative growth rate of *V. parahaemolyticus* in laboratory broth cultures versus that in oysters has been characterized as an uncertainty. This uncertainty was specified to appropriately represent the present lack of knowledge as to the true growth rate versus temperature relationship in oysters and the uncertainty inherent in extrapolating from studies of the relationship in axenic culture. With additional study this uncertainty could be reduced. Variations in day-to-day water temperatures on the other hand can not be reduced by further study. The result of making a distinction between model inputs that are uncertain and model inputs that are variable is that the effect of reducing the uncertainty of each of the uncertainty variables can be assessed separately. Based on such an analysis, uncertainties can be prioritized in order to help identify research efforts that are most likely to help reduce the total uncertainty that has been identified in the risk assessment.

The model can be improved. At present, the modeling approach simulates individual exposures and risks with defined variability largely based on the relationship of total *V. parahaemolyticus* levels to air/water temperature and a random variation (within defined limits) of the percentage of total *V. parahaemolyticus* that are pathogenic. However, within region/season groupings the model is not temporal and thus the structure of the

model does not allow for a complete and quantitative evaluation of the likely reduction in risk resulting from implementation of the FDA/ISSC *V. parahaemolyticus* interim control plan (adopted by the ISSC in July 1999 and revised in 2001). This is because, implicitly, the interim control plan operates at a finer level of spatial resolution (e.g. harvest areas) and is time-sensitive in the sense that there is prescribed closure to harvesting after measuring an unsafe level of *V. parahaemolyticus* and then re-opening once exposure levels have been demonstrated to have subsided. In order to develop an assessment model applicable to an evaluation of this control plan additional data and consequent restructuring of the present assessment would be needed. First the model would need to be scaled to the level of individual shellfish harvesting areas. To accomplish this, further data (e.g. water temperature) are needed with respect to the individual harvesting areas. Second, sensitivity and specificity characteristics of the pathogenic *V. parahaemolyticus* gene probe methodology used by the individual laboratories (doing the tests) are needed. Third, the model needs to be extended to encompass putative factors responsible for or affecting the rapidity by which pathogenic *V. parahaemolyticus* levels may change in specific areas and not in others. At present the model predictions are primarily based on temperature. Although variation of the percentage of total pathogenic *V. parahaemolyticus* has been incorporated in the assessment, this variation has not been modeled (or linked) to any environmental factor(s). The model might be improved by considering the rapidity of turnover of water in shellfish harvesting areas based on levels of freshwater flows, tide changes, wind direction, and depth of harvesting area if these environmental factors have an effect on persistence of pathogenic *V. parahaemolyticus*. It is, however, unknown at the present time how these factors may affect the persistence of pathogenic *V. parahaemolyticus* and hence this remains an area for future study.

If and when such model refinement is feasible, more sophisticated approaches to modeling of the data may be appropriate. For example, whereas normal distribution approximations of water/air temperatures were found to be sufficient at the regional/seasonal level, stochastic weather models (e.g., Richardson, 1981), which better represent skewness of temperature distributions as a consequence of precipitation patterns, may help facilitate a more unified approach that is not based on segmentation by season (and region).

Appendix 4: Details of the Data Analysis for the Hazard Characterization Component of the *Vibrio parahaemolyticus* Risk Assessment Model

Two illness endpoints were evaluated in the hazard characterization: (1) gastrointestinal illness and (2) septicemia. A dose-response for the probability of illness was determined by fitting selected parametric dose-response models to the available feeding trials and then comparing model-based predictions of illness based on these dose-response models to CDC's best estimate of the average annual number of illnesses occurring due to raw oyster consumption. The occurrence of septicemia was modeled as an event conditional on the occurrence of illness with a frequency that was assumed to be independent of dose. The population (of oyster consumers) was assumed to be homogeneous with respect to susceptibility to gastrointestinal illness but not septicemia. Based on evaluation of the available data, a subset of the population with predisposing (immunocompromised) health conditions was estimated to be at higher risk of developing septicemia.

Dose-Response for Probability of Illness

As a starting point, a dose-response for illness was initially estimated by fitting selected dose-response models to pooled data on the incidence of gastrointestinal illness from human volunteer studies. The pooled data were taken from the studies by Takikawa (1958), Aiso and Fujiwara (1963), and Sanyal and Sen (1974). Collectively, a total of 20 healthy volunteers were administered Kanagawa positive *V. parahaemolyticus* at doses ranging from 2.3 to 9-$\log_{10}$ cfu in bicarbonate buffer. The dose-response observed is shown in Table A4-1.

Table A4-1. Observed Incidence of Gastroenteritis in Healthy Human Subjects Fed Kanagawa-positive *Vibrio parahaemolyticus* Strains Administered with Bicarbonate

Dose ($\log_{10}$ cfu)	Number of Illnesses	Number of Subjects	Percentage Responding
2.3	0	4	0%
5	0	4	0%
6	1	2	50%
7	4	6	67%
9	4	4	100%

Data from Takikawa (1958), Aiso and Fujiwara (1963), and Sanyal and Sen (1974)

The dose response models that were used in the evaluation (Beta-Poisson, Probit, and Gompertz) were selected *a priori* to span a range of steepness in the dose-response and consequent differences in predictions when extrapolating away from the relatively high dose region where some adverse response was observed in the feeding trials (e.g., extrapolation of the dose-response below 3-$\log_{10}$ cfu). The functional form of the selected models is shown in Table A4-2.

Table A4-2. Selected Dose-Response Models Fit to the Observed Incidence of Illness (Gastroenteritis) in Healthy Human Subjects Administered Vibrio parahaemolyticus in Feeding Trials Studies

Dose-Response Model	Risk of Illness (Gastroenteritis) as a Function of Dose [a]
Beta-Poisson	$\Pr(ill \mid d) = 1-(1+\frac{d}{\beta})^{-\alpha}$
Probit[a]	$\Pr(ill \mid d) = \Phi(\alpha+\beta * f(d))$
Gompertz[1]	$\Pr(ill \mid d) = 1-\exp[-\exp[\alpha+\beta * f(d)]]$

[a] $f(d) = \log_{10}(d)$ is the effective dose corresponding to an ingested dose level d

For both the Probit and the Gompertz models an effective dose was defined based on the ingested dose (number of microorganisms). Some appropriate transformation of the ingested number of microorganisms is necessary for both of these models to ensure that the probability of illness approaches zero as the ingested dose approaches zero. Although a number of transformations exist for which this property will hold, a log transformation was adopted here. Transformation of the ingested dose is not applicable with respect to the Beta-Poisson model. An approximate formula for the Beta-Poisson dose-response (which is shown in Table A4-2) was found to be an appropriate alternative to the exact formula (based on the hypergeometric function) because the data set to which the model was fit was such that parameter estimates obtained generally satisfied the necessary condition that $\beta >> \alpha$ and $\beta >> 1$. The approximate formula for this model was used for both parameter estimation and in the simulation of the risk assessment model.

The three dose-response models were fit to the data shown in Table A4-1 by the method of maximum likelihood (MLE). All of the models provided an adequate statistical fit to the data. Based on the deviance between the MLE model fits and the observed data (a likelihood-ratio based goodness-of-fit measure), none of the model fits could be statistically rejected. The deviances between model fits and observed data were 1.0 for the Beta-Poisson, 0.85 for the Probit, and 1.17 for the Gompertz. Given 5 data points and 2 parameters for each model, these goodness-of-fit statistics are distributed as a Chi-square with 3 degrees of freedom. Thus, the p-values associated with the fit of the models to the data are 0.80, 0.84 and 0.75 for the Beta-Poisson, the Probit, and the Gompertz, respectively; all well above a rejection threshold of 0.05.

A nonparametric bootstrap method was used to characterize uncertainty distributions of model parameters about the MLE fit to the observed data. Bootstrap distributions of parameter estimates obtained by this procedure are shown in Table A4-3. Following the nonparametric bootstrap procedure, a probability is associated with alternative (hypothetical) outcomes for the experimental data based on the assumption that the true probability of response at each experimental dose level is equal to that empirically observed. For example, at the dose level of 6-$\log_{10}$, 1 illness was observed in 2 dosed subjects. Assuming a true probability of response of 50% at this dose level, alternative

outcomes of 0, 1, and 2 illnesses would be expected to occur at this dose level with frequencies of 25%, 50% and 25%, respectively, under hypothetical replication of the experiment. The probability of alternative outcomes for the experiment as a whole is the product of the probabilities associated with each dose level in the combined data set. For each possible outcome, the dose-response models were refit to obtain best estimates of the parameters, again by the method of maximum likelihood. Collectively, the set of parameter estimates obtained, weighted by the associated probabilities of the outcomes, were used to define a bootstrap uncertainty distribution for the parameters of each of the dose response models.

The bootstrapping approach was utilized here to characterize the uncertainty distribution of the dose-response parameters due to the relatively small sample size of the data. As a consequence of the small samples size asymptotic methods, such as the Wald or likelihood-ratio based methods, were considered inappropriate. The nonparametric bootstrap approach was chosen over a parametric approach for simplicity, since the probability of alternative outcomes is determined solely by the observed data. Under a parametric bootstrap approach the probabilities of alternative outcomes would differ for different dose-response models. The MLE fits of all three models predict low probability of illness below 5-$\log_{10}$ and high probability of illness at or above the highest dose level of 9-$\log_{10}$. Consequently, use of a parametric bootstrap approach would not give substantially different results compared to the nonparametric approach. Most of the parameter uncertainty is associated with variability of the outcome response at the mid-dose levels of 6 and 7-$\log_{10}$ cfu (under hypothetical replication of the experiment).

The SAS NLIN procedure was used to obtain maximum likelihood estimates by the method of iteratively re-weighted least squares. For some of the bootstrap outcomes, including the first 7 listed in Table A4-3, the likelihood function of the data was relatively flat and convergence of the estimation procedure was not obtained. More detailed results of refitting the Beta-Poisson dose-response to bootstrap outcomes are shown in Table A4-4. Although converged estimates of MLEs were not obtained for the first 7 outcomes listed in Table A4-4, the (unconverged) model fits to the outcomes were adequate based on the p-values of the deviance statistic. The probability associated with all of the unconverged estimates is a relatively high 31.5%. Rescaling of the likelihood was attempted to obtain better convergence of the estimation algorithm for this model and these outcomes but definitive estimates were not obtained. It may be that the lack of convergence is a consequence of the lack of existence of an MLE for the model parameters for some outcomes, rather than just a numerical scaling problem.

Table A4-3. Non-Parametric Bootstrap Estimates of Parameter Uncertainty Distributions for the Beta-Poisson, Probit and Gompertz Dose-Response Model Fits to Human Feeding Trials Data

Probability	Beta-Poisson		Probit		Gompertz	
	α	β	α	β	α	β
0.00034	1.47×10^{6}	3.53×10^{14}	-52.75	6.59	-51.12	5.95
0.00412	1.26×10^{7}	7.20×10^{14}	-33.21	4.61	-20.45	2.68
0.02058	636.53	1.65×10^{10}	-30.79	4.34	-16.94	2.29
0.05487	35.81	5.42×10^{8}	-28.85	4.12	-14.64	2.03
0.08230	20.84	1.99×10^{8}	-26.92	3.91	-12.76	1.82
0.06584	14.87	8.78×10^{7}	-24.53	3.64	-10.96	1.62
0.02195	10.58	2.99×10^{7}	-20.19	3.16	-8.94	1.40
0.00069	3.89	2.28×10^{8}	-7.11	0.93	-11.43	1.41
0.00823	1.31	2.93×10^{7}	-6.64	0.90	-9.49	1.21
0.04115	0.52	3.61×10^{6}	-6.51	0.92	-8.53	1.13
0.10974	0.47	1.50×10^{6}	-6.73	0.99	-8.57	1.19
0.16461 [a]	**0.60**	$\mathbf{1.31 \times 10^{6}}$	**-7.54**	**1.16**	**-9.80**	**1.43**
0.13169	1.00	1.80×10^{6}	-9.35	1.49	-9.97	1.51
0.04390	8.59	1.30×10^{7}	-16.44	2.74	-7.82	1.27
0.00034	0.15	2.33×10^{5}	-5.05	0.68	-7.96	1.00
0.00412	0.19	2.29×10^{5}	-4.94	0.69	-7.05	0.92
0.02058	0.25	2.36×10^{5}	-4.99	0.73	-6.52	0.88
0.05487	0.32	2.57×10^{5}	-5.27	0.81	-6.38	0.90
0.08230	0.43	3.04×10^{5}	-5.98	0.96	-6.96	1.04
0.06584	0.69	4.34×10^{5}	-7.67	1.28	-8.15	1.27
0.02195	6.92	4.49×10^{6}	-13.49	2.41	-6.98	1.18

[a] bootstrap probability and MLEs corresponding to the observed data *per se*

Table A4-4. Maximum Likelihood Parameter Estimates and Goodness-of-Fit Statistics for Beta-Poisson Dose-Response Model Fits to Nonparametric Bootstrap Outcomes

	Bootstrap Outcome [a]					MLEs of Parameters		Likelihood of Bootstrap Outcome	MLE of Log_{10} ID_{50}	Deviance of Fit to Bootstrap Outcome	P-Value of Fit to Bootstrap Outcome
	x_1	x_2	x_3	x_4	x_5	α	β				
1*	0	0	0	0	4	1.47×10^{6}	3.53×10^{14}	0.00034	8.22	0.6450	0.8861
2*	0	0	0	1	4	1.26×10^{7}	7.20×10^{14}	0.00412	7.60	0.0857	0.9935
3*	0	0	0	2	4	636.53	1.65×10^{10}	0.02058	7.26	0.1901	0.9792
4*	0	0	0	3	4	35.81	5.42×10^{8}	0.05487	7.03	0.3262	0.9550
5*	0	0	0	4	4	20.84	1.99×10^{8}	0.08230	6.83	0.5204	0.9144
6*	0	0	0	5	4	14.87	8.78×10^{7}	0.06584	6.62	0.8557	0.8361
7*	0	0	0	6	4	10.58	2.99×10^{7}	0.02195	6.31	2.2562	0.5210
8	0	0	1	0	4	3.89	2.28×10^{8}	0.00069	7.65	7.4536	0.0588
9	0	0	1	0	4	1.31	2.93×10^{7}	0.00823	7.31	4.4426	0.2175
10	0	0	1	0	4	0.52	3.61×10^{6}	0.04115	7.00	2.9538	0.3988
11	0	0	1	0	4	0.47	1.50×10^{6}	0.10974	6.70	1.7571	0.6243
12	0	0	1	0	4	0.60	1.31×10^{6}	0.16461	6.46	0.9994	0.8014
13	0	0	1	0	4	1.00	1.80×10^{6}	0.13169	6.26	0.6272	0.8902
14*	0	0	1	0	4	8.59	1.30×10^{7}	0.04390	6.04	0.6242	0.8909
15	0	0	2	0	4	0.15	2.33×10^{5}	0.00034	7.32	15.9553	0.0012
16	0	0	2	1	4	0.19	2.29×10^{5}	0.00412	6.90	10.6999	0.0135
17	0	0	2	2	4	0.25	2.36×10^{5}	0.02058	6.57	7.9684	0.0467
18	0	0	2	3	4	0.32	2.57×10^{5}	0.05487	6.30	6.0785	0.1079
19	0	0	2	4	4	0.43	3.04×10^{5}	0.08230	6.08	4.6970	0.1954
20	0	0	2	5	4	0.69	4.34×10^{5}	0.06584	5.88	3.6564	0.3010
21*	0	0	2	6	4	6.92	4.49×10^{6}	0.02195	5.68	2.3697	0.4993

* unconverged estimates

[a] bootstrap outcomes where x_1 denotes the (hypothetical) number of illnesses in the 1st dose group (2.3-log_{10} cfu), x_2 denotes the number of illnesses in the 2nd dose group (5.0-log_{10} cfu), x_5 denotes the number of illnesses in the 5th dose group (9.0-log_{10} cfu)

The MLEs and bootstrap uncertainty distributions of the parameters of the selected dose-response models shown in Table A4-3 were subsequently used in risk assessment model simulations to obtain predictions of illness based on the model-predicted distributions of dose to which raw oyster consumers are exposed. The resulting predictions of illness were compared to the CDC's best estimate of the annual illness rate (2,800 cases/year), which is based on the assumption that only 5% of illness is culture-confirmed (Mead *et al.*, 1999). The model-based predictions of illness rates were found to be inconsistent with the CDC estimate for all three dose-response models (Beta-Poisson, Probit, and Gompertz) that were considered as part of the assessment. Possible reasons for this inconsistency include differences in the food matrix and host effects in the feeding trials

studies compared to that associated with a diverse population of consumers exposed to *V. parahaemolyticus* via raw oyster consumption.

As a consequence of the identified inconsistency, the CDC's best estimate of the annual illness rate (~2,800 cases/year) was taken to be an additional data point for the purpose of dose-response estimation. A nonspecific location parameter was introduced into each dose-response model and this parameter was then adjusted until resulting risk assessment predictions of illness were centered (or "anchored") to the CDC estimate of 2,800 cases/year based on simulations using the estimated distributions of pathogenic dose consumed as derived in the exposure assessment. The resulting dose-response adjustment corresponded to a change in the location of each model (i.e., a change in the β parameter of the Beta-Poisson model and the α parameter of the Probit and the Gompertz models) relative to that estimated based on the feeding trials data alone. Estimates of 1.4, 1.3, and 3.3 greater $\log_{10}$ ID_{50} under conditions of population exposure versus that of controlled exposure with antacid in human volunteers were obtained for the Beta-Poisson, Probit, and Gompertz models, respectively. Given the uncertainties associated with the CDC's best estimate of the average yearly illness burden (e.g., due to uncertainty of underreporting of illness), no formal statistical criteria was used in the process of anchoring each dose-response to this estimate.

After anchoring each of the dose-response models (in turn) to the CDC's best estimate of annual illness burden, the unconverged bootstrap estimates of dose-response uncertainty for two of the three models (the Probit and the Gompertz) were found to correspond to extremely low levels of risks. When applying these two models for the purpose of Risk Characterization, these tails of the uncertainty distributions, driven by unconverged estimates, were found to be generally inconsistent with CDC estimates of annual illness for any reasonable magnitude of the frequency by which illnesses are reported. This is evident in Figure A4-1, which shows the mean and central 95% of the uncertainty distribution of $\log_{10}$ ID_{50} and $\log_{10}$ ID_{001} (i.e., the infectious dose levels corresponding to 50% and 0.1% illness rates, respectively). The wider range of the uncertainty distributions of $\log_{10}$ ID_{001} for the Probit and Gompertz models is evident and is a consequence of the substantial impact of the unconverged estimates for these two models. That is, the unconverged parameter estimates for both the Probit and Gompertz model correspond to the upper portion of the 95% uncertainty range. Consequently, unconverged estimates for these two models (Probit and Gompertz) were considered implausible and were not retained with respect to characterizing the dose-response uncertainty and the suitability of these models for the purpose of Risk Characterization. The effect of dropping the unconverged estimates is shown in Figure A4-1. The impact of unconverged estimates for the Beta-Poisson model was found to be much less substantial and therefore uncertainty of model predictions were based on retaining all of the bootstrap estimates of uncertainty in the characterization of the dose-response using this model.

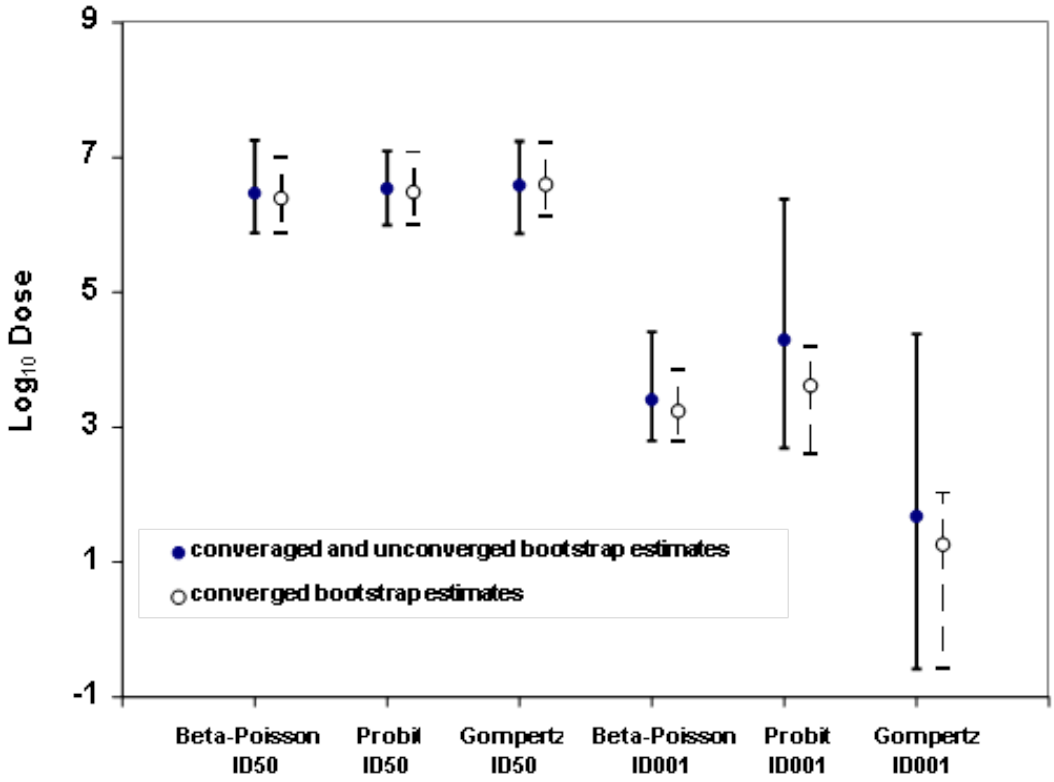


Figure A4-1. Bootstrap Estimates of the 2.5%-tile, the Mean, and the 97.5%-tile of the Uncertainty Distribution of ID_{50} and ID_{001} Based on Fit of the Beta-Poisson, Probit and Gompertz Models to the Human Feeding Trials Data (With and Without Unconverged Parameter Estimates Being Retained)

Probability of Septicemia Given the Occurrence of Illness

Probabilities of septicemia occurring in healthy and immunocompromised individuals were estimated based on an evaluation of the frequency of putatively predisposing health conditions and illness outcome types (gastroenteritis versus septicemia) in a CDC case series of culture-confirmed illnesses. The dataset selected for analysis consisted of oyster-related culture-confirmed cases that were reported in Gulf Coast states during 1997 and 1998 (Angulo and Evans, 1999). This data set was considered particularly relevant as a basis for estimation because the data collected in this region during this period of time was less likely to be biased, due to a heightened awareness of *V. parahaemolyticus* illness following the outbreaks that occurred at that time. The CDC dataset consisted of a total of 107 oyster-related culture-confirmed *V. parahaemolyticus* cases (sporadic- and outbreak-related) with 102 identified cases of gastroenteritis, 5 cases of septicemia and one death. Among those cases in the series with available information on health conditions, 23 of 79 (29%) illnesses occurred in individuals with an identified underlying chronic (immunocompromising) health condition; 27 of 90 (30%) gastroenteritis illnesses were hospitalized and 3 of 4 (75%) septicemia illnesses occurred in individuals with an underlying chronic (immunocompromising) health condition.

These identified conditional probabilities of health conditions given illness outcome type can be used to obtain corresponding conditional probabilities of illness outcome type given health condition by an application of Bayes' theorem. Specifically, based on Bayes' theorem, the frequency of an illness outcome type in a (homogeneous) subpopulation defined by the presence of a predisposing health condition is related to the frequency of the predisposing condition among individuals with that illness outcome and the marginal probabilities of the outcome type and the predisposing health condition with respect to the overall population. This relationship is:

$$\Pr(\text{illness outcome} \mid \text{health status}) = \frac{\Pr(\text{health status} \mid \text{illness outcome}) * \Pr(\text{illness outcome})}{\Pr(\text{health status})}$$

where, for example, 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 the epidemiological case series data.

Substituting appropriate observed frequencies (based on the CDC case series data) into the above equation provides point estimates, with respect to the population of culture-confirmed illness, of the probability of septicemia occurring within any appropriately defined subpopulation identified by a common health status. For the assessment of risk of septicemia, the population was considered to consist of two risk subgroups defined by the presence (or absence) of a predisposing "immunocompromising" health condition. Implicitly it is assumed that the risk within each subgroup is relatively homogeneous. Thus, for the subpopulation identified as having an "immunocompromised" chronic health condition the probability of septicemia (given that illness occurs and is culture-confirmed) was estimated from Bayes' theorem and the CDC data as follows:

$$\Pr(\textit{septicemia} \mid \text{immunocompromised}) = \frac{\Pr(\text{immunocompromised} \mid \textit{septicemia}) * \Pr(\textit{septicemia})}{\Pr(\text{immunocompromised})}$$

$$= \frac{3/4 * 5/107}{23/79} = 0.12$$

The probability of septicemia occurring consequent to culture-confirmed illness in healthy individuals was estimated in a similar fashion. The conditional probabilities obtained for progression of illness to septicemia are:

$$\Pr(\text{septicemia} \mid \text{immunocompromised}) = 0.12$$
$$\Pr(\text{septicemia} \mid \text{healthy}) = 0.0165$$
$$\Pr(\text{septicemia}) = 0.047$$
$$\Pr(\text{death} \mid \text{septicemia}) = 0.2$$

It is important to recognize that these estimated probabilities pertain to the population of 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. Thus for example, given that a culture-confirmed illness occurs there is an overall 4.7% chance that the illness outcome will be septicemia. The two primary illness outcomes (i.e., gastroenteritis only or gastroenteritis with progression to septicemia) are mutually exclusive. Death was considered a separate outcome subsequent only to the occurrence of septicemia.

In order to obtain estimates of the probabilities of septicemia applicable to all *V. parahaemolyticus* illness, regardless as to whether culture-confirmed or not, consideration needs to be given to apparent selection biases in the case series data. While cases of septicemia are unlikely to go undiagnosed (i.e., unconfirmed) as a function of a patient's health status, this may not be true of gastroenteritis. If the frequency by which illnesses are culture-confirmed increases with the severity of illness and an immunocompromising health condition predisposes to more severe gastroenteritis (as well as increased risk of septicemia) then one would expect immunocompromised individuals to be over-represented in case series of gastroenteritis. Based on analysis of the 1997-1998 CDC case series data this would appear to be the case, although differences in consumption behavior could also be partially responsible.

Differential reporting rates for culture-confirmed gastroenteritis occurring in immunocompromised versus healthy individuals can be estimated from the case series data based on relationships between conditional probabilities and marginal probabilities that are implied by Bayes' theorem. The appropriate relationships are:

$$\Pr(CC) = \Pr(CC \mid S) * \Pr(S) + \Pr(CC \mid \overline{S}) * \Pr(\overline{S})$$

and

$$\Pr(S \mid CC) = \frac{\Pr(CC \mid S) * \Pr(S)}{\Pr(CC \mid S) * \Pr(S) + \Pr(CC \mid \overline{S}) * \Pr(\overline{S})}$$

where

$\Pr(CC) = \Pr(\text{culture - confirmed})$

$\Pr(S) = \Pr(\text{immunocompromised})$

$\Pr(\overline{S}) = \Pr(\text{healthy})$

$\Pr(S \mid CC) = \Pr(\text{immunocompromised} \mid \text{culture - confirmed})$

$\Pr(CC \mid S) = \Pr(\text{culture - confirmed} \mid \text{immunocompromised})$

$\Pr(CC \mid \overline{S}) = \Pr(\text{culture - confirmed} \mid \text{healthy})$

These two equations stipulate that the weighted average of differential reporting rates in immunocompromised versus otherwise healthy subpopulations is equal to the overall aggregate reporting rate subject to a restriction, or constraint, that these differential reporting rates are consistent with the frequency of individuals with underlying immunocompromising (chronic) health conditions in the population of culture-confirmed illness.

By assumption, the aggregate probability of illness being culture-confirmed (i.e., "reported") has been taken to 1 in 20 cases (5%) based on the study by Mead *et al*. (1999). The frequency of immunocompromised individuals in the CDC (gastroenteritis) case series data is 29%. Two additional unknowns in the equations are the marginal frequencies of immunocompromised and healthy statuses. In this regard, it has been estimated that approximately 7% of the general population has an underlying health condition predisposing to *V. vulnificus* infection (Klontz, 1997). The same set of health conditions would likely predispose to more severe *V. parahaemolyticus* illness and are, in fact, the types of health conditions reported in the 1997-1998 CDC case series data of *V. parahaemolyticus* illness. Based on this observation, it is assumed the same frequency of predisposing health conditions (7%) applies to *V. parahaemolyticus* and that immunocompromised individuals consume raw oysters at the same frequency as the general population.

Substituting these estimates into the relationships above gives a system of two equations in two unknowns. Solving for these two unknowns yields point estimates of the rate by which illnesses are culture-confirmed for immunocompromised versus healthy subpopulations:

$\Pr(CC \mid S) = \Pr(\text{culture - confirmed} \mid \text{immunocompromised}) = 0.21$

$\Pr(CC \mid \overline{S}) = \Pr(\text{culture- confirmed} \mid \text{healthy}) = 0.038$

Based on these estimates, predicted probabilities for occurrence of septicemia among all *V. parahaemolyticus* illness, both culture-confirmed and unreported, are:

$$\Pr(\textit{septicemia} \mid S) = \Pr(\textit{septicemia} \mid S \ \& \ CC) * \Pr(CC \mid S) = 0.12 * 0.21 = 0.025$$

$$\Pr(\textit{septicemia} \mid \bar{S}) = \Pr(\textit{septicemia} \mid \bar{S} \ \& \ CC) * \Pr(CC \mid \bar{S}) = 0.0165 * 0.038 = 0.00063$$

Finally, the overall risk of illness progressing to septicemia among the population of all *V. parahaemolyticus* illness is the weighted average of the conditional probabilities of septicemia for immunocompromised and healthy individuals:

$$\Pr(\text{septicemia} \mid \text{illness})$$

$$= \Pr(\text{immunocompromised}) * \Pr(\text{septicemia} \mid \text{illness} \ \& \ \text{immunocompromised}) + \Pr(\text{healthy}) * \Pr(\text{septecemia} \mid \text{illness} \ \& \ \text{healthy})$$

$$= \ 0.07 * 0.025 + 0.93 * 0.00063 \ = \ 0.0023$$

Based on this analysis, a combined variability/uncertainty distribution for the probable number of septicemia which may occur in a given year was defined as a binomial distribution with size parameter equal to the total number of illnesses predicted (i.e., in each individual simulation of the risk assessment model) and probability parameter equal to the estimated aggregate risk of septicemia following illness (0.0023). A tacit assumption here is that the probability of septicemia occurring is independent of the dose leading to infection and illness. The uncertainty associated with estimated progression rates in immunocompromised and healthy individuals obtained via Bayes' theorem have not been fully evaluated here. However, the uncertainties are considered to be substantially less than that already characterized with respect to the number of illnesses occurring.

Appendix 5: Details of the Data Analysis for the Exposure Assessment Component of the *Vibrio parahaemolyticus* Risk Assessment Model

Relationship between Levels of *Vibrio parahaemolyticus* in Oysters At-Harvest and Environmental Conditions

There have been a number of extensive 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 here with regard to their utility for developing or estimating an appropriate predictive relationship between total *V. parahaemolyticus* densities in oysters at the time of harvest and environmental conditions; specifically water temperature and salinity. Most of the older studies did not provide sufficient information with respect to a quantitative relationship, primarily because these studies were either limited to specific seasons with correspondingly 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 following sixteen studies that were evaluated are listed below:

- Tepedino (1982). This survey of Long Island oysters from October 1979 to June 1980 found 33% of oysters analyzed contained detectable levels ($\geq$10 organisms/g) of total *V. parahaemolyticus* with range of 3.6 to 23 organisms/g.

- Kelly and Stroh (1988a). In this study *V. parahaemolyticus* were found in 44% of natural and 21% of cultivated oysters from British Columbia under warm conditions (July and August) but was not found in any oysters in cooler conditions (March and April).

- Kelly and Stroh (1988b). A seasonal association with *V. parahaemolyticus* illness and total *V. parahaemolyticus* density was reported in the estuarine waters of British Columbia. *Vibrio parahaemolyticus* was isolated in 11-33% of water samples collected during the summer months, when warm, low-salinity water conditions prevail in the coastal marine environment. Peak densities of 70 cfu/ml were found. Oysters were not examined.

- Chan *et al*. (1989). This study examined total *V. parahaemolyticus* levels in seafood from Hong Kong from June through October. Mean *V. parahaemolyticus* densities in oysters (harvest), mussels (market) and clams (market) were $3.4x10^4$, $4.6x10^4$, and $6.5x10^3$ cfu per gram, respectively.

- Kiiyukia *et al*. (1989). Total *V. parahaemolyticus* in water and sediments of Japan were enumerated in this study. *Vibrio parahaemolyticus* was isolated in 2 out of 8 market oyster samples. The *V. parahaemolyticus* levels in oysters were not determined.

- Ogawa *et al*. (1989). In this study the ecology of total *V. parahaemolyticus* in Hiroshima Bay was investigated from July 1987 through June 1988. The highest incidence of detectable *V. parahaemolyticus* (68.8%) was found from May to October when water temperature ranged from 19.3 to 22.0° C. *V. parahaemolyticus* levels in oysters were seasonal and ranged

from 10^3 *V. parahaemolyticus* /100g oyster in June to July, 10^2/100g in May and August to September, and less than 10^2/100g in the other months.

- DePaola *et al*. (1990). In this study total and TDH^+ *V. parahaemolyticus* were enumerated in seawater and oyster samples collected seasonally from May 1984 through April 1985 from shellfish growing areas on the Pacific, Gulf and Atlantic coasts. Total *V. parahaemolyticus* levels were found to be related to water temperature, with highest densities in samples collected in the spring and summer from the Gulf Coast.

- Kaysner *et al.* (1990a). Water, sediment and oysters of Grays Bay, WA were sampled during September when salinity ranged from 0.0 to 30.6 ppt and temperature from 13.5 to18.0° C. Highest total *V. parahaemolyticus* densities were found in sediments (8 to 1,500 MPN/g), followed by oysters (0.4 to 15 MPN/g) and water (0.001 to 0.4 MPN/g).

- Hariharan *et al*. (1995). A yearlong survey of mussels and oysters was conducted in Prince Edward Island, Canada. *Vibrio parahaemolyticus* was isolated from 4.7% and 6.7%, respectively. Pathogenic *V. parahaemolyticus* were isolated in the fall and summer only (4 from 85 mussels and 3 from 45 oysters).

- FDA/ISSC (2001). In 1999 and 2000, the Interstate Shellfish Sanitation Conference (ISSC) and the FDA conducted a large survey of *V. parahaemolyticus* densities in oyster samples collected from 14 harvest areas in 7 states (Cook *et al*., 2002b). A total of 671 samples were collected from growing areas on the Atlantic and Gulf coasts over a period of 18 months. Total and pathogenic *Vibrio parahaemolyticus* densities in these samples were determined by a direct plating procedure in which colonies are identified by gene probe. This study compared well with that of DePaola *et al*. (1990); both studies found that *V. parahaemolyticus* densities were related to water temperature with the highest densities being obtained in samples collected in the Gulf Coast.

- DePaola *et al*. (2000). Environmental investigations were conducted in the weeks following the 1997/1998 outbreaks in Washington State, Texas, and New York. *Vibrio parahaemolyticus* was found to be prevalent in oysters from these areas. A small but significant salinity effect was observed in Galveston Bay with areas of low salinity (~20 ppt) having slightly higher levels of total *V. parahaemolyticus* than areas of high salinity (~25 ppt).

- Washington State Department of Health (1999; 2000; 2001). In the fall of 1997, in response to the outbreak of *V. parahaemolyticus* cases that occurred that summer, the Washington State Department of Health initiated an ongoing *V. parahaemolyticus* monitoring program. Samples collected for analysis are submitted voluntarily by participating harvesters and reflect the effects of normal harvest practice at each particular collection site. Data obtained from 1988 through 1999 and in 2001, totaling 262 oyster samples, were provided to the risk assessment team. These data show a strong seasonal effect on total *V. parahaemolyticus* with the highest levels obtained in July and August.

- Herwig and Cheney (2001). The effect of intertidal exposure on total and pathogenic *V. parahaemolyticus* densities was investigated in oysters, sediment and water collected from selected sites on Puget Sound and Hood Canal from June through November 1999. *V. parahaemolyticus* was enumerated by a PCR-based MPN procedure. *Vibrio parahaemolyticus* densities were found to be correlated with the rise and fall of water temperature from late spring through early fall. Up to a 100-fold increase in *V. parahaemolyticus* densities in oysters was observed during intertidal cycles. There was considerable variation in the magnitude of the increases across different sampling sites.

- DePaola *et al*. (2002). Another study on the effect of intertidal exposure on total and pathogenic *V. parahaemolyticus* densities in the Pacific Northwest was conducted in August 2001. Oyster and sediment samples were collected from selected sites in Hood Canal over the course of several intertidal exposure cycles. Densities were determined by a direct plating procedure. A 4- to 8-fold increase of the density of total *V. parahaemolyticus* in oysters was observed between the time the oysters immediately emerged from the receding tide and just before they submerged in the rising tide. Like the study by Herwig and Cheney, (2001), considerable variation of the intertidal effect across different sites was evident. Little or no change in *V. parahaemolyticus* densities during intertidal cycles was observed in some areas.

- Kaufman *et al*. (2003). Total and pathogenic *V. parahaemolyticus* densities were determined in a Gulf Coast study conducted from June through September 2001. The variability of total and pathogenic *V. parahaemolyticus* densities in individual oysters was examined at time of harvest and after 24 hours of storage at 26 °C. At time of harvest, pathogenic *V. parahaemolyticus* was detected in 8 of 30 (27%) samples at levels ranging from 10 to 20 CFU/g. Both total and pathogenic densities increased after storage at 26 °C with pathogenic *V. parahaemolyticus* detected in some oysters at levels >100 CFU/g. At the time of harvest, pathogenic *V. parahaemolyticus* were detected in 40% of the oysters collected (10 to 20 cfu/g). After storage pathogenic V. parahaemolyticus was detected in some oysters at levels of >100 cfu/g.

- DePaola *et al*., 2003a. Oyster samples collected in Alabama were examined. *Vibrio parahaemolyticus* isolates were screened for the presence of TDH+ by direct plating and following enrichment. The results of this study suggest that there may be a relationship between water temperature and the relative prevalence of pathogenic *V. parahaemolyticus* with a higher ratio of number of pathogenic to total number strains during the winter than in the summer. However, samples analyzed in this study were collected from only two sites and a statistically significant site-to-site difference in the abundance of pathogenic strains was also observed. The apparent significance of the relationship between water temperature and prevalence of pathogenic V. *parahaemolyticus* was problematic (i.e., not robust with respect to alternative statistical analyses).

Harvest Module

Water Temperatures

With the exception of the Pacific Northwest region, distributions of regional/seasonal water temperatures were developed based on accumulated records from selected coastal water buoys maintained by the National Buoy Data Center (NBDC). Hourly water temperature measurements were generally available from 1984 through 1998 from several buoys in each region. However, given intermittent records and lack of both water temperature and air measurements for some buoys, a single representative buoy was selected for each region that had both water and air temperature measurements. The data from these selected buoys were analyzed to determine an appropriate summary distribution for temperature in the Monte Carlo simulation model. After examination of these data, implementation of temperature distributions in the Monte Carlo simulation by resampling from empirical distributions was determined to be overly cumbersome. Although there is some error associated with simpler distribution summaries that have been used, and are discussed here, the differences appear to be minor in consideration of the natural variation of *V. parahaemolyticus* levels at any given temperature and the other factors/uncertainties identified in the risk assessment process.

Although there is a diurnal cycle in water (and air) temperature, the effects of hourly changes in water temperatures were not considered in predicting *V. parahaemolyticus* levels at the time of harvest. Examination of selected NBDC buoy datasets indicated that the hourly water temperature variations were minor in comparison to the variations across days or weeks. This is illustrated in Figure A5-1, which shows the mean, the 2.5% and 97.5%-percentiles of the hourly water temperature measurements recorded at the NBDC Dauphin Island, Alabama buoy during the summer of 1997. As is evident in the figure, the variation of mean water temperature by time of the day is only slightly greater than 1 °C; much less than the variation across different days or weeks as indicated by the percentiles.

The day-to-day variation in temperature is temporally correlated as weather patterns determining air and water temperatures persist over time spans varying from several days to several weeks. Figure A5-2 shows the temporal pattern of daily (midday) water temperatures recorded at Dauphin Island in the summer of 1997. Figure A5-3 shows a histogram plot of the same temperature data with an approximate normal distribution summarizing the variation about the mean. A normal distribution was fit to the data by the method of "moments." As evident in Figure A5-3, the actual temperature data are skewed and the normal distribution summary does not capture this facet of the data since only the 1st two moments of the fitted distribution match that of the empirical distribution.

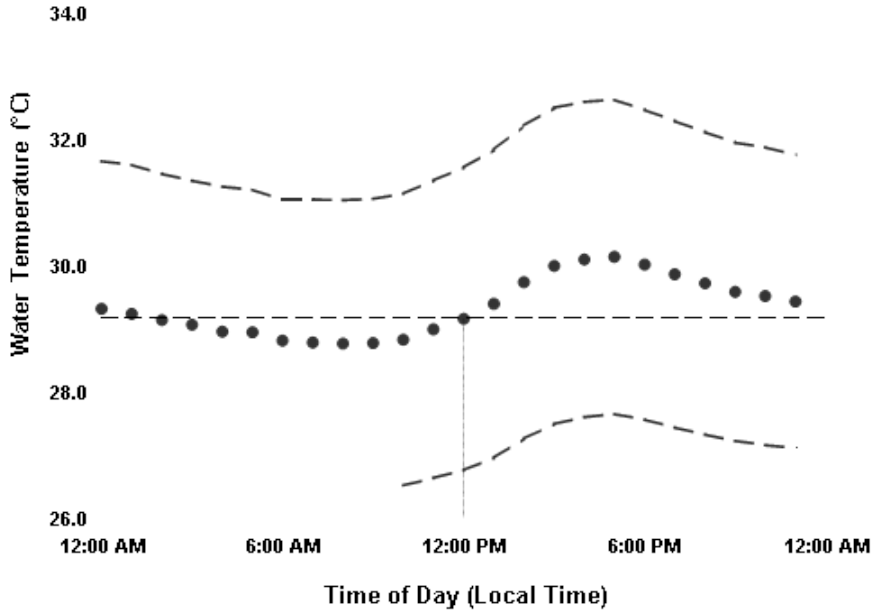


Figure A5-1. Mean and Percentiles (2.5% and 97.5%) of Hourly Water Temperature Profile for Dauphin Island, AL (NBDC, July – Sept 1997)

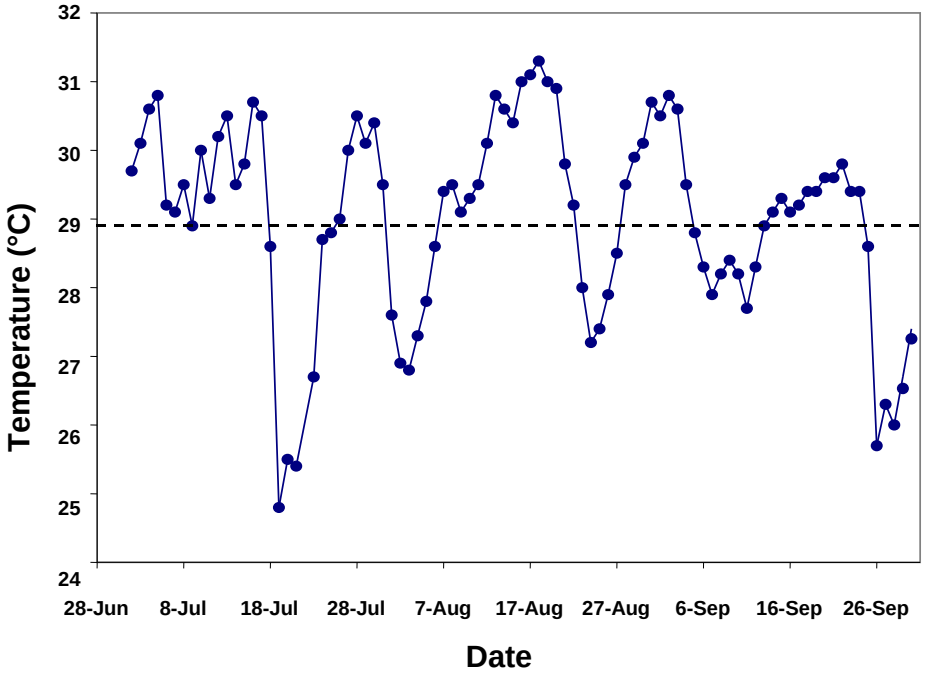


Figure A5-2. Temporal Pattern of Day-to-Day Variation of MiddayWater Temperature Profile for Dauphin Island, AL (NBDC, July – Sept 1997)

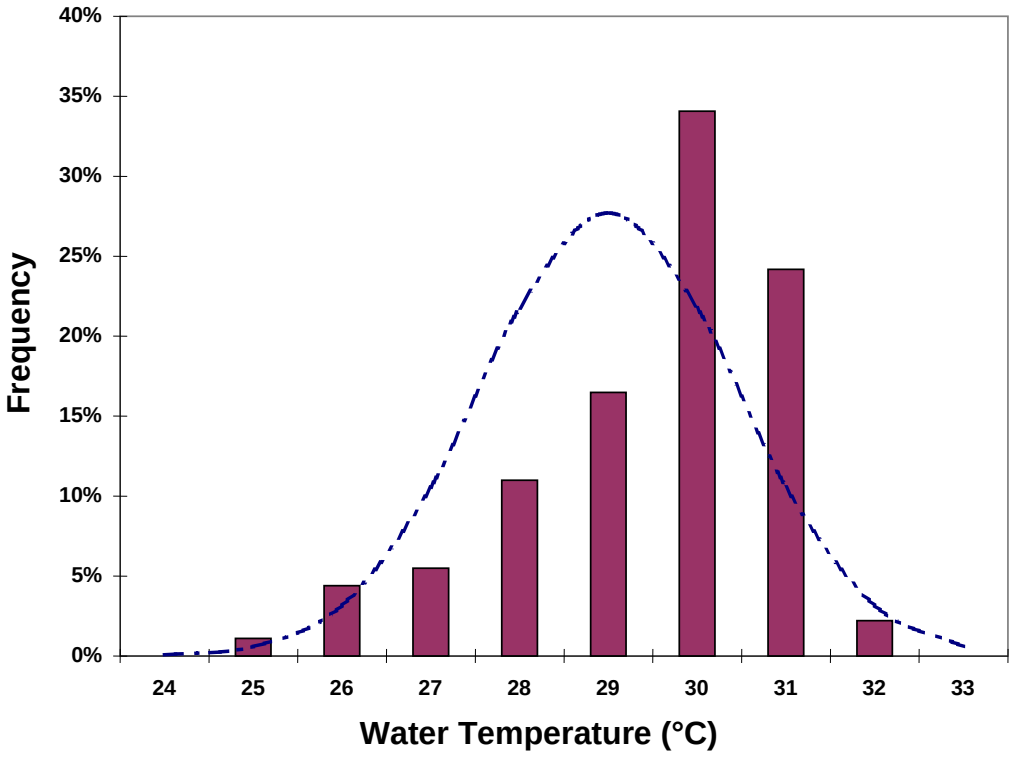


Figure A5-3. Histogram of Day-to-Day Variation of Midday Water Temperature Profile and Approximating Normal Distribution Summary for Dauphin Island, AL (NBDC, July – Sept 1997)

Overall, considering the patterns in the observed distributions of (midday) water temperatures in the other regions and in other seasons for all years of data available from NBDC (1984 – 1998), the normal distribution approximation (as shown in Figure A5-3) was judged to be a reasonable summary for within season water temperature variation. This summary distribution was chosen for simplicity and in consideration of larger determinant factors and uncertainties in the risk assessment model. Within season air temperature, and consequently water temperature of shallow water bodies, is known to be slightly skewed, primarily as a consequence of precipitation patterns. This is reflected in more complex temporal modeling of weather patterns and their effect on temperature (Richardson, 1981). The skewness in the NBDC temperature data for other regions and seasons, when present in a given year, is typical of that shown in Figure A5-3. For these data the approximating Gaussian distribution underestimates the median temperature slightly (approximately 1 °C). Within the context of the risk assessment model, the effect of this bias would be to underestimate levels of total *V. parahaemolyticus* at harvest.

In addition to the variation of daily water temperature in the NBDC data, the variation of water temperature distributions across multiple years was also evaluated for incorporation as a factor in the risk assessment. Figure A5-4 shows a plot of the means and standard deviations of within year and season daily (midday) water temperature distributions across multiple years of data for the Dauphin Island buoy. The data represented here are from 1989 through 1998 (with 1995 excluded due to instrument malfunction). There are four clusters of points based on the definitions of the four seasons used to categorize the data. As evident in Figure A5-4,

temperature distributions were more variable in the spring and fall (middle two clusters of points in the plot) and the least variable in the summer.

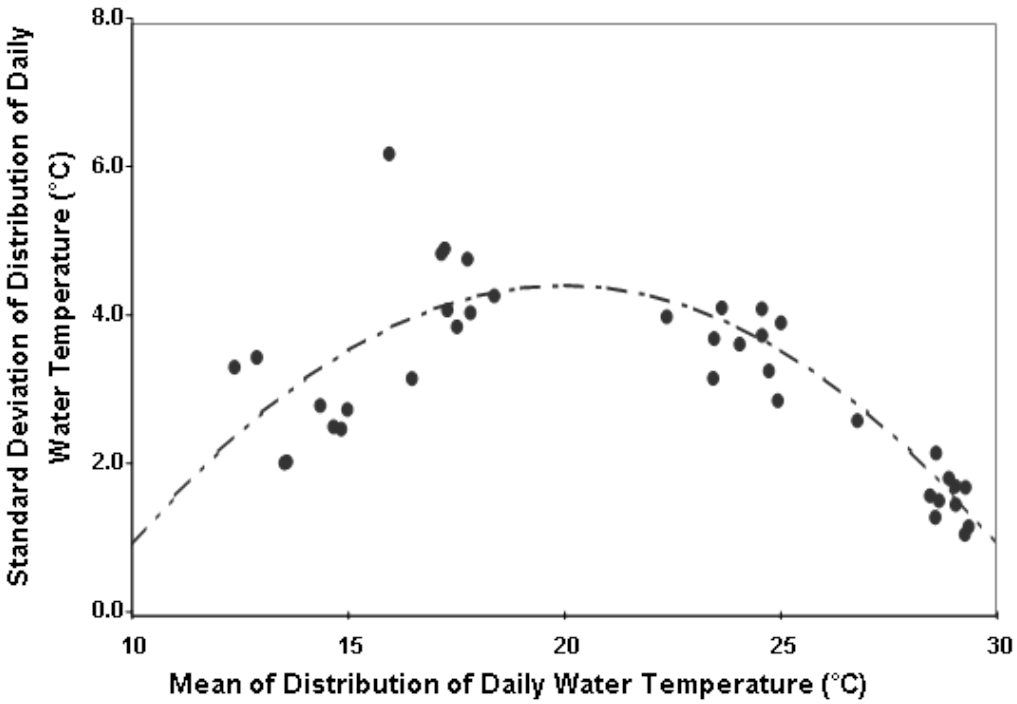


Figure A5-4. Interannual Variation of the Mean and Standard Deviation of Within Season Water Temperature Distributions for Dauphin Island, AL (NBDC, 1997).

The data suggest that a relationship between the mean and variance of daily water temperatures applies not only to comparison of temperature variation in one season versus another within the same year but also to the variation of temperature in the same season across different years. That is, there appears to be a tendency for a warmer than average summer to be less variable day-to-day than a cooler than average summer. Rather than using an approximating relationship to model or summarize this characteristic of the data (e.g., such as by a quadratic), the year-to-year variation of the means and standard deviations of the within year seasonal distributions were summarized by means, variances and correlations. These summaries are shown in Table IV-2.

The number of years of water temperature data available from NDBC sites was limited to at most 15. The statistical precision associated with estimates of the variation and correlation between the mean and standard deviation across different years is low. Nevertheless, some degree of correlation seems reasonable *a priori*, and therefore the apparent correlations were used in Monte Carlo simulations as part of evaluating the effect of year-to-year variations in temperature on the total illness rate predictions. To obtain model predictions, the parameters for within year and season water temperature distributions were obtained by sampling from the bivariate normal distributions with means, standard deviations and correlation specified in Table IV-2. The parameters of the bivariate normal distributions were obtained by a method of moments fit to the relevant summary statistics.

NDBC water temperature data were not available for the Pacific Northwest region. However, the same analysis was conducted using a set of approximately 50,000 water temperatures measurements collected from 1988 through 1999 during the course of various harvest water monitoring programs (e.g., for fecal coliforms, vibrios, etc). These data were made available to the *V. parahaemolyticus* QRA Team by the Washington State Department of Health (1999). The temperature measurements were generally taken at the time of sample collection (e.g., for water or shellfish). The monitoring programs from which these data were abstracted were conducted in multiple oyster harvesting areas of Washington State. Frequently, the dataset had multiple measurements from different sites within the same estuary on the same day. The time of temperature measurement was not reported. Prior to developing the summary statistic described above, the data were averaged over measurements in the same estuary on the same day. It was assumed that variation of time of water temperature measurement across the data set was of minor concern (i.e., in consideration of the discussion of Figure A5-1 above).

Total *V. parahaemolyticus* per gram versus Water Temperature Relationship

Given water temperature distributions, the prediction of the distribution of the density of total *V. parahaemolyticus* in oysters at the time of harvest was obtained based on fitted regression relationships between *V. parahaemolyticus* density and water temperature. Data from three sources were evaluated to determine the relationship. These data were considered the most appropriate for determining the regression relationship because oyster sampling was conducted year round. Two of the studies (DePaola *et al*., 1990, FDA/ISSC, 2001) were nationwide studies with samples being obtained from geographically diverse harvest areas.

Additional data, specific to the Pacific Northwest, were made available to the *V. parahaemolyticus* QRA Team. These data were collected during Washington State monitoring programs at selected times from 1997 through 2001 ((Washington State Department of Health, 2000; 2001). Because the ecology of vibrios is notably different in the Pacific Northwest (e.g., possibly as a consequence of higher salinities) and this area is underrepresented in the two studies above, a separate analysis of the *V. parahaemolyticus* versus water temperature relationship was considered appropriate for this region. However, it was apparent that data collected by Washington State were influenced by the nature of commercial harvesting (e.g., intertidal collection versus dredged). Examination of the Washington State data shows high levels of *V. parahaemolyticus*/g for areas of Hood Canal and South Puget Sound during the summer. These areas are known for intertidal harvesting and the high observed densities are not appropriately predicted based on water temperature alone.

With respect to other regions, the risk assessment model is structured to define "at-harvest densities" as those occurring when oysters are submerged in water. This definition is less obvious for the Pacific Northwest but, to maintain consistency, "at-harvest densities" are defined to be the same as that of the other regions. By this definition, elevation in *V. parahaemolyticus*/g due to intertidal collection is a Post Harvest effect and is most appropriately modeled as separate from the effect of water temperature. Thus, an estimate of the relationship of *V. parahaemolyticus*/g versus water temperature in "at-harvest" (i.e., submerged) oysters was considered a necessary component of the risk assessment model construction.

In this regard, it was determined that subsetting of the data from Washington State was appropriate by excluding from "at-harvest" estimation any data that was likely to have been collected intertidally. Areas of Hood Canal and Southern Puget Sound in the Washington State dataset were considered predominantly intertidal harvest areas and were therefore excluded from "at-harvest" density estimation. The remaining data collected from harvest areas in Willapa Bay and Northern Puget Sound were considered to be predominantly dredged and thus the most appropriate with respect evaluating the relationship between water temperature and "at-harvest" densities (i.e., in order to predict *V. parahaemolyticus*/g in submerged oysters). Appropriate modeling of the effect of the intertidal collection on *V. parahaemolyticus*/g is discussed further under the sections of this appendix pertaining to modeling of the Post Harvest module.

All three of the data sets that were evaluated are subject to some limitations of measurement. When water temperatures are low, total *V. parahaemolyticus* levels are generally below the limit of detection of currently available methods (MPN procedure, direct plating). Thus, microbiological analysis of the oyster samples obtained during the winter frequently yields an outcome that is "nondetect" or nondetectable (i.e., the microorganism is not found in the samples). Statistically, the outcome of such measurements are said to be left censored at the limit of detection (LOD), since failure to isolate *V. parahaemolyticus* from a relatively small analytical portion of a sample places an upper limit on the density in the sample rather than an estimate of that density *per se*. Depending on the size of analytical portion relative to the sample, a conclusion that the microorganism is not present in the sample is generally not warranted. Appropriate statistical analysis of data sets with censored values depends on the overall extent or proportion of samples that are censored. When the extent of censoring is relatively small (<10% of samples), a mean imputation of half the LOD is a commonly used strategy and has been shown not to overly bias parameter estimates. However, when the extent of censoring is large (e.g., >40%), it is generally accepted that mean imputation is not appropriate (EPA, 2000). The degree of censoring is approximately 40% for all three of the data sets considered here. Thus, given that mean imputation is questionable, alternative methods of analysis were considered (see for example, Carabin *et al*., 2001).

The method of analysis used here to determine the regression relationship is the censored or Tobit regression method (Tobin, 1958). The method is appropriate when censoring occurs in the context of regression with normally distributed and homogeneous variance about the mean. Following the observation that the distribution of *V. parahaemolyticus* densities at a given temperature is positively skewed and asymmetric, the $\log_{10}$ transformation of densities is approximately normally distributed. This is not particular to *V. parahaemolyticus* but is common to exposure assessment of other microbial pathogens, possibly due to the exponential characteristics of birth and death of microbial populations. Thus, the Tobit regression method was found to apply to an analysis of $\log_{10}$ *V. parahaemolyticus*/g versus water temperature and was chosen because it is a relatively simple procedure.

Assuming a linear relationship between mean $\log_{10}$ *V. parahaemolyticus*/g and water temperature, both above and below the censoring point or limit of detection (LOD), the regression model assumed for parameter estimation is of the form

$$\text{mean } \log_{10} V.\ parahaemolyticus/g = \alpha + \beta * \text{WTEMP} + \varepsilon$$

where WTEMP is the water temperature, α and β are parameters for the linear relationship of $\log_{10}$ *V. parahaemolyticus* /g versus temperature, and ε is a normally distributed variate with mean zero and variance σ^2. Estimation of parameters in the Tobit regression method is commonly obtained by the frequentist procedure of maximizing the likelihood function, which models the probability of obtaining nondetectable outcomes as well as quantified values. Specifically, when an observation is found to be quantifiable with value X_i (i.e., a value above the LOD) at a water temperature $WTEMP_i$ the contribution to the likelihood is

$$L_i(\alpha,\beta,\sigma \mid X_i > LOD) = \varphi\left(\frac{X_i - (\alpha + \beta * WTEMP_i)}{\sigma}\right)$$

and when an observation is nondetect (at a water temperature $WTEMP_i$) the contribution to the likelihood is

$$L_i(\alpha,\beta,\sigma \mid X_i < LOD) = \Phi\left(\frac{LOD - (\alpha + \beta * WTEMP_i)}{\sigma}\right)$$

where ϕ is the probability density function and Φ is the cumulative distribution function of the standard normal. The total likelihood is the product of the likelihood components for each datum. Maximum likelihood estimates (MLEs) of the parameters are those values of the parameters that maximize this function (i.e., the values of the parameters for which the observed data are most probable in the context of the model).

The SAS (SAS Institute, 1999) procedure LIFEREG was used to obtain the estimates of the regression parameters based on these criteria for each of the three data sets being analyzed. The parameter estimates obtained are given in Table A5-1. The variance-covariance matrix of the MLEs obtained is given in Table A5-2. These variance-covariance estimates were used to construct an approximate uncertainty distribution for the parameter estimates. Based on asymptotic normal distribution theory, the parameter uncertainty was taken to be multivariate normal with mean equal to the MLEs obtained and variance-covariance matrix equal to that estimated based on the data.

Table A5-1. Maximum Likelihood Estimates of the Parameters of the Tobit Regression of $\log_{10}$ *Vibrio parahaemolyticus* per gram Versus Water Temperature (MLEs and 95% Confidence Intervals of Parameters of Temperature-only Regression)

Study	α	β	σ
DePaola *et al*., 1990	-1.03 (-2.14,0.08)	0.12 (0.072,0.17)	1.07 (0.83,1.37)
FDA/ISSC, 2001	-0.63 (-0.87,-0.39)	0.10 (0.092,0.11)	0.76 (0.71,0.82)
Washington State DOH, 2000; 2001	-4.32 (-5.77,-2.88)	0.24 (0.16,0.32)	0.78 (0.61,0.99)

Table A5-2. Estimated Variance-Covariance Matrix for MLEs of $\log_{10}$ *Vibrio parahaemolyticus* per gram Versus Water Temperature Regression Parameters

Study		α	β	σ
DePaola *et al*., 1990	α	0.32	-0.014	-0.032
	β	-0.014	0.00062	0.0012
	σ	-0.032	0.0012	0.019
FDA/ISSC, 2001	α	0.015	-0.00063	0.0012
	β	-0.00063	0.000028	0.000044
	σ	0.0012	0.000044	0.00081
Washington State DOH, 2000; 2001	α	0.543	-0.031	-0.029
	β	-0.031	0.0018	0.0015
	σ	-0.029	0.0015	0.0092

Alternative methods of parameter estimation that could have been applied to appropriately estimate parameter values in the presence of censoring include logistic regression of data classified as >LOD versus <LOD and multiple imputation of left censored data according to estimated distributions. Analyses of the three data sets by these methods do not give substantially different parameter estimates than that obtained by the Tobit regression method.

As assessed by likelihood ratio statistics and measures of goodness-of-fit appropriate to the Tobit regression model (Cameron and Windmeijer, 1996, 1997), the fits of the models to the data were good with temperature being a highly significant effect ($p<0.001$). Based on McFadden's R^2 (a likelihood-based extension of the usual R^2) which is appropriate to the Tobit model, the proportion of the variance in $\log_{10}$ *V. parahaemolyticus* densities which is explained by the effect of temperature is approximately 50%.

The effect of the uncertainties in the parameter estimates obtained by these regression analyses was incorporated into risk assessment evaluation. Although the uncertainty is ostensibly an uncertainty with respect to the relationship existing at the time samples were collected, this was assumed a reasonable surrogate for the potential variation of the relationship across different years due to the possibility of changes in other environmental conditions affecting *V. parahaemolyticus* densities (e.g. oyster physiology, oyster disease, nutrient levels). The effect of regression parameter uncertainty was implemented in the risk assessment by using a multivariate normal approximation for parameter uncertainty (i.e., asymptotic normality of MLEs with sufficiently large sample size). A multivariate approach was necessary due to the fact that the parameter estimates for the slope and intercept of the regressions were highly correlated. The effect of this uncertainty was implemented in Monte Carlo simulations by taking a sample of 1,000 sets of parameters from the uncertainty distributions (a multivariate normal with mean equal to the MLEs and variance-covariance equal to the estimated variance-covariance matrix).

Independent estimates of method error were then used to correct the estimated variance about the regression lines to predict the population variance of the density per gram. The FDA/ISSC (Cook *et al.*, 2002b) study utilized a direct plating procedure with DNA probes. The method

error variance associated with this method has been estimated to be 0.03 based on the difference between counts on replicate analyses of sample aliquots (Ellison *et al*., 2001). A method error variance of 0.03 for $\log_{10}$ *V. parahaemolyticus* (Vp)/g corresponds to a standard deviation of 0.17 $\log_{10}$ Vp/g between replicate analyses. The FDA-BAM method with 3 tubes per dilution was the standard method of analysis for the Washington State data (Garthwright, 1995). The FDA-BAM method has a method error variance of 0.35. In the DePaola *et al.* study (1990) the HGMF procedure as developed by Watkins *et al.* (1976) and later revised by Entis and Boleszczuk (1983) was used. When all suspect colonies are tested for confirmation, the precision of the hydrophobic grid membrane filtration (HGMF) procedure has been shown to be somewhat greater than the 3 tube MPN (most probable number) procedure (Entis and Boleszczuk, 1983; Watkins *et al.*, 1976). In the DePaola *et al.* study (1990), enumeration of *V. parahaemolyticus* colonies was based on testing of five suspect colonies. Consequently, enumeration was not as precise as possible and overall method error associated with estimating *V. parahaemolyticus* densities may have been more comparable to that of a 3 tube MPN procedure. Therefore the method error variance of the FDA-BAM was considered a reasonable estimate of the method error for the HGMF method used in the DePaola *et al.* study (1990).

Analysis of Effects Other than Water Temperature on Mean $\log_{10}$ *Vibrio parahaemolyticus* per Gram

The effect of salinity was considered as a potential predictor of *V. parahaemolyticus* densities in addition to water temperature. For the three datasets considered here, a regression model that is linear in the effect of temperature and quadratic in the effect of salinity was fit to estimate the additional effect of salinity. This regression model is of the form:

$$\log_{10}(\text{V. parahaemolyticus/g}) = \alpha + \beta * WTEMP + \gamma_1 * SAL + \gamma_2 * SAL^2 + \varepsilon$$

where WTEMP denotes water temperature in °C and SAL denotes salinity in parts per thousand (ppt). The parameters α and β are the regression parameters for the temperature effect, γ_1 and γ_2 are parameters for the salinity effect, and ε is a random normal deviate with zero mean and variance σ^2 corresponding to the combined effects of population and method error variation. The maximum likelihood estimates for the fit of this model to the data are shown in Table A5-3.

Table A5-3. Maximum Likelihood Estimates of Tobit Regression of $\log_{10}$ *Vibrio parahaemolyticus* per gram Versus Water Temperature and Salinity (MLEs and 95% Confidence Interval of Parameters of Temperature and Salinity Regression)

Study	α	β	γ_1	γ_2	σ
DePaola *et al*., 1990	-2.63 (-2.14,0.08)	0.12 (0.075,0.17)	0.18 (0.016,0.34)	-0.0042 (-0.0084,0)	1.00 (0.78,1.28)
FDA/ISSC, 2001	-2.05 (-2.76,-1.34)	0.097 (0.087,0.11)	0.20 (0.13,0.27)	-0.0055 (-0.0073, -0.0038)	0.73 (0.68,0.79)
Washington State DOH, 2000; 2001	-1.02 (-34.3,35.0)	0.30 (0.18,0.42)	-0.39 (-3.0,2.2)	0.0084 (-0.04,0.06)	0.87 (0.64,1.16)

For the DePaola *et al*. (1990) data set the effect of temperature is highly significant ($p<0.0001$) and the effect of salinity is marginally significant ($p=0.03$ for the linear term and $p=0.05$ for the quadratic term). The MLE of the optimal salinity level ($-\gamma_1/(2*\gamma_2)$) based on this model and data set is 21.4 ppt.

For the FDA/ISSC (2001) data set, the effects of both temperature and salinity were highly significant ($p<0.0001$). This is a consequence of the much larger sample size of this study compared to that of DePaola *et al*. (1990). The MLE of the optimal salinity level was 18.1 ppt. For the Washington State data, the effect of water temperature was also highly significant ($p<0.0001$) but salinity was not a significant effect. The range of salinities associated with samples in this data set was much narrower compared to the other two data sets and this would appear to be the most obvious reason for lack of significance.

The added value of prediction based on salinity as well as temperature is shown in Figure A5-5 as estimated by both the temperature-only and temperature/salinity regression fits to the FDA/ISSC (2001) data. The relative difference plotted in Figure A5-5 is the difference in the prediction based on temperature and salinity versus temperature-only divided by the prediction based on temperature alone. A relative difference greater than zero indicates that predictions based on water temperature and salinity are higher than based on water temperature alone. When salinity is in a nominal range of 10 to 25 ppt and water temperature is high (>25 °C), the relative difference in predicted values of mean $\log_{10}$ *V. parahaemolyticus* density is relatively small (i.e., an absolute difference of <10%). However, when water temperature is low the difference in predictions is more substantial (up to 40% at 15 °C). Water salinities in harvest areas are more variable than water temperature and no sufficiently comprehensive data sources were identified with respect to including this as a predictive factor in the assessment. Figure A5-5 indicates that the effect on model predictions of neglecting salinity effects is likely to be minor when water temperature is high (e.g., Gulf Coast summer). Furthermore, salinity may not be a strong effect in estuaries of the Pacific Northwest due to the fact that the range of salinities is narrower there than in other harvest areas. Thus, although salinity was identified as a significant effect in the regression analysis, its impact on predicted risk was not judged to be substantial and as such was not included as a parameter/component of the risk assessment model based on these considerations.

For the Washington State data, the possibility of additional effects such as year-to-year differences or differences between sampling areas were also considered. There was an apparent difference in the estimated regression relationship in 1998 when water temperatures were warmer than average but the difference was not large and regression parameter estimates obtained by fit to all available data were used in the assessment.

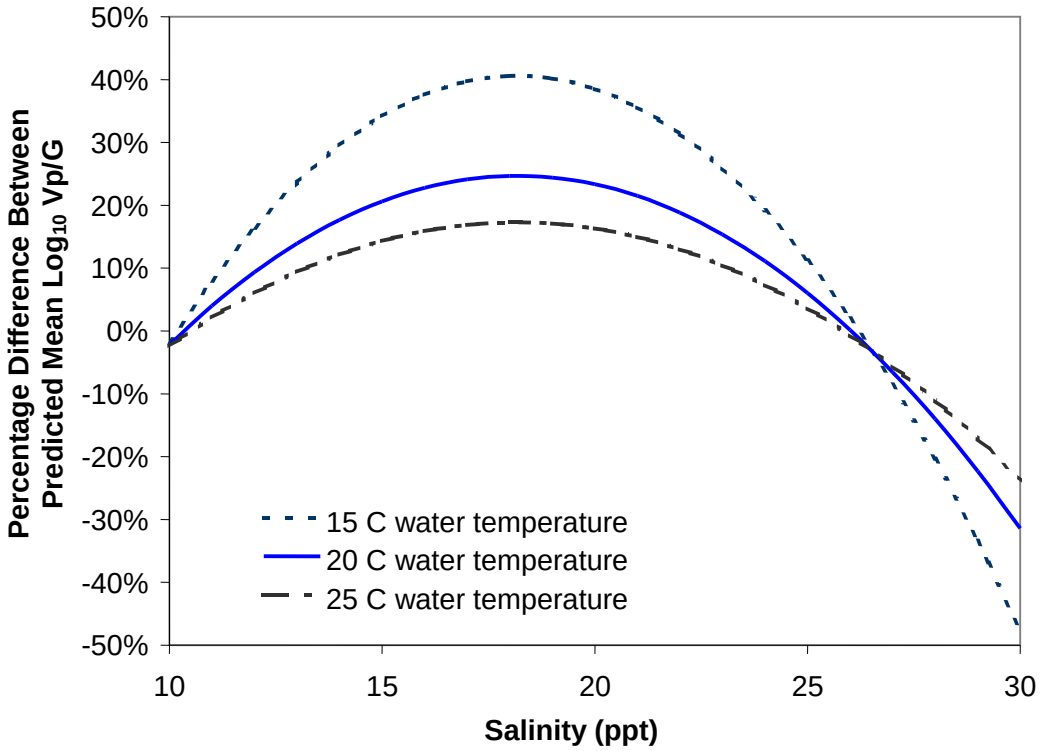


Figure A5-5. Effect of Salinity on Predicted Mean $\log_{10}$ *Vibrio parahaemolyticus* Density in Oysters Relative to Predicted Density at Optimal Salinity (22 ppt).

Percentage of Total *Vibrio parahaemolyticus* that are Pathogenic

Studies of the distribution of total and pathogenic *V. parahaemolyticus* at harvest (Kaufman *et al*., 2003; DePaola *et al*., 2002, DePaola *et al*., 2003a) provide the best information available on both the mean and the variation of the relative abundance of pathogenic (*tdh*+) strains across samples. The information available from older studies is less detailed because the proportion of pathogenic strains in individual samples was generally not reported. Only the average percentage pathogenic, aggregated across multiple samples, can be inferred from the data reported in the older studies that were identified.

The study by DePaola *et al*. (2003a) was a component of the collaborative ISSC/FDA *V. parahaemolyticus* harvest study (FDA/ISSC, 2001). This study collected a total of 156 oyster samples from two harvest areas in Alabama over a period of 14 months in 1999 and 2000. The study by Kaufman *et al*. (2003) was conducted in the summer of 2001 with samples taken from a single Gulf Coast harvest area. A total of 60 individual oysters were sampled with half of these being analyzed immediately after harvest and the other half analyzed after 24 hours of storage at 26 °C. The DePaola *et al*., (2002) study analyzed samples from selected areas of Hood Canal collected in August 2001. Approximately 60 samples were analyzed for pathogenic and total *V. parahaemolyticus* in this study. All three studies utilized a direct plating procedure and gene probes to obtain paired counts of both pathogenic (*tdh*+) and total *V. parahaemolyticus* in analytical portions of each sample. The paired analytical portions assayed for pathogenic versus total *V. parahaemolyticus* were not necessarily the same volume (or weight).

The Beta-Binomial model was assumed as a model for the distribution of observed counts of pathogenic *V. parahaemolyticus* in analytical sample portions. This model implies an overall average percentage pathogenic but the percentage pathogenic in individual samples is also assumed to vary about the average according to a Beta distribution. The extent of the variation about the average that would occur in samples of oysters is likely dependent upon the size of the sample. In the context of the risk assessment, it is the variation of percentage pathogenic between servings that is of particular interest. The serving size varies with 6 and 12 oysters being typical. Environmental studies typically composite oysters, with 6 or 12 oysters per composite for microbiological analysis. Consequently, there is reasonable agreement as to definition of sample versus serving and it was judged that there was little need to correct the distribution of percentage pathogenic on the basis of a substantial difference between the number of oysters per "sample" versus per "serving". Implicitly, it is assumed that the oysters in a typical serving are harvested from the same location (i.e., come from the same harvest collection).

The variation of percentage pathogenic across samples was not estimated as the distribution of the ratio x/n, where x is the observed count of pathogenic *V. parahaemolyticus* (i.e., in an analytical sample portion) and n is a corresponding observed or estimated count of total *V. parahaemolyticus* (in a comparable volume) from the same oyster sample because, for most samples, the pathogenic count was zero. Estimation based on summarizing the data in this manner would potentially bias the estimate of percentage pathogenic distribution towards zero. Instead the Beta-Binomial distribution was fit directly to model the counts of pathogenic in the sample data and obtain estimates of the parameters of the Beta distribution assumed for variation of percentage pathogenic across samples.

Estimation of the distribution of percentage pathogenic was obtained conditional on the observed number of total *V. parahaemolyticus* in each sample and the volumes of sample (i.e., size of analytical portions) examined for both pathogenic and total, respectively. Specifically, given an observation of "n" total *V. parahaemolyticus* in an analytical portion of a sample of volume "V_t" and a count of "x" pathogenic *V. parahaemolyticus* in a replicate analytical portion of volume "V_p", it was assumed that the pathogenic count corresponded to the outcome of a (random) Binomial trial of size "integer(n*(V_p / V_t))" with probability of success equal to the percentage pathogenic in the sample. That is, the density of total *V. parahaemolyticus* in the sample portion examined for pathogenic was assumed known and equal to the estimate obtained from the sample portion assayed for total *V. parahaemolyticus*. The variability of total *V. parahaemolyticus* across analytical portions taken from the same sample was not considered for the purpose of estimation of percentage pathogenic.

The SAS procedure NLP (NonLinear Programming) was used to obtain the MLEs of the Beta-Binomial model for each of the data sets considered. Under the Beta-Binomial model the likelihood of the data is:

$$L = \prod_i \left[\int \binom{n_i}{x_i} p^{x_i} (1-p)^{n_i - x_i} \, dF_p \right]$$

$$= \prod_i \left[\binom{n_i}{x_i} \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} \frac{\Gamma(x_i+\alpha)\Gamma(n_i - x_i + \beta)}{\Gamma(n_i+\alpha+\beta)} \right]$$

where F_p is the assumed Beta distribution (with parameters α and β) for variation of the relative abundance (p) of pathogenic strains in different samples.

Reparameterization facilitated a more numerically stable estimation of the parameters of the Beta-Binomial model by the procedure NLP (e.g., to mitigate effects of excessively large values in the Gamma functions). The reparameterized likelihood used to obtain parameter estimates is of the form:

$$L = \prod_i \left[\binom{n_i}{x_i} \frac{\left\{\prod_{r=0}^{x_i-1}(P+r\theta)\right\} * \left\{\prod_{r=0}^{n_i-x_i-1}(1-P+r\theta)\right\}}{\left\{\prod_{r=0}^{n_i-1}(1+r\theta)\right\}} \right]$$

where P is the average percentage pathogenic and θ is a transformation of the overdispersion parameter (ϕ) of the Beta-Binomial:

$$P = \frac{\alpha}{\alpha+\beta}, \quad \varphi = \frac{1}{\alpha+\beta+1}, \quad \theta = \frac{\varphi}{1+\varphi}$$

The MLEs for P and θ were obtained by NLP subject to the constraints that $0<P<1$ and $0<\theta<1$. The corresponding MLEs for the original parameters α and β were then obtained by the inverse of the defining transformations of the reparameterization. The estimates obtained are shown in Table A5-4.

Table A5-4. Estimates of the Distribution of Percentage Pathogenic *Vibrio parahaemolyticus*

Study Data	P[a]	ϕ	α	β	95% Confidence Interval for P
DePaola *et al*., 2002 (Hood Canal)[b]	2.33%	0.076	0.283	11.86	(1.05%, 5.47%)
Kaufman *et al*., 2003 (0 hr)	0.51%	0.0114	0.442	86.2	(0.21%, 1.47%)
Kaufman *et al*., 2003 (24 hr)	0.08%	0.0011	0.681	907	(0.03%, 0.16%)
Kaufman *et al*., 2003 (all data)[c]	0.18%	0.0045	0.394	221	(0.09%, 0.44%)
DePaola *et al*., 2003a	0.44%	0.0146	0.297	67.2	(0.24%, 0.82%)

[a] α and β denote the parameters, ϕ denotes the overdispersion and P denotes the average of the assumed Beta distribution

[b] estimate used in the risk assessment model for the Pacific Northwest region

[c] estimate used in the risk assessment model for regions outside of the Pacific Northwest

Given the discrete nature of the observed count data, and small samples sizes relative to the mean percentage pathogenic being estimated, a parametric bootstrap procedure (Garren *et al*., 2001) was used to estimate an uncertainty distribution for the parameters α and β. The parametric model assumed was the Beta-Binomial with the parameter values equal to the MLEs obtained based on the observed data. With respect to each study, replicate bootstrap samples of the count of *tdh*+ colonies in analytical sample portions were generated by random sampling of percentage pathogenic from the fitted Beta distributions followed by random sampling from Binomial distributions with size parameter equal to the number of total *V. parahaemolyticus* observed (or estimated) in a volume of sample comparable to that assayed for *tdh*+ colonies. A total of 1,000 bootstrapped data sets were generated for each study and MLEs for the parameters α and β were obtained for each bootstrap by the NLP procedure as described above. On a few rare occasions the NLP procedure did not converge for a bootstrapped outcome, suggesting the lack of existence of an MLE. When this occurred the bootstrapped outcome was dropped from the set defining uncertainty in the estimates of α and β.

All model fits of the Beta-Poisson to the observed data were found to be adequate. Goodness-of-fit was assessed by the method of Brooks *et al*. (1997). Briefly, the maximum likelihood of the fit of the Beta-Binomial model to the observed data (of each study separately) was compared to a null distribution of maximum likelihood values generated by parametric bootstrapping of hypothetical outcomes and obtaining maximum likelihood values for each bootstrap by refitting the Beta-Binomial model. If the maximum likelihood value of the fit of the Beta-Binomial model to the observed data lies at either extreme of the null distribution obtained by bootstrapping then the fit is questionable. As shown in Table A5-5, all of the maximum likelihood values obtained with respect to the observed data are not extreme (e.g., not < 2.5%-tile or > 97.5%-tile). The nature of the variation of relative abundance of pathogenic strains could be other than Beta-Binomial, but the fit of this model to the datasets was not rejected or marginal in any way. The hypothesis that there is no extra-binomial variation in the data (i.e., fit of a binomial model to the data would be adequate) was rejected at the 95% confidence level for all three data sets based on the deviance statistic of the fits.

Table A5-5. Goodness of Fit of Beta-Binomial Model

Study Data	Log_{10} of Maximum Likelihood of Fit	Percentile of the Null Distribution [a]
DePaola *et al*., 2002 (Hood Canal)	-34.35	0.44
Kaufman *et al*., 2003 (0 hr)	-24.50	0.27
Kaufman *et al*., 2003 (24 hr)	-25.63	0.59
Kaufman *et al*., 2003 (all data)	-55.77	0.38
DePaola *et al*., 2003a	-62.94	0.13

[a]the null distribution of maximum likelihood values was estimated by 1,000 Monte Carlo samples of hypothetical (bootstrap) outcomes based on the MLE of the parameters to the observed data

Of the three data sets analyzed, the Hood Canal study (DePaola *et al*., 2002) was the only study with samples taken from the Pacific Northwest. The results of model fits to this data set were taken to be representative of the Pacific Northwest region. Estimates based on the pooled 0 and 24-hour time points of the Kaufman *et al*. (2003) study were used to model all other areas of the country. Collectively, the estimate of the mean based on combined 0 and 24-hour time points of the Kaufman *et al*. study (2003) was lower than that of the DePaola *et al*. (2003a) study, but not significantly so. Furthermore, although the 0 versus 24-hour time points suggested differences in the percentage pathogenic distribution, an estimated mean of 0.18% based on pooling of the data was used for the purpose of risk assessment. Previous studies of the percentage of *V. parahaemolyticus* isolates that are pathogenic in retail samples (FDA/ISSC, 2000) and studies of the growth rate of pathogenic versus nonpathogenic strains (Cook, 2002a) do not support the hypothesis that there is any appreciable difference in percentage pathogenic at retail versus at harvest levels. The inferred uncertainty distributions of mean percentage pathogenic and the underlying bootstrap uncertainty distributions for α and β are shown in Figures A5-6 and A5-7, respectively.

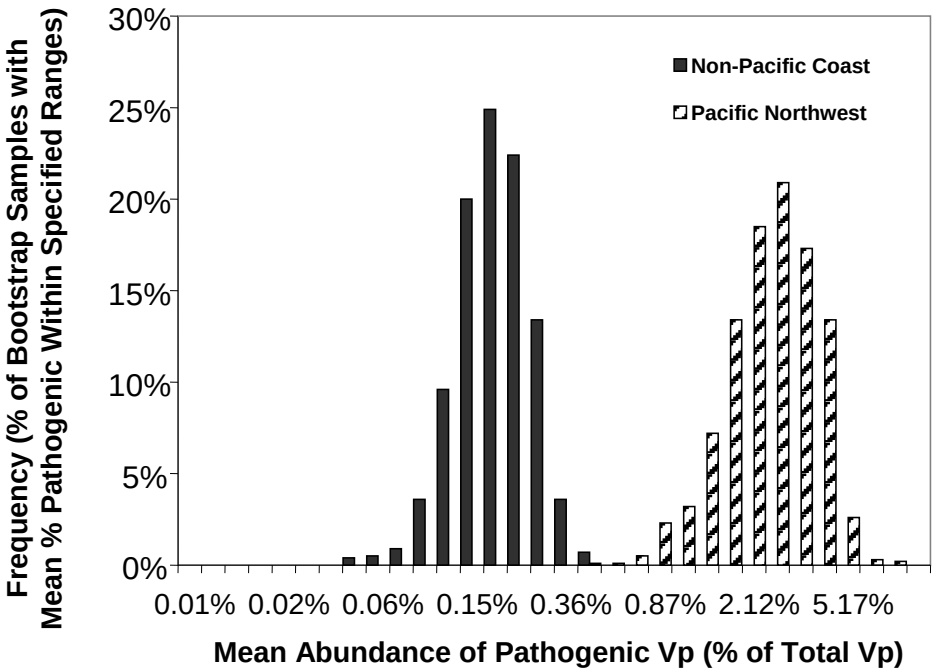


Figure A5-6. Histograms of Bootstrap Uncertainty Distributions of Mean Percentage Pathogenic Based on the Beta Distribution Model of Sample-to-Sample Variation of Percentage Pathogenic *Vibrio parahaemolyticus* (Pacific Northwest and Non-Pacific Coastal regions).

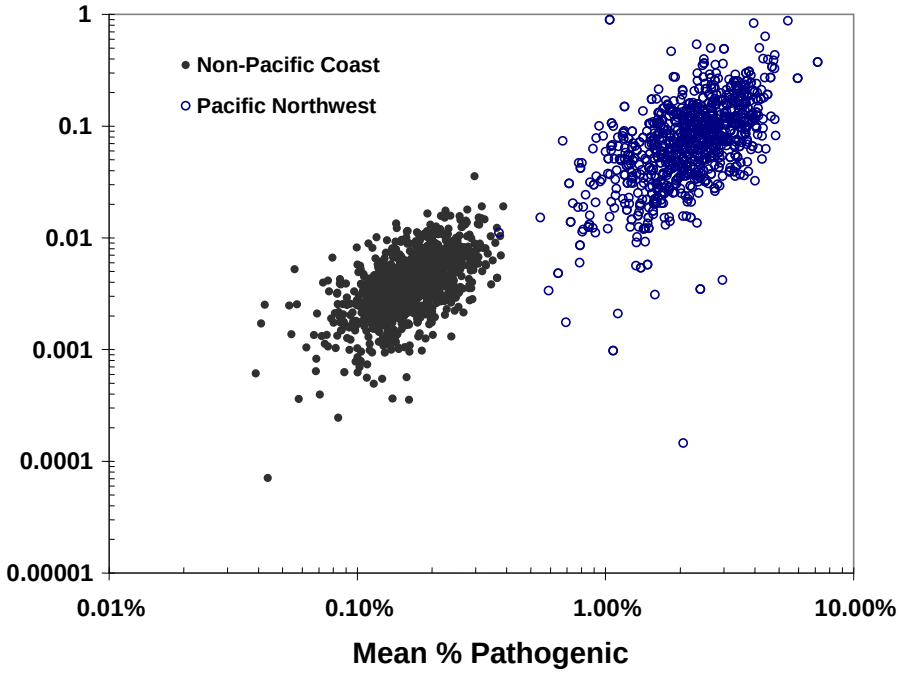


Figure A5-7. Bootstrap Samples of Uncertainty of Mean Percentage Pathogenic and Dispersion Parameter of the Beta Distribution Model of Sample-to-Sample Variation of Percentage Pathogenic Vibrio parahaemolyticus (Pacific Northwest and non-Pacific Coastal regions).

Post Harvest Module

Based on the distribution of *V. parahaemolyticus*/g at harvest, the Post-Harvest Module predicts the distribution of *V. parahaemolyticus*/g at the time of consumption by modeling the influence of various factors on the outgrowth and die-off likely to occur during storage. Principally, this is determined by distributions of time that oysters are exposed to ambient temperature during harvest before first refrigeration, various times of transport and storage together with distributions of temperature of exposure and rates of growth or decline versus temperature.

Distribution of Air Temperature at Oyster Harvest

Oysters are typically harvested from shallow water estuaries (e.g. 1-3 m depth). Consequent to the action of wind and tides, one would expect a correlation between the day-to-day variations of the water temperature and that of the air temperatures. This was confirmed from examination of the data from the NBDC buoys selected as being representative of the four harvest regions.

To facilitate the Monte Carlo simulation of the risk assessment model, the correlation between the air and water temperatures that oysters are exposed to was implemented by using the distribution of the difference between air and water temperature as a model parameter. This difference and water temperature were then used as inputs to determine the distribution of air temperatures that oysters are exposed to during harvesting. This approach was adopted in order to assure proper correlation between water and air temperatures.

Analysis of the NBDC (1997) data revealed that the relationship between air and water temperature changes during the course of the day. This is due to the fact that the temperature of air is more variable over a 24-hour period than that of the water. Figure A5-8 shows the mean difference between air and water temperature as a function of the time of day at the Dauphin Island Buoy (1997 data). The relationship between air and water temperatures in the Gulf are different from more northern areas of the country in that the mean water temperature is always warmer than that of the air. In northern areas of the country, i.e., the other 3 harvest regions in the assessment, the mean water temperature is cooler than that of the air during the spring and summer, and the reverse is true during the fall and winter.

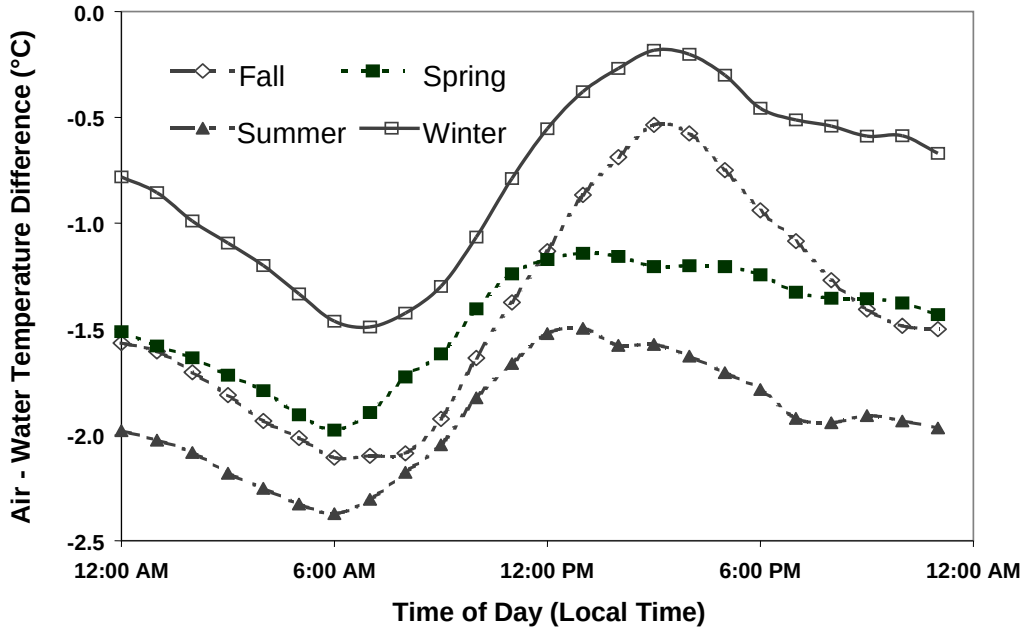


Figure A5-8. Variation of mean hourly air-water temperature differences for Dauphin Island, Alabama Buoy (NBDC, 1997).

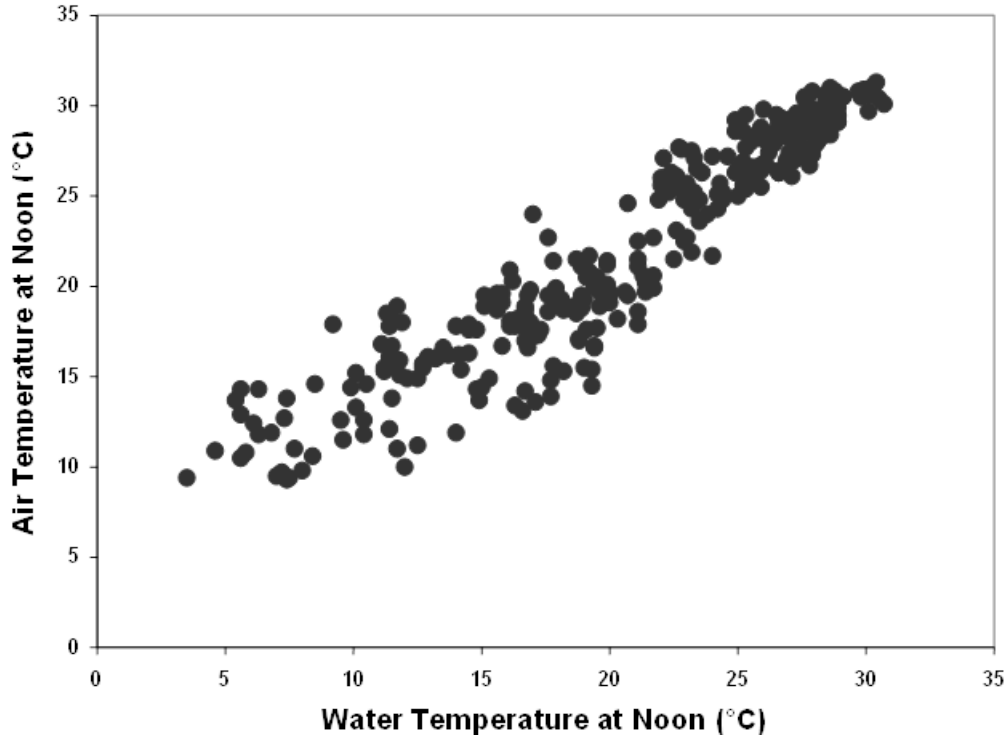


Figure A5-9. Correlation of Daily Midday Air and Water Temperature Measurements for Dauphin Island, AL (NBDC, 1997).

To minimize complexity, the relationship of air versus water temperature at 12:00 pm (midday) was assumed to be a reasonable average for the purpose of estimating a distribution of temperatures that oysters are typically exposed to during the course of oyster harvesting (i.e., harvesting starts in the early to mid morning and may last through mid-afternoon). Figure A5-9 shows the correlation of air versus water temperatures measured at midday at the Dauphin Island Buoy in 1997. Figure A5-10 shows the distribution of the difference between the same air and water temperature data as a function of water temperature. Clearly, the difference between air and water temperature is more variable when the water temperature is lower.

There were no noticeable differences in this relationship between air versus water temperatures within the same season across different years, either for Dauphin Island or any of the other NBDC sites utilized for the assessment. Consequently, seasonal distributions of the difference between air and water temperature were obtained by pooling all available years of data. The distribution of the difference in air temperature versus water temperature was then approximated as being Gaussian within each region/season classification, with mean and standard deviation estimated by the method of moments. As was the case with water temperature, this summary distribution is only an approximation since the air temperatures in the NBDC data exhibit the same degree of skewness as was discussed above with regard to water temperatures (Figure A5-3). The air-water temperature difference is also slightly skewed but less than that of either air or water temperature alone.

Distribution of Time to 1st Refrigeration

The distribution of time that oysters are exposed to ambient air temperatures during harvest (i.e., prior to refrigeration) was derived based on the distribution of duration of harvest and a distribution for the time when individual oyster lots are collected during the harvest. For the first distribution, the only information identified was minimum, maximum and most likely durations obtained by interviews with harvesters in several Gulf Coast states (GCSL, 1997). Based on this information estimated distributions of duration of harvest were taken to be Beta-PERT distributions with specified minimums, maximums and modes for each region and season combination.

The relative proportion of the harvest caught during the course of harvesting operations may vary somewhat from one harvest area to the next. However, with the exception of time required to return to dock from the harvest area, a constant harvesting operation was assumed to be typical of the majority of harvest areas. A time of 1 hour was considered typical of time to return to dock from the harvest areas. Constant harvesting operation implies that the distribution of the catch within the harvest period is uniform. Thus the time of collection of oyster lots, relative to time of 1st refrigeration, was taken to be a continuous uniform random variable with minimum time equal to 1 hour and maximum equal to the duration of harvesting operation. Distribution of time to 1st refrigeration for individual lots is the mixture of the distribution functions for duration of harvesting operation and the distribution of time of collection for a given duration of harvesting.

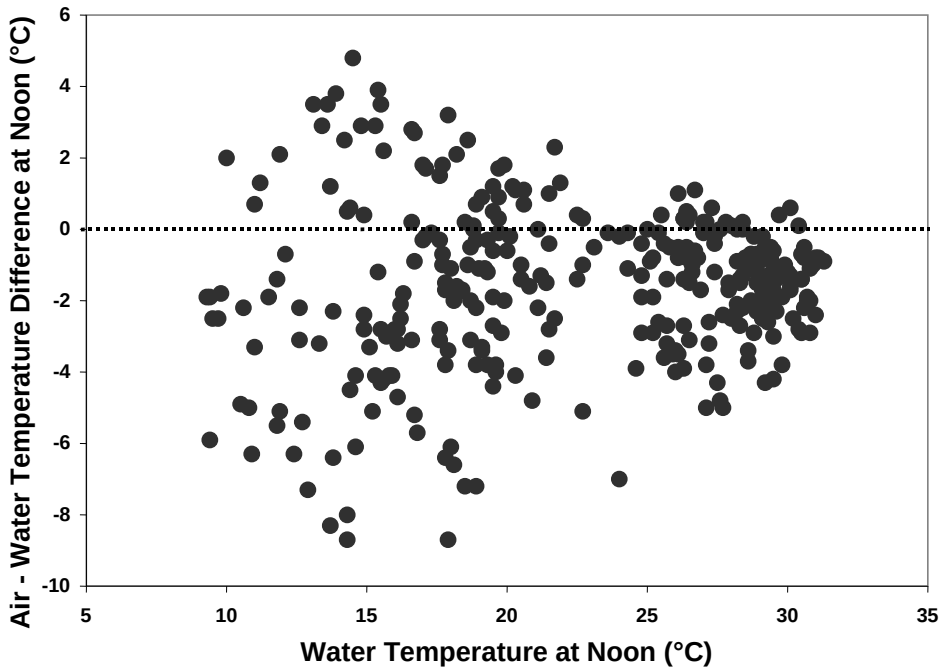


Figure A5-10. Differences Between Midday Air and Water Temperatures as a Function of Water Temperature for Dauphin Island, AL (NBDC, 1997).

Growth Rates

The growth and die-off rates were based on estimates obtained from the published literature (Miles *et al*., 1997, Gooch *et al*., 2002, FDA/ISSC, 2000). Statistical criteria used to obtain these estimates are described in the respective references. The growth rate study in oysters (Gooch *et al*., 2002) was conducted at only one temperature (26 °C). Therefore, a study of growth rate in broth (axenic) culture (Miles *et al*., 1997) across a range of temperatures (and water activities) was used as a basis to extrapolate growth rate in oysters to temperatures higher and lower than 26 °C. The uncertainty associated with this prediction was addressed in the assessment by incorporating an uncertainty distribution for the relative growth rate in broth culture versus in oysters. The uncertainty distribution selected for this ratio was taken to be a triangle distribution with minimum of 3, mode of 4 and maximum of 5. The mode of 4 corresponds to the best estimate of the ratio of the predicted growth rate in broth culture at 26 °C, using the Miles *et al*. (1997) model, compared to the growth actually observed in oysters held at 26 °C (Gooch *et al*., 2002).

More specifically, with respect to the growth rate, Miles *et al*. (1997) obtained worse case estimates based on the fastest growing of four strains that were studied. For each combination of temperature and water activity, the extent of bacterial growth observed was modeled using the Gompertz function and an estimate of the maximal rate of growth was obtained. A secondary model was then used to estimate the effect of environmental parameters (temperature and water activity) on the maximal growth rate. The model that was assumed by Miles *et al*. (1997) was of the square root type:

$$\sqrt{\mu_m} = \frac{b*(T - T_{min})*\left[\{1 - \exp(c*(T - T_{max}))\}*\sqrt{(a_w - a_{w,min})*[1 - \exp(d*(a_w - a_{w,max}))]}\right]}{\sqrt{\ln(10)}}$$

where

μ_m = maximal growth rate ($\log_{10}$ per minute)
a_w = water activity
T = temperature (in degree Kelvin)

The estimates of the parameters that were obtained are:

b = 0.0356
c = 0.34
T_{min} = 278.5
T_{max}= = 319.6
$a_{w,min}$ = 0.921
$a_{w,max}$ = 0.998
d = 263.64

The parameters T_{min}, T_{max}, $a_{w,min}$, and $a_{w,max}$ denote the range of temperatures and water activity over which growth can occur. The authors validated their model by comparison of model predictions with observed rates in eight other studies of growth in broth model systems obtained from the literature.

To use the (1997) *et al.* equation as a prediction of growth rate in oysters 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 ^{o}C (78.8 °F) is 0.84 $\log_{10}$ per hour, which is approximately a 7-fold increase in density per hour. This is four times greater than the rate of growth observed for *V. parahaemolyticus* in oysters held at 26 ^{o}C (78.8 °F) (Gooch *et al*., 1999). Therefore, based on this observation, prediction of the growth rate in oysters at temperatures other than 26 °C (78.8 °F) was obtained by dividing the predicted rate for broth culture by a factor of four. This assumes that the growth rate in oysters is a constant fraction of the growth rate in broth at all temperatures. The influence of this assumption in the risk assessment was evaluated by considering this factor as an uncertainty parameter varying according to a triangle distribution in the range of 3 to 5 with a mean of 4. This gives an indication of the sensitivity of our conclusions to the magnitude of the relative growth rate in oysters versus broth culture but does not fully address the uncertainty in so far as it is conceivable that the relative growth rate could be temperature dependent. Although the appropriateness of the assumption has not been fully validated, the ambient temperature of Gulf Coast is close to 26 °C (78.8 °F) from April through October and this is a region and season for which the largest number of *V. parahaemolyticus* cases is associated.

A plot of the resulting model prediction for μ_m as a function of either temperature or water activity is a unimodal function with a maximum value and zero growth rate outside of the predicted range of temperatures and water activity favorable for growth. To use this equation as

a prediction of growth rate in oysters 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 ^{o}C (78.8 °F) is 0.84 $\log_{10}$ per hour, which is approximately a 7-fold increase in density per hour. This is four times greater than the rate of growth observed for *V. parahaemolyticus* in oysters held at 26 ^{o}C (78.8 °F) (Gooch *et al*., 1999; Gooch *et al*., 2002).

Oyster meat temperature

Air temperature was used as a surrogate for oyster meat temperature for oysters harvested by dredging and intertidal. For oysters harvested in intertidal areas, additional growth of *V. parahaemolyticus* was considered as described below.

Effect of Intertidal Exposure in the Pacific Northwest

Unlike other areas of the country, a significant fraction of oysters harvested in the Pacific Northwest are collected when oyster reefs are exposed during the course of the tide cycle. Exposure to the air and consequent radiative heating of oysters in bright sunlight can elevate oyster temperatures substantially above that of the water temperature. To model the effect of intertidal harvesting on *V. parahaemolyticus* densities in the Pacific Northwest, a distribution of oyster temperature during intertidal exposure was developed based on the observational data available and an estimate of the proportion of days that are subject to cloudy, partly cloudy or sunny conditions. Radiative heating, leading to oyster temperatures well above ambient air temperature was considered likely for sunny days and unlikely for cloudy days.

Estimates of the fraction of the Pacific Northwest catch that are harvested during intertidal cycles were obtained based on data for harvest volume from selected areas of Washington State. This information was combined with expert opinion concerning the fraction of harvest from each area that is collected intertidally rather than dredged (i.e., from submerged reefs) (Kaysner, 2002). The harvest volumes of selected areas of Washington State are shown in Table A5-6.

Table A5-6. Average Total (Dredged and Intertidally Picked) Oyster Shellfish Harvest (in pounds) in Selected Areas of Washington State by Season (Yearly Averages Based on 1990-2001 data)

Area	Winter	Spring	Summer	Fall
Hood Canal	389,000	480,000	378,000	416,000
North Sound	328,000	308,000	254,000	245,000
South Sound	844,000	574,000	437,000	595,000
Willapa Bay/Grays Harbor	324,000	259,000	205,000	198,000
Total	1,886,000	1,620,000	1,274,000	1,454,000

Note: Harvest intended for the shucked market was excluded since this market is typically not intended for raw consumption

Table A5-7. Average Area-Specific Oyster Shellfish Production Expressed as a Percentage of the Total Oyster Shellfish Harvest (in pounds) for Selected Areas of Washington State by Season (yearly averages based on 1990-2001 data)

Area	Winter	Spring	Summer	Fall
Hood Canal	20.6%	29.6%	29.7%	28.6%
North Sound	17.4%	19.0%	19.9%	16.8%
South Sound	44.8%	35.4%	34.3%	40.9%
Willapa Bay/Grays Harbor	17.2%	16.0%	16.1%	13.6%

Table A5-8. Percentage of Area-Specific Oyster Shellfish Harvest Collected During Intertidal Exposure

Area	Percentage Intertidal	Percentage Dredged
North Sound	75%	25%
Hood Canal	95%	5%
South Sound	90%	10%
Willapa Bay/Grays Harbor	10%	90%

An estimate of the overall percentage of the harvest collected intertidally in Washington State for each season was obtained by weighting the expert opinion on area-specific percentages for intertidal collection (Table A5-8) by the percentages that each area contributes to the total shellfish harvest (Table A5-7). The shellfish harvest excludes the portion of the total harvest intended for the shucked market. The harvest statistics indicated that virtually all oysters

intended for the shucked market were harvested by dredging. In these calculations it was assumed that the area-specific percentages for intertidal versus dredged harvest (Table A5-8) do not vary by season. Thus, the seasonal fraction of the total oyster harvest collected intertidally was calculated as:

$$\% \text{ intertidal } = \sum_i h_i * p_i$$

where h_i is the percentage of total harvest collected in the i^{th} area and p_i is the percentage of the harvest in that area collected during intertidal exposure. The results of these calculations give an estimate of 75% of the total harvest being collected intertidally. There was very little variation in this estimated percentage across different seasons; therefore the aggregate average was used as an estimate for all seasons in the risk assessment model. The remaining 25% of the harvest, being dredged, was not subject to predictions of growth that may occur due to elevated oyster temperatures. The Post-Harvest growth of *V. parahaemolyticus* in this latter portion of the harvest was treated in a manner similar to that applied to the other 3 regions of the country.

Studies of *V. parahaemolyticus* densities in Washington State (DePaola *et al*., 2002; Herwig and Cheney, 2001) provide some observational data bearing on the extent to which oyster temperatures are elevated during intertidal exposure. In the DePaola *et al*. (2002) study, a total of 17 temperature measurements were taken over a period of a week in conjunction with the microbiological analysis of oyster samples collected at the end of the exposure cycle (i.e., after full or "maximum" exposure to ambient air temperatures and sunlight). Across this set of measurements, the minimum, maximum, and mean oyster temperatures were 23.3, 32.6, and 27.5 °C, respectively. The distribution of temperatures was almost uniform over the range from minimum to maximum with the 25%-tile and 75%-tile of the distribution being 25 and 30 °C, respectively. Compared to air temperatures, oyster temperatures after maximum exposure were an average of 5 °C (9 °F) greater than air temperature. The maximum difference was 8 °C (14.4 °F). The mean oyster temperature was equal to the median and thus was not a consequence of any extreme observations. At the time of the study the water temperature in the Hood Canal estuary was slightly less than 17 °C. Elevated oyster temperatures, relative to that of the air were also reported by Herwig and Cheney (2001).

National Weather Service (NWS) historical data indicate that during the summer, in the Pacific Northwest, meteorological conditions are evenly divided between cloudy, partly cloudy and sunny conditions. A higher proportion of cloudy days occur during the winter but, given that summer is the higher risk season, the proportion of sunny versus cloudy days during the summer was considered to be more pertinent. Based on this information and the range of oyster temperatures observed in the study by DePaola *et al*. (2002), average difference between oyster temperatures versus air during intertidal exposure was modeled as being uniform in the range of 0 to 10 °C. The duration of exposure to ambient air and radiative heat was assumed to be uniform in the range of 4 to 8 hours; e.g., in consideration of the likely variation in the depth of oyster beds in relation to tide height and flows. The duration of oyster harvesting for intertidal was assumed to commence at the start of oyster collection. Given the estimate of a minimum of 2, mean of 8 and maximum of 11 hours for duration of harvest for the Pacific Northwest (i.e., in regard to ISSC time-to-refrigeration guidelines, ISSC&FDA, 1997), it was assumed that oysters harvested intertidally would reach refrigeration in a maximum of 11 hours from the start of

collection. The duration of transport time (in hours) after intertidal exposure was therefore taken as:

Max(Beta-PERT(2,8,11) – Intertidal Exposure Time, 1)

Growth during the period of transport was assumed to occur at a rate commensurate with air temperature, as oysters are typically collected by boat or barge after being briefly cooled by water when the tide comes in and the oysters are retrieved for transport to processing facilities. A minimum transport time of 1 hour was assumed.

Distribution of Time to Reach No-growth Temperatures and Duration of Cold Storage

There is little data available to precisely quantify the distribution of the length of time required for oyster lots to reach no-growth temperatures after being placed in cold storage after transport from the harvest areas. Therefore it has been assumed that the distribution is uniform between 1 and 10 hours. This distribution was chosen to represent a mixture of both variability and uncertainty. A maximum time of 10 hours was selected based on literature of cooling studies with other food products, primarily meat. Studies of oyster equilibration to air temperature were undertaken by GCSL, in part to validate the reasonableness of this distribution (Cook, 2002b). The component of these temperature studies pertinent to the distribution of time to reach no growth temperature during 1st refrigeration was conducted with initial oyster temperatures of 25 °C and cooler temperature of 4 °C. In this study the temperatures of selected oysters within a "sack" were continuously recorded during cooldown. Oysters located toward the center of the sack/lot cooled more slowly than those on the outside. Temperatures of individual oysters decreased exponentially, reaching the cooler temperature at times ranging from 7 to 8.5 hours. This was considered confirmatory of the maximum of 10 hours assumed for the distribution given that the loading of commercial coolers is likely to be heavier and more variable than that typified by conditions in the experimental study. Furthermore, all commercial coolers may not be consistently at or below 4 °C.

Consumption Module

The distribution of the dose of pathogenic *V. parahaemolyticus* ingested per serving was estimated based on combining distributions of (a) the number of oysters consumed; (b) the weight of oysters consumed; and (c) the density of total *V. parahaemolyticus* per g and (d) the percentage of total *V. parahaemolyticus* per g that are pathogenic. Estimated distributions of the number of oysters consumed and the weight of oysters consumed is addressed here.

Number of Oysters per Serving

The modeled distribution of the number of oysters per serving was taken to be equal to the empirical distribution observed in response to a consumer survey conducted in 1994 by the Florida Agricultural Market Research Center (Degner and Petrone, 1994). In this study, the average number of oysters eaten per occasion was reported to be 13.8 with a range of 1 to 60. Given the relatively large number of respondents in the survey (n=319) and the evident multimodal characteristics of this distribution (6, 12, and 24 oysters/serving being the most probable), the empirical distribution was taken as an estimate rather than attempting to summarize the data by fit of a parametric distribution.

The survey was conducted in a coastal area, where consumption of oysters per eating occasion may be expected to be higher than in inland areas of the country. However, no other suitable sources of data were identified with respect to consumption patterns nationwide and it was judged that the potential bias in using the distribution as a nationwide estimate was minimal in comparison to other modeling uncertainties impacting estimated dose per serving.

Distribution of Meat Weight per Oyster

Given a distribution of the number of oysters per serving, an estimate of meat weight per oyster is needed to determine a distribution of the meat weight consumed per serving. The most relevant data identified to estimate the gram weight of oysters was the ISSC/FDA retail data (FDA/ISSC, 2000; DePaola, 2002). In this study, 339 of the 370 oyster samples collected from wholesale and retail locations were weighed prior to microbiological analysis. Samples generally consisted of composites of 12 oysters (range, 4-15) and this included both the oyster meat and the mantle fluid. The average oyster (i.e., meat and mantle fluid) weight per sample was calculated by dividing the total gram weight of the composite sample by the number of oysters in the sample. The resulting distribution of average oyster weight per sample was found to be positively skewed. The distribution is shown in Figure A5-11.

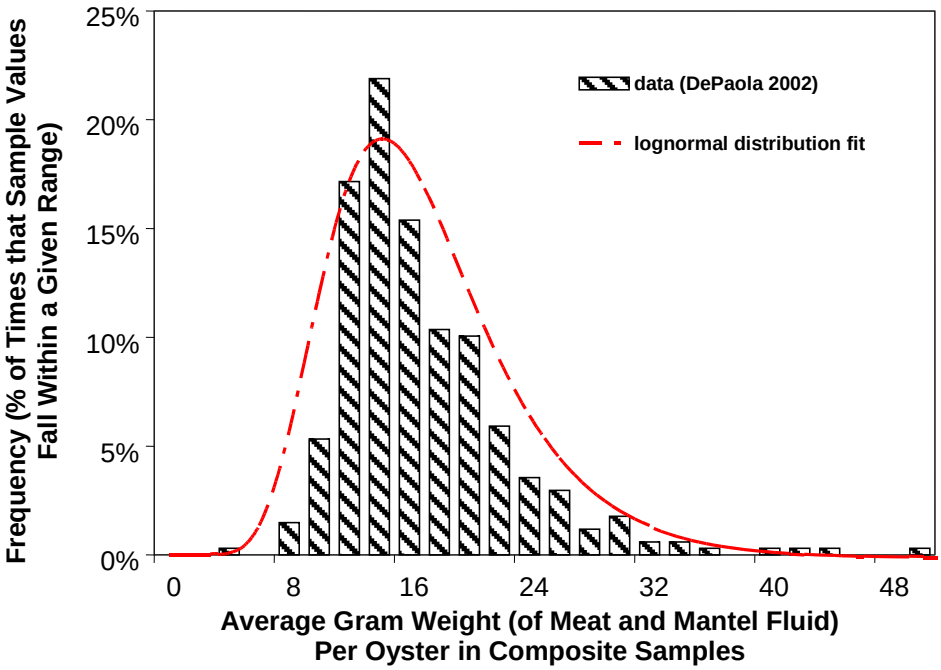


Figure A5-11. Distribution of Average Oyster (Meat and Mantle Fluid) Weight Over Samples of Composites of 4-15 Oysters Collected From Retail Establishments (FDA/ISSC, 2000; DePaola, 2002).

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 per sample of 15.2 grams and a geometric standard deviation of 1.4 grams (Figure A5-11).

Samples in the retail study consisted of composites of both oyster meat and mantle fluid. Accordingly, a correction was applied to infer the average meat weight per oyster consumed. Oyster mantle fluid is typically not consumed with the oyster meat. The distribution of the ratio of meat weight to total (meat and mantle fluid) oyster weight based on measurements of individual Gulf Coast oysters collected during the Kaufman *et al*. (2003) study is shown in Figure A5-12. Although there is a distribution of percentage meat weight per oyster the coefficient of variation is very small. The mean of the distribution is 90%. Given the relatively small coefficient of variation, an average percentage was used, rather than the distribution, to determine a distribution of oyster meat weight consumed from the distribution of oyster weights shown in Figure A5-11.

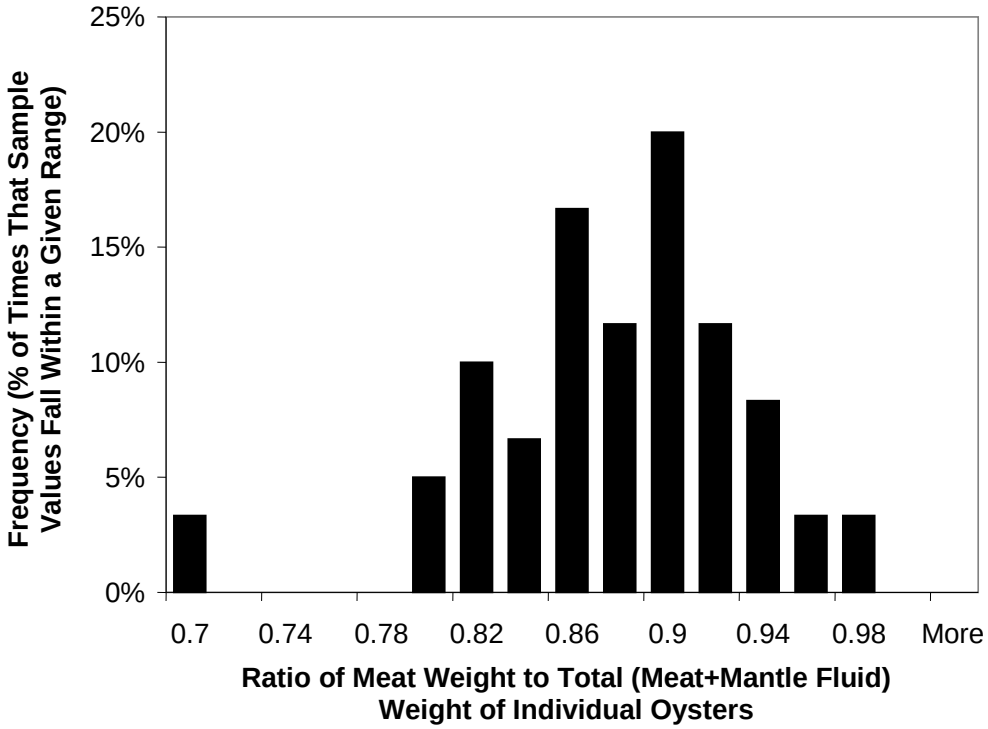


Figure A5-12. Distribution of Oyster Meat Weight as the Percentage of Total Oyster (Meat and Mantle Fluid) Weight over Samples of Individual Oysters (Kaufman et al., 2003).

Appendix 6: Regression-Based Sensitivity Analyses to Determine Influential Variability and Uncertainty Parameters

Sensitivity Analysis of Variability Parameters

A deficiency associated with sensitivity analysis via Tornado plots (i.e., pairwise correlations) is that the importance of various factors is evaluated one at a time. Correlation or multicollinearity between input factors can confound the interpretation of importance via a Tornado plot. An alternative method of influence or importance assessment is based on estimation of the percentage of variation of the output variable (e.g., $\log_{10}$ risk per serving) attributable to selected factors and combinations of factors. A variety of parametric and nonparametric methods have been developed to estimate importance based on the concept of variance decomposition (i.e., attribution of variance to selected factors) (McKay, 1995; Saltelli *et al*., 2000; Archer *et al*., 1997; Chan *et al*., 1997).

Parametric, or regression-based methods, are the easiest to implement and do not entail substantial error when the fit of the regression model used to assess importance is a reasonable approximation of the model simulation output (Manteufel, 1996). For the *V. parahaemolyticus* risk assessment model, simple regression models were found to be reasonable and therefore appropriate for the assessment of importance based on variance decomposition. Table A6-1 gives the results of one such analysis, with a measure of sensitivity based on relative partial sums of squares (Rose *et al*., 1991), applied to assess importance of variability parameters on the $\log_{10}$ of individual risk per serving for both the Gulf Coast summer harvest and the Pacific Northwest intertidal summer harvest. The log transformation of risk per serving was used as the output variable in this evaluation given the observation that individual risk per serving was a highly asymmetric distribution.

Both a linear regression and a quadratic response surface were considered as approximations of model simulation output. However, a quadratic response surface did not provide a substantially better fit than a simple linear regression. Hence, only the results of the linear regression are presented in Table A6-1. Sensitivity coefficients based on the proportion of total variation explained by each factor/parameter were calculated from regression fits according to the formula

$$Sensitivity\ coefficient = RPSS_i = \frac{(RSS - RSS_{-i})}{TSS} \times 100$$

where:

$RPSS_i$ is the relative partial sum of squares attributable to factor i.
RSS_{-i} is the regression sum of squares for a regression model with factor i not present as a predictor.

RSS is the regression sum of squares of a full regression model with all factors present.
TSS is the total variation (total sum of squares) of the output variable (i.e., $\log_{10}$ of risk per serving).

The difference between RSS and RSS_{-i} is the amount of variation in the output variable that can be explained by inclusion of i-th factor and its (potential) interaction with other factors depending on the form of the approximating regression. Thus, the relative partial sum of squares is an indication of the additional percentage of variance of the output variable explained by a parameter, given that all other parameters are included in the regression model. The sum of the percentage of additional variation explained by each parameter is not, however, exactly equal the total amount of variation explained by the full approximating regression since partial (or type III) sums of squares do not add up to the total regression sum of squares.

Table A6-1. Importance of Within Region/Season Variability Parameters on $\log_{10}$ Individual Risk per Serving for the Gulf Coast (Louisiana) Summer and the Pacific Northwest (Intertidal) Summer Harvests Based on Linear Regression Analysis of Monte Carlo Simulation Output

Region / Season	Parameter	Sensitivity Coefficient [a]
Gulf (Louisiana) / summer	Log_{10} *V. parahaemolyticus* per g at harvest	21.4%
	Percentage pathogenic	16.2%
	Time unrefrigerated	9.6%
	Duration of cooldown	3.9%
	Grams of oysters consumed	3.2%
	Length of refrigeration time	2.1%
	Ambient air temperature	1.7%
	R^2 of full model of $\log_{10}$ risk per serving	64%
Pacific Northwest (intertidal) / summer	Percentage pathogenic	20.1%
	Log_{10} *V. parahaemolyticus* per g at harvest	12.2%
	Oyster temperature	5.4%
	Grams of oysters consumed	4.2%
	Length of refrigeration time	2.3%
	Duration of intertidal exposure	1.7%
	Duration of cooldown	1%
	Time unrefrigerated (after collection)	0.7%
	Air temperature	0.6%
	R^2 of full model of $\log_{10}$ risk per serving	72%

[a] mean of sensitivity coefficients (or the R^2 of the full linear regression model approximating simulation model output) over 200 uncertainty sample realizations

As indicated by the results shown in Table A6-1, for the Gulf Coast summer harvest an approximating linear regression, with seven variability parameters as predictors, explains 64% of the variation in $\log_{10}$ risk per serving (the RSS of the full model divided by TSS). The results of the variance decomposition under the linear regression model indicate that the variation of $\log_{10}$ *V. parahaemolyticus*/g at time of harvest is the single most important determinant of the variation of $\log_{10}$ risk per serving for this region/season. The variation of the percentage pathogenic (across individual servings) is also identified as an important component of the variation of $\log_{10}$ risk per serving, as is the time unrefrigerated. The relative ranking of importance of these parameters by the regression-based approach is the same as that obtained by the Tornado plot (i.e., pairwise correlation) analysis shown in the Risk Characterization section. The effect of grams of oysters consumed is not as strong on the basis of this analysis compared to the correlation analysis; possibly due to the fact that consumption in excess of two dozen oysters is infrequent (<2%) and therefore extremes of the variability distribution of grams of oysters consumed is not a strong determinant of the total variation of $\log_{10}$ risk per serving. Variation in the length of refrigeration time and ambient air temperature during harvest do not have strong effects on the variation of risk.

With respect to the Pacific Northwest intertidal summer harvest, the fit of a linear regression with nine variability parameters as predictors explained 72% of the overall variation of $\log_{10}$ risk per serving. Based on the relative partial sums of squares sensitivity measure, the percentage pathogenic is a more influential parameter than the level of $\log_{10}$ *V. parahaemolyticus*/g at time of harvest for this region, season, and harvest type. The sensitivity coefficient for percentage pathogenic was 20% compared to 12% for $\log_{10}$ *V. parahaemolyticus*/g at time of harvest. The influence of other factors was much less pronounced. Grams of oysters consumed and oyster temperature during intertidal exposure were the next most influential factors with each being associated with approximately 5% of the variation in $\log_{10}$ risk per serving.

For both of these examples of region/season combinations, the regression-based sensitivity analysis was repeated using a quadratic response-surface model to determine the effect of interaction of factors on estimates of importance. Although the quadratic response-surface regression indicated that there are significant interactions between factors in the model, the resulting estimates of variance attributable to the variability parameters did not differ substantially from that estimated based on the linear regression for either region.

Sensitivity Analysis of Uncertainty Parameters

A regression-based sensitivity analysis approach was also applied to the uncertainty parameters in order to compare the results to and validate the estimates of importance of uncertainty parameters obtained by the method of fixing parameter values to nominal levels, one at a time, and calculating conditional variances of the output variable (mean risk per serving), as described in the Risk Characterization section.

Both a linear and a quadratic response surface were considered as approximating regressions with $\log_{10}$ mean risk per serving (over variability factors) as the response variable of the regression. Similar to the results obtained in the analysis of importance of variability factors on individual risk per serving, a $\log_{10}$ transformation of mean risk as the output variable was appropriate and the linear regression approximation was found to be generally sufficient for the purpose of importance assessment. The influence of dose-response uncertainty was assessed in the regression-based approach by using dose-response model parameter uncertainty realizations to calculate the uncertainty of $\log_{10}$ ID_{01} (the dose level corresponding to a probability of infection of 1%). This was used as a regression predictor, rather than the $\log_{10}$ ID_{50} or some other summary of the dose-response uncertainty that might be less pertinent. Similarly, mean percentage pathogenic (or the relative abundance of pathogenic strains) was used as a regression predictor since this is the most direct and pertinent summary of the variability distribution of percentage pathogenic. For the effect of year-to-year variations in water temperature, both the mean and the standard deviation of the temperature distribution were used as predictors. Similarly, the effect of uncertainty of prediction of *V. parahaemolyticus*/g at time of harvest based on water temperature was assessed by using both the mean and the standard deviation of the prediction uncertainty as regression predictors of model simulation output. The results of the regression-based sensitivity analysis of uncertainty parameters for the two examples described above (i.e., Gulf Coast (Louisiana)/Summer and Pacific Northwest (Intertidal)/Summer) are shown in Table A6-2.

For the Gulf Coast (Louisiana)/ Summer harvest, the fit of a linear regression of $\log_{10}$ mean risk per serving versus seven selected input uncertainty factors explained 97% of the variation of the output variable. Based on the relative partial sums of squares sensitivity measure, the parameter uncertainty of the Beta-Poisson dose-response model is associated with ~78% of the variation in $\log_{10}$ mean risk per serving. The 2^{nd} and 3^{rd} most influential factors were identified as the uncertainty of mean percentage pathogenic and the growth rate uncertainty, which are associated with 8% and 7% of the total variation, respectively. The effect of the other uncertainties were minimal, particularly the variation in the mean and standard deviation of water temperature distributions (i.e., year-to-year variations of water temperature).

The effect of uncertainty parameters on mean $\log_{10}$ risk per serving for the Pacific Northwest (Intertidal)/Summer harvest was noticeably different than that obtained for the Gulf Coast. An approximating linear regression explained only 80% of the variation in mean $\log_{10}$ risk per serving. With the inclusion of 1^{st} order interaction terms (quadratic regression) the proportion of the variance explained was only marginally higher at 82%. Although the dose-response and the growth rate prediction uncertainties are identified as important, the influence of uncertainty of mean percentage pathogenic was much less substantial in comparison to the results obtained for the Gulf Coast (Louisiana)/Summer harvest. This may be a consequence of the fact that the percentage pathogenic is generally an order of magnitude greater in the Pacific Northwest in comparison to the Gulf Coast and/or the relative effect of other types of uncertainties is more substantial.

Table A6-2. Importance of Uncertainty Parameters on $\log_{10}$ Mean Risk per Serving for the Gulf Coast (Louisiana) Summer and the Pacific Northwest (Intertidal) Summer Harvests Based on Linear Regression Analysis of Monte Carlo Simulation Output

Region	Parameter Uncertainty	Sensitivity Coefficient
Gulf Coast (Louisiana) / Summer	Dose-response (uncertainty of $\log_{10}$ ID_{01})	78%
	Mean percentage pathogenic	8.4%
	Growth rate in oysters vs. broth culture	7.0%
	Predicted mean $\log_{10}$ *Vibrio parahaemolyticus*/g [a]	1.5%
	Predicted std dev of $\log_{10}$ *Vibrio parahaemolyticus*/g [b]	0.6%
	Mean of water temperature distribution	0.5%
	Std Dev of water temperature distribution	0.1%
	R^2 of full model of $\log_{10}$ mean risk per serving	97%
Pacific Northwest (Intertidal) / Summer	Dose-response (uncertainty of $\log_{10}$ ID_{01})	31%
	Std dev of water temperature distribution	15%
	Growth rate in oysters vs. broth culture	13%
	Mean percentage pathogenic	3.8%
	Predicted std dev of $\log_{10}$ *Vibrio parahaemolyticus*/g [b]	3.4%
	Predicted mean $\log_{10}$ *Vibrio parahaemolyticus*/g [a]	1.4%
	Mean of water temperature distribution	0.7%
	R^2 of full model of $\log_{10}$ risk per serving	80%

[a] uncertainty of the regression estimate of mean $\log_{10}$ *V. parahaemolyticus*/g at mean water temperature
[b] uncertainty of the regression estimate of variation of $\log_{10}$ *V. parahaemolyticus*/g

The most striking difference between the results obtained for the Pacific Northwest (Intertidal) compared to that obtained for the Gulf Coast (Louisiana) is the apparent importance of year-to-year variations in water temperature for this region/season. Summary statistics of year-to-year variations in water temperature distributions used for model construction indicate greater year-to-year variability in the Pacific Northwest (Intertidal)/Summer compared to the Gulf Coast (Louisiana)/ Summer. Although the differences may not appear substantial, the results of the sensitivity analysis shown in Table A6-2 suggest that small differences in predicted year-to-year variations of temperature distributions across different regions and seasons imply relatively larger variability of risk and/or uncertainty of the number of illnesses that may occur in a given year due to temperature extremes. For the Pacific Northwest (Intertidal), the influence of year-to-year variation in spread of temperature distributions (as measured by the standard deviation of daily water temperatures) is particularly influential with approximately 15% of the variation in mean $\log_{10}$ risk per serving being attributable to this aspect of year-to-year variation of water temperature distributions.

Appendix 7: Actual Values Predicted by the Risk Assessment Model

Table A7-1. Mean total *Vibrio parahaemolyticus* /g at time of harvest

Region	Season	Mean	Median	5th %-tile	95th %-tile
Gulf LA	winter	51.85	37.19	17.69	128.86
	spring	937.38	483.59	273.81	3055.82
	summer	2103.32	979.05	630.53	7302.99
	fall	220.55	130.23	61.02	644.27
Gulf non-LA	winter	51.98	37.22	18.36	130.85
	spring	936.04	483.60	275.10	3092.20
	summer	2103.35	971.96	627.47	7675.75
	fall	218.02	130.63	62.36	602.14
Northeast Atlantic	winter	3.73	2.88	0.83	8.73
	spring	42.25	28.85	14.84	111.23
	summer	229.45	147.53	82.72	593.89
	fall	32.58	23.94	12.78	80.86
Mid-Atlantic	winter	3.45	2.71	0.73	8.73
	spring	195.75	115.04	67.23	575.09
	summer	775.35	425.29	229.72	2194.98
	fall	50.94	33.60	16.76	136.33
PNW dredged	winter	0.0188	0.0132	0.0028	0.0556
	spring	0.8124	0.4805	0.1163	2.2556
	summer	5.0399	3.4513	1.2915	13.9599
	fall	0.1455	0.1259	0.0496	0.3021
PNW intertidal	winter	0.0386	0.0253	0.0047	0.1174
	spring	60.70	11.05	0.86	292.93
	summer	652.21	293.18	50.51	2571.28
	fall	2.32	0.99	0.24	6.90

Column 3 = mean of the uncertainty distribution of "mean Vp/g"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5^{th} %-tile of uncertainty

Table A7-2. Mean pathogenic Vibrio parahaemolyticus /g at time of harvest

Region	Season	Mean	Median	5th %-tile	95th %-tile
Gulf LA	winter	0.0874	0.0617	0.0249	0.2212
	spring	1.6027	0.8652	0.3286	5.3897
	summer	3.5558	1.8377	0.7433	12.1087
	fall	0.3835	0.2256	0.0767	1.1911
Gulf non-LA	winter	0.0927	0.0630	0.0245	0.2293
	spring	1.5858	0.8730	0.3182	5.2191
	summer	3.5840	1.8337	0.7299	11.8884
	fall	0.3793	0.2242	0.0766	1.1289
Northeast Atlantic	winter	0.0064	0.0048	0.0012	0.0164
	spring	0.0707	0.0499	0.0187	0.1845
	summer	0.3928	0.2616	0.1035	1.0930
	fall	0.0568	0.0403	0.0160	0.1448
Mid-Atlantic	winter	0.0059	0.0043	0.0011	0.0139
	spring	0.3325	0.2030	0.0837	1.0098
	summer	1.3110	0.7580	0.2834	3.8896
	fall	0.0873	0.0575	0.0229	0.2391
PNW dredged	winter	0.0004	0.0003	0.0001	0.0014
	spring	0.0193	0.0106	0.0019	0.0536
	summer	0.1152	0.0751	0.0221	0.3445
	fall	0.0034	0.0027	0.0008	0.0081
PNW intertidal	winter	0.0009	0.0005	0.0001	0.0031
	spring	1.4495	0.2279	0.0166	6.0919
	summer	14.9062	6.0430	0.8674	63.2740
	fall	0.0507	0.0197	0.0040	0.1493

Column 3 = mean of the uncertainty distribution of "mean Vp/g"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5^{th} %-tile of uncertainty

Table A7-3. Mean total *Vibrio parahaemolyticus*/g at time of cooldown

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	787.24	464.68	79.53	2434.14
	spring	62061.82	56490.03	23563.18	118882.04
	summer	165199.14	153525.28	74182.70	293861.75
	fall	15654.30	12528.95	3513.43	37311.71
Gulf non-LA	winter	372.24	221.60	53.03	1215.18
	spring	44226.14	39066.31	15734.72	88768.61
	summer	116622.74	105585.07	48795.43	225993.76
	fall	6881.04	5084.78	1206.76	18331.58
Northeast Atlantic	winter	4.01	3.06	0.89	9.40
	spring	1403.94	893.51	140.33	4506.91
	summer	6787.28	4907.13	1391.13	18321.50
	fall	144.31	84.68	26.00	432.34
Mid-Atlantic	winter	3.86	2.96	0.80	10.00
	spring	11674.22	10137.35	3379.63	25474.28
	summer	34342.94	27305.00	7394.09	84762.05
	fall	839.96	470.69	63.20	2758.27
PNW dredged	winter	0.022	0.015	0.003	0.066
	spring	25.717	3.465	0.317	138.135
	summer	287.581	108.962	17.667	1221.362
	fall	0.645	0.364	0.101	1.868
PNW intertidal	winter	0.047	0.028	0.005	0.153
	spring	415.228	52.572	1.879	2172.929
	summer	4566.77	2422.75	329.45	16931.88
	fall	10.74	2.73	0.42	45.15

Column 3 = mean of the uncertainty distribution of "mean Vp/g"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5^{th} %-tile of uncertainty

Table A7-4. Mean pathogenic *Vibrio parahaemolyticus*/g at time of cooldown

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	1.317	0.697	0.110	4.506
	spring	108.460	92.385	32.186	244.923
	summer	287.154	253.152	103.010	597.996
	fall	27.512	20.997	4.968	68.499
Gulf non-LA	winter	0.644	0.341	0.071	2.141
	spring	77.027	63.539	21.026	179.791
	summer	202.870	173.521	66.229	437.251
	fall	11.979	8.297	1.737	32.402
Northeast Atlantic	winter	0.007	0.005	0.001	0.017
	spring	2.398	1.390	0.182	8.199
	summer	11.814	8.184	1.838	32.201
	fall	0.250	0.137	0.034	0.858
Mid-Atlantic	winter	0.007	0.005	0.001	0.016
	spring	20.040	16.709	4.641	47.741
	summer	59.295	45.427	10.835	154.094
	fall	1.4779	0.7598	0.0959	5.3229
PNW dredged	winter	0.0005	0.0003	0.0001	0.0017
	spring	0.6363	0.0721	0.0054	2.6605
	summer	6.4807	2.1624	0.2836	29.1563
	fall	0.0162	0.0072	0.0018	0.0507
PNW Intertidal	winter	0.0011	0.0006	0.0001	0.0038
	spring	10.1514	1.0690	0.0363	52.6528
	summer	105.049	52.515	5.167	388.076
	fall	0.240	0.052	0.007	0.989

Column 3 = mean of the uncertainty distribution of "mean Vp/g"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5^{th} %-tile of uncertainty

Table A7-5. Mean total *Vibrio parahaemolyticus* /g at-retail (post-harvest)

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	286.70	165.04	29.52	924.17
	spring	22508.79	20412.66	8491.07	43159.94
	summer	59882.85	55813.92	27055.13	106638.93
	fall	5670.00	4506.72	1284.31	13710.14
Gulf non-LA	winter	135.38	78.42	18.84	429.52
	spring	16033.78	14157.30	5681.66	32574.90
	summer	42273.90	38369.08	17786.53	81881.76
	fall	2496.79	1845.00	443.81	6622.02
Northeast Atlantic	winter	1.49	1.11	0.31	3.42
	spring	510.58	319.67	50.57	1667.50
	summer	2458.30	1782.36	501.60	6808.45
	fall	51.94	30.37	9.45	160.20
Mid-Atlantic	winter	1.40	1.07	0.29	3.56
	spring	4225.24	3676.15	1244.48	9341.05
	summer	12456.42	9914.27	2677.74	30933.37
	fall	305.85	171.56	22.89	994.08
PNW dredged	winter	0.0080	0.0054	0.0011	0.0242
	spring	9.1392	1.2599	0.1133	43.0935
	summer	104.8961	38.6175	6.3163	428.4740
	fall	0.2302	0.1316	0.0369	0.6721
PNW intertidal	winter	0.0169	0.0102	0.0019	0.0556
	spring	150.14	18.99	0.66	778.34
	summer	1651.17	870.28	117.41	6080.52
	fall	3.9387	0.9567	0.1526	17.1876

Column 3 = mean of the uncertainty distribution of "mean Vp/g"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5^{th} %-tile of uncertainty

Table A7-6. Mean pathogenic *Vibrio parahaemolyticus* /g at retail (post-harvest)

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	0.4750	0.2478	0.0401	1.6221
	spring	39.2890	33.4437	11.5471	87.6192
	summer	104.0239	91.2896	37.4281	217.9466
	fall	9.9519	7.5803	1.7550	25.0750
Gulf non-LA	winter	0.2294	0.1224	0.0257	0.7961
	spring	27.9565	23.0971	7.6225	64.8276
	summer	73.4609	62.9898	23.9565	158.4772
	fall	4.3620	3.0104	0.6378	12.0473
Northeast Atlantic	winter	0.0025	0.0018	0.0004	0.0063
	spring	0.8777	0.5108	0.0638	3.0233
	summer	4.2858	2.9966	0.6799	11.7178
	fall	0.0882	0.0484	0.0121	0.2943
Mid-Atlantic	winter	0.0024	0.0017	0.0004	0.0058
	spring	7.2760	6.0325	1.7164	17.6758
	summer	21.4864	16.4224	3.7599	54.2711
	fall	0.5410	0.2587	0.0348	1.9754
PNW dredged	winter	0.00019	0.00012	0.00002	0.00061
	spring	0.22433	0.02632	0.00196	0.87405
	summer	2.32247	0.77659	0.10017	10.78517
	fall	0.00577	0.00263	0.00064	0.01792
PNW intertidal	winter	0.00040	0.00021	0.00003	0.00135
	spring	3.7098	0.3843	0.0137	18.7625
	summer	37.7557	18.9119	1.8714	139.5608
	fall	0.0860	0.0177	0.0026	0.3021

Column 3 = mean of the uncertainty distribution of "mean Vp/g"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5^{th} %-tile of uncertainty

Table A7-7. Mean dose total *Vibrio parahaemolyticus* per serving

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	5.79E+04	3.33E+04	5.96E+03	1.87E+05
	spring	4.55E+06	4.12E+06	1.72E+06	8.72E+06
	summer	1.21E+07	1.13E+07	5.47E+06	2.15E+07
	fall	1.15E+06	9.10E+05	2.59E+05	2.77E+06
Gulf non-LA	winter	2.73E+04	1.58E+04	3.81E+03	8.68E+04
	spring	3.24E+06	2.86E+06	1.15E+06	6.58E+06
	summer	8.54E+06	7.75E+06	3.59E+06	1.65E+07
	fall	5.04E+05	3.73E+05	8.96E+04	1.34E+06
Northeast Atlantic	winter	3.02E+02	2.24E+02	6.33E+01	6.90E+02
	spring	1.03E+05	6.46E+04	1.02E+04	3.37E+05
	summer	4.97E+05	3.60E+05	1.01E+05	1.38E+06
	fall	1.05E+04	6.13E+03	1.91E+03	3.24E+04
Mid-Atlantic	winter	2.82E+02	2.15E+02	5.92E+01	7.19E+02
	spring	8.53E+05	7.43E+05	2.51E+05	1.89E+06
	summer	2.52E+06	2.00E+06	5.41E+05	6.25E+06
	fall	6.18E+04	3.47E+04	4.62E+03	2.01E+05
PNW dredged	winter	1.61E+00	1.09E+00	2.20E-01	4.88E+00
	spring	1.85E+03	2.54E+02	2.29E+01	8.70E+03
	summer	2.12E+04	7.80E+03	1.28E+03	8.66E+04
	fall	4.65E+01	2.66E+01	7.45E+00	1.36E+02
PNW intertidal	winter	3.41E+00	2.07E+00	3.81E-01	1.12E+01
	spring	3.03E+04	3.84E+03	1.33E+02	1.57E+05
	summer	3.34E+05	1.76E+05	2.37E+04	1.23E+06
	fall	7.96E+02	1.93E+02	3.08E+01	3.47E+03

Column 3 = mean of the uncertainty distribution of "mean Vp/serving"; column 4 = median of the uncertainty distribution of "mean Vp/serving"; column 5 = 5^{th} %-tile of uncertainty

Table A7-8. Mean dose pathogenic *Vibrio parahaemolyticus* per serving

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	97.54	50.32	8.06	332.70
	spring	7878.70	6669.40	2320.26	17773.88
	summer	20816.05	18319.93	7506.42	43704.33
	fall	1992.93	1525.87	322.83	5054.04
Gulf non-LA	winter	46.67	23.76	5.09	163.87
	spring	5619.13	4633.61	1484.91	13117.20
	summer	14731.34	12505.11	4904.38	31955.61
	fall	875.78	612.42	111.24	2506.69
Northeast Atlantic	winter	0.50	0.36	0.09	1.23
	spring	178.64	99.19	12.25	616.43
	summer	862.05	583.28	130.28	2462.78
	fall	17.49	9.66	2.42	57.16
Mid-Atlantic	winter	0.48	0.35	0.09	1.17
	spring	1456.03	1204.63	327.89	3468.49
	summer	4308.16	3341.36	754.41	10836.96
	fall	109.42	49.01	7.09	412.18
PNW dredged	winter	0.04	0.02	0.00	0.12
	spring	42.86	5.28	0.40	164.75
	summer	456.71	147.64	20.82	2087.10
	fall	1.18	0.52	0.12	3.63
PNW intertidal	winter	0.08	0.04	0.01	0.28
	spring	739.13	72.20	2.59	3665.94
	summer	7498.71	3679.84	374.39	29915.03
	fall	16.87	3.45	0.50	74.14

Column 3 = mean of the uncertainty distribution of "mean Vp/serving"; column 4 = median of the uncertainty distribution of "mean Vp/serving"; column 5 = 5^{th} %-tile of uncertainty

Table A7-9. Mean risk per serving

Region	Season	Mean	Median	5th %-tile	95th %-tile
Gulf LA	winter	2.14E-06	7.64E-07	5.20E-08	8.26E-06
	spring	1.68E-04	1.04E-04	1.20E-05	5.41E-04
	summer	4.39E-04	3.00E-04	3.40E-05	1.39E-03
	fall	4.27E-05	2.36E-05	2.07E-06	1.51E-04
Gulf non-LA	winter	1.05E-06	3.63E-07	3.05E-08	4.17E-06
	spring	1.21E-04	7.19E-05	8.31E-06	3.94E-04
	summer	3.11E-04	2.03E-04	2.32E-05	1.03E-03
	fall	1.88E-05	9.64E-06	7.44E-07	6.65E-05
Northeast Atlantic	winter	1.11E-08	5.81E-09	4.92E-10	3.47E-08
	spring	3.64E-06	1.49E-06	8.35E-08	1.49E-05
	summer	1.78E-05	9.08E-06	8.37E-07	6.86E-05
	fall	3.98E-07	1.65E-07	1.25E-08	1.62E-06
Mid-Atlantic	winter	1.05E-08	5.61E-09	4.93E-10	3.75E-08
	spring	3.11E-05	1.84E-05	1.81E-06	1.05E-04
	summer	9.24E-05	4.88E-05	4.86E-06	3.31E-04
	fall	2.21E-06	7.82E-07	4.94E-08	1.02E-05
PNW dredged	winter	8.11E-10	3.66E-10	3.19E-11	3.22E-09
	spring	8.68E-07	8.83E-08	3.96E-09	3.08E-06
	summer	1.01E-05	2.21E-06	1.58E-07	4.15E-05
	fall	2.65E-08	8.38E-09	6.86E-10	9.46E-08
PNW intertidal	winter	1.67E-09	6.41E-10	5.50E-11	6.47E-09
	spring	1.30E-05	1.22E-06	2.27E-08	5.83E-05
	summer	1.44E-04	5.05E-05	3.17E-06	6.22E-04
	fall	3.87E-07	5.71E-08	3.05E-09	1.59E-06

Column 3 = mean of the uncertainty distribution of "mean risk"; column 4 = median of the uncertainty distribution of "mean risk"; column 5 = 5^{th} %-tile of uncertainty

Table A7-10. Number of Illnesses Associated with *V. parahaemolyticus*

Region	Season	Mean	Median	5th %-tile	95th %-tile
Gulf LA	winter	6.65	2.37	0.16	25.60
	spring	505.02	313.01	35.96	1623.72
	summer	1406.36	960.87	108.92	4435.45
	fall	132.25	73.22	6.42	468.44
Gulf non-LA	winter	2.85	0.98	0.08	11.26
	spring	192.96	115.04	13.30	630.61
	summer	298.59	194.55	22.27	984.84
	fall	50.71	26.03	2.01	179.53
Northeast Atlantic	winter	0.027	0.014	0.001	0.083
	spring	2.95	1.21	0.07	12.08
	summer	13.72	6.99	0.64	52.84
	fall	1.67	0.69	0.05	6.79
Mid-Atlantic	winter	0.012	0.006	0.001	0.041
	spring	4.35	2.58	0.25	14.76
	summer	6.93	3.66	0.36	24.84
	fall	3.76	1.33	0.08	17.38
PNW dredged	winter	0.0006	0.0002	0.0000	0.0022
	spring	0.4165	0.0424	0.0019	1.4795
	summer	3.93	0.86	0.06	16.19
	fall	0.0238	0.0075	0.0006	0.0852
PNW intertidal	winter	0.0033	0.0013	0.0001	0.0129
	spring	18.24	1.71	0.03	81.56
	summer	172.69	60.66	3.80	746.14
	fall	1.05	0.15	0.01	4.28

Column 3 = mean of the uncertainty distribution of "mean number illnesses"; column 4 = median of the uncertainty distribution of "mean number illnesses"; column 5 = 5th %-tile of uncertainty

Table A7-11. Number of septicemia cases

Region	Season	Mean	Median	5th %-tile	95th %-tile
Gulf LA	winter	0.0155	0.0055	0.0004	0.0598
	spring	1.1797	0.7312	0.0840	3.7930
	summer	3.2853	2.2446	0.2544	10.3612
	fall	0.3089	0.1710	0.0150	1.0943
Gulf non-LA	winter	0.0067	0.0023	0.0002	0.0263
	spring	0.4508	0.2687	0.0311	1.4731
	summer	0.6975	0.4545	0.0520	2.3006
	fall	0.1185	0.0608	0.0047	0.4194
Northeast Atlantic	winter	0.000062	0.000033	0.000003	0.000194
	spring	0.0069	0.0028	0.0002	0.0282
	summer	0.0321	0.0163	0.0015	0.1234
	fall	0.0039	0.0016	0.0001	0.0159
Mid-Atlantic	winter	0.000027	0.000014	0.000001	0.000096
	spring	0.0102	0.0060	0.0006	0.0345
	summer	0.0162	0.0086	0.0009	0.0580
	fall	0.0088	0.0031	0.0002	0.0406
PNW dredged	winter	0.0000013	0.0000006	0.0000001	0.0000051
	spring	0.0010	0.0001	0.0000	0.0035
	summer	0.0092	0.0020	0.0001	0.0378
	fall	0.000056	0.000018	0.000001	0.000199
PNW intertidal	winter	0.0000078	0.0000030	0.0000003	0.0000302
	spring	0.0426	0.0040	0.0001	0.1905
	summer	0.4034	0.1417	0.0089	1.7430
	fall	0.002443	0.000360	0.000019	0.010002

Column 3 = mean of the uncertainty distribution of "mean number septicemia cases"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5th %-tile of uncertainty

Table A7-12. Mean pathogenic *V. parahaemolyticus* per gram at-cooldown after immediate refrigeration, i.e., ~1 log reduction

Region	Season	Mean (no mitigation)	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	1.317	0.0591	0.0397	0.0130	0.1650
	spring	108.460	4.1855	2.7837	0.8359	11.7680
	summer	287.154	10.0785	6.6973	2.3462	28.6955
	fall	27.512	0.6541	0.3875	0.0903	2.0872
Gulf non-LA	winter	0.644	0.0603	0.0383	0.0137	0.1721
	spring	77.027	4.2041	2.8754	0.8177	11.9167
	summer	202.870	10.0933	6.7617	2.4139	28.4286
	fall	11.979	0.6574	0.3790	0.0927	2.1358
Northeast Atlantic	winter	0.007	0.00234	0.00175	0.00042	0.00591
	spring	2.398	0.0971	0.0527	0.0146	0.2941
	summer	11.814	0.5217	0.3361	0.1089	1.4607
	fall	0.250	0.0302	0.0204	0.0071	0.0801
Mid-Atlantic	winter	0.007	0.00228	0.00160	0.00040	0.00544
	spring	20.040	0.8753	0.5677	0.1350	2.7134
	summer	59.295	2.5533	1.5811	0.4567	7.6112
	fall	1.4779	0.0898	0.0449	0.0137	0.3155
PNW dredged	winter	0.0005	0.000169	0.000105	0.00002	0.000557
	spring	0.6363	0.0223	0.0079	0.0011	0.0760
	summer	6.4807	0.1973	0.0977	0.0232	0.6755
	fall	0.0162	0.00195	0.00138	0.00040	0.00498
PNW intertidal	winter	0.0011	0.00037	0.00020	0.00003	0.00131
	spring	10.1514	1.8835	0.2277	0.0092	9.7423
	summer	105.049	20.4757	9.4094	0.9484	84.2898
	fall	0.240	0.0380	0.0130	0.0022	0.1329

Column 3 = mean of the uncertainty distribution of "mean Vp/g"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5^{th} %-tile of uncertainty

Table A7-13. Mean pathogenic *V. parahaemolyticus* per serving at-retail after immediate refrigeration, i.e., ~1 log reduction mitigation

Region	Season	Mean (no mitigation)	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	0.4750	11.9386	8.0146	2.6278	33.3291
	spring	39.2890	845.4704	562.3106	168.8508	2377.1382
	summer	104.0239	2035.86	1352.85	473.9361	5796.4965
	fall	9.9519	132.1342	78.2792	18.2489	421.6187
Gulf non-LA	winter	0.2294	12.1846	7.7457	2.7649	34.7622
	spring	27.9565	849.2298	580.8270	165.1785	2407.1684
	summer	73.4609	2038.84	1365.86	487.6049	5742.5852
	fall	4.3620	132.7878	76.5543	18.7229	431.4337
Northeast Atlantic	winter	0.0025	0.4734	0.3536	0.0846	1.1934
	spring	0.8777	19.6208	10.6371	2.9497	59.4099
	summer	4.2858	105.3749	67.8978	22.0022	295.0676
	fall	0.0882	6.1050	4.1237	1.4353	16.1709
Mid-Atlantic	winter	0.0024	0.4614	0.3241	0.0804	1.0997
	spring	7.2760	176.8161	114.6784	27.2646	548.1126
	summer	21.4864	515.7639	319.3810	92.2551	1537.4702
	fall	0.5410	18.1424	9.0685	2.7665	63.7350
PNW dredged	winter	0.00019	0.0341	0.0212	0.0039	0.1124
	spring	0.22433	4.4984	1.5888	0.2293	15.3516
	summer	2.32247	39.8579	19.7436	4.6772	136.4576
	fall	0.00577	0.3931	0.2785	0.0812	1.0063
PNW intertidal	winter	0.00040	0.0748	0.0409	0.0066	0.2647
	spring	3.7098	380.47	46.00	1.85	1967.93
	summer	37.7557	4136.10	1900.71	191.57	17026.55
	fall	0.0860	7.6694	2.6215	0.4500	26.8481

Column 3 = mean of the uncertainty distribution of "mean Vp/serving"; column 4 = median of the uncertainty distribution of "mean Vp/serving"; column 5 = 5^{th} %-tile of uncertainty

Table A7-14. Mean pathogenic *V. parahaemolyticus* per gram at-cooldown after 2 log reduction mitigation

Region	Season	Mean (no mitigation)	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	1.317	0.00501	0.00251	0.00039	0.01795
	spring	108.460	0.38858	0.32658	0.11234	0.88743
	summer	287.154	1.03056	0.90900	0.36279	2.15488
	fall	27.512	0.09750	0.07328	0.01609	0.24394
Gulf non-LA	winter	0.644	0.00228	0.00114	0.00027	0.00753
	spring	77.027	0.27724	0.22999	0.07549	0.65012
	summer	202.870	0.72828	0.63702	0.24142	1.56452
	fall	11.979	0.04253	0.02950	0.00558	0.12375
Northeast Atlantic	winter	0.007	2.44E-05	1.78E-05	3.47E-06	6.04E-05
	spring	2.398	0.00887	0.00492	0.00062	0.03239
	summer	11.814	0.04225	0.02914	0.00682	0.11437
	fall	0.250	0.00099	0.00045	0.00012	0.00337
Mid-Atlantic	winter	0.007	2.44E-05	1.73E-05	3.47E-06	6.09E-05
	spring	20.040	0.07301	0.06030	0.01548	0.17157
	summer	59.295	0.21194	0.16272	0.03601	0.53950
	fall	1.4779	0.00506	0.00232	0.00033	0.01933
PNW dredged	winter	0.0005	1.87E-06	9.90E-07	0.00E+00	6.44E-06
	spring	0.6363	0.00213	0.00025	0.00002	0.00918
	summer	6.4807	0.02332	0.00758	0.00099	0.09652
	fall	0.0162	4.92E-05	2.60E-05	5.94E-06	1.47E-04
PNW intertidal	winter	0.0011	3.98E-06	2.12E-06	3.36E-07	1.36E-05
	spring	10.1514	0.03490	0.00405	0.00012	0.19899
	summer	105.049	0.37985	0.18354	0.01770	1.53891
	fall	0.240	0.00069	0.00019	0.00003	0.00231

Column 3 = mean of the uncertainty distribution of "mean Vp/g"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5^{th} %-tile of uncertainty

Table A7-15. Mean pathogenic *V. parahaemolyticus* at-retail per serving after 2 log reduction mitigation

Region	Season	Mean (no mitigation)	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	0.4750	1.012	0.506	0.078	3.625
	spring	39.2890	78.49	65.97	22.69	179.26
	summer	104.0239	208.17	183.62	73.28	435.29
	fall	9.9519	19.70	14.80	3.25	49.28
Gulf non-LA	winter	0.2294	0.461	0.230	0.054	1.520
	spring	27.9565	56.00	46.46	15.25	131.32
	summer	73.4609	147.11	128.68	48.77	316.03
	fall	4.3620	8.59	5.96	1.13	25.00
Northeast Atlantic	winter	0.0025	0.0049	0.0036	0.0007	0.0122
	spring	0.8777	1.79	0.99	0.13	6.54
	summer	4.2858	8.54	5.89	1.38	23.10
	fall	0.0882	0.2000	0.0900	0.0243	0.6803
Mid-Atlantic	winter	0.0024	0.0049	0.0035	0.0007	0.0123
	spring	7.2760	14.75	12.18	3.13	34.66
	summer	21.4864	42.81	32.87	7.27	108.98
	fall	0.5410	1.02	0.47	0.07	3.90
PNW dredged	winter	0.00019	0.00038	0.00020	0.00000	0.00130
	spring	0.22433	0.4308	0.0501	0.0041	1.8535
	summer	2.32247	4.7107	1.5304	0.1997	19.4966
	fall	0.00577	0.0099	0.0053	0.0012	0.0297
PNW intertidal	winter	0.00040	0.00080	0.00043	0.00007	0.00276
	spring	3.7098	7.0507	0.8184	0.0250	40.1965
	summer	37.7557	76.7307	37.0752	3.5762	310.8602
	fall	0.0860	0.1384	0.0379	0.0056	0.4666

Column 3 = mean of the uncertainty distribution of "mean Vp/serving"; column 4 = median of the uncertainty distribution of "mean Vp/serving"; column 5 = 5th %-tile of uncertainty

Table A7-16. Mean pathogenic *V.parahaemolyticus* per gram at cooldown after 4.5 log reduction mitigation

Region	Season	Mean (no mitigation)	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	1.317	1.58E-05	8.42E-06	9.90E-07	5.74E-05
	spring	108.460	1.23E-03	1.04E-03	3.55E-04	2.80E-03
	summer	287.154	3.26E-03	2.87E-03	1.15E-03	6.80E-03
	fall	27.512	3.08E-04	2.37E-04	5.00E-05	7.68E-04
Gulf non-LA	winter	0.644	7.19E-06	3.47E-06	4.95E-07	2.43E-05
	spring	77.027	8.76E-04	7.24E-04	2.39E-04	2.04E-03
	summer	202.870	2.30E-03	2.01E-03	7.52E-04	4.95E-03
	fall	11.979	1.35E-04	9.21E-05	1.83E-05	3.95E-04
Northeast Atlantic	winter	0.007	8.32E-08	0.00E+0	0.00E+00	4.95E-07
	spring	2.398	2.80E-05	1.53E-05	1.49E-06	1.01E-04
	summer	11.814	1.34E-04	9.11E-05	2.08E-05	3.67E-04
	fall	0.250	3.15E-06	1.49E-06	0.00E+00	1.19E-05
Mid-Atlantic	winter	0.007	7.52E-08	0.00E+0	0.00E+00	4.95E-07
	spring	20.040	2.31E-04	1.92E-04	5.05E-05	5.44E-04
	summer	59.295	6.70E-04	5.20E-04	1.13E-04	1.70E-03
	fall	1.4779	1.60E-05	7.67E-06	9.65E-07	6.04E-05
PNW dredged	winter	0.0005	5.45E-09	0.00E+0	0.00E+00	0.00E+00
	spring	0.6363	6.88E-06	9.90E-07	0.00E+00	2.99E-05
	summer	6.4807	7.40E-05	2.40E-05	2.97E-06	3.07E-04
	fall	0.0162	1.65E-07	0.00E+0	0.00E+00	9.90E-07
PNW intertidal	winter	0.0011	1.26E-08	6.70E-09	1.06E-09	4.32E-08
	spring	10.1514	1.10E-04	1.28E-05	3.92E-07	6.29E-04
	summer	105.049	1.20E-03	5.80E-04	5.60E-05	4.87E-03
	fall	0.240	2.17E-06	5.94E-07	8.71E-08	7.31E-06

Column 3 = mean of the uncertainty distribution of "mean Vp/g"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5^{th} %-tile of uncertainty

Table A7-17. Mean pathogenic *V.parahaemolyticus* per serving at-retail after 4.5 log reduction mitigation

Region	Season	Mean (no mitigation)	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	0.4750	0.00320	0.00170	0.00020	0.01160
	spring	39.2890	0.24828	0.20925	0.07178	0.56649
	summer	104.0239	0.65827	0.57880	0.23236	1.37454
	fall	9.9519	0.06224	0.04785	0.01010	0.15521
Gulf non-LA	winter	0.2294	0.00145	0.00070	0.00010	0.00491
	spring	27.9565	0.17697	0.14630	0.04819	0.41267
	summer	73.4609	0.46534	0.40625	0.15198	0.99969
	fall	4.3620	0.02724	0.01860	0.00370	0.07971
Northeast Atlantic	winter	0.0025	1.68E-05	0.00E+00	0.00E+00	1.00E-04
	spring	0.8777	0.00565	0.00310	0.00030	0.02041
	summer	4.2858	0.02706	0.01840	0.00420	0.07422
	fall	0.0882	0.00064	0.00030	0.00000	0.00240
Mid-Atlantic	winter	0.0024	1.52E-05	0.00E+00	0.00E+00	1.00E-04
	spring	7.2760	0.04668	0.03870	0.01020	0.10990
	summer	21.4864	0.13534	0.10510	0.02289	0.34281
	fall	0.5410	0.00324	0.00155	0.00020	0.01221
PNW dredged	winter	0.00019	1.10E-06	0.00E+00	0.00E+00	0.00E+00
	spring	0.22433	0.00139	0.00020	0.00000	0.00603
	summer	2.32247	0.01494	0.00485	0.00060	0.06202
	fall	0.00577	3.33E-05	0.00E+00	0.00E+00	2.00E-04
PNW intertidal	winter	0.00040	2.54E-06	1.35E-06	2.15E-07	8.72E-06
	spring	3.7098	0.02230	0.00259	0.00008	0.12711
	summer	37.7557	0.24264	0.11724	0.01131	0.98303
	fall	0.0860	0.00044	0.00012	0.00002	0.00148

Column 3 = mean of the uncertainty distribution of "mean Vp/serving"; column 4 = median of the uncertainty distribution of "mean Vp/g"; column 5 = 5^{th} %-tile of uncertainty

Table A7-18. # of annual illnesses after immediate refrigeration, i.e. ~1 log reduction

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	0.80	0.41	0.04	2.53
	spring	54.06	28.50	2.98	185.27
	summer	138.87	73.65	7.64	492.47
	fall	8.81	3.99	0.34	33.51
Gulf non-LA	winter	0.72	0.35	0.04	2.31
	spring	28.95	14.38	1.52	98.14
	summer	41.62	22.05	2.55	144.08
	fall	7.65	3.43	0.32	27.77
Northeast Atlantic	winter	0.0244	0.0134	0.0011	0.0814
	spring	0.3305	0.1374	0.0129	1.2325
	summer	1.7132	0.8781	0.0986	6.1876
	fall	0.5514	0.2955	0.0290	1.8030
Mid-Atlantic	winter	0.0106	0.0058	0.0005	0.0372
	spring	0.5267	0.2528	0.0242	2.0308
	summer	0.8264	0.3839	0.0395	3.1570
	fall	0.6352	0.2707	0.0250	2.4228
PNW dredged	winter	4.97E-04	2.27E-04	1.89E-05	1.97E-03
	spring	0.0509	0.0124	0.0009	0.1594
	summer	0.3689	0.1216	0.0102	1.4661
	fall	0.0081	0.0040	0.0004	0.0307
PNW intertidal	winter	0.0032	0.0013	0.0001	0.0126
	spring	9.98	1.04	0.02	49.94
	summer	95.71	31.73	1.94	422.48
	fall	0.49	0.11	0.01	1.70

Column 3 = mean of the uncertainty distribution of "mean number illnesses"; column 4 = median of the uncertainty distribution of "mean number illnesses"; column 5 = 5th %-tile of uncertainty

Table A7-19. # of annual illnesses after 2 log reduction mitigation

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	0.0704	0.0245	0.0017	0.2957
	spring	5.20	3.15	0.35	16.69
	summer	14.56	9.67	1.10	46.88
	fall	1.34	0.72	0.06	4.98
Gulf non-LA	winter	0.0279	0.0101	0.0009	0.1085
	spring	1.9684	1.1501	0.1299	6.3499
	summer	3.0684	1.9492	0.2188	10.3400
	fall	0.5134	0.2563	0.0209	1.8016
Northeast Atlantic	winter	2.54E-04	1.38E-04	1.13E-05	8.67E-04
	spring	0.0307	0.0120	0.0008	0.1254
	summer	0.1383	0.0707	0.0070	0.5312
	fall	0.0180	0.0064	0.0005	0.0732
Mid-Atlantic	winter	1.13E-04	5.87E-05	5.36E-06	4.08E-04
	spring	0.0449	0.0254	0.0027	0.1599
	summer	0.0704	0.0366	0.0038	0.2553
	fall	0.0367	0.0125	0.0008	0.1579
PNW dredged	winter	5.51E-06	2.29E-06	0.00E+00	2.19E-05
	spring	4.73E-03	4.07E-04	1.72E-05	1.69E-02
	summer	0.0441	0.0091	0.0006	0.1983
	fall	2.10E-04	7.20E-05	6.61E-06	7.42E-04
PNW intertidal	winter	3.35E-05	1.17E-05	0.00E+00	1.39E-04
	spring	0.2209	0.0178	0.0003	1.0663
	summer	2.1265	0.6351	0.0394	9.3803
	fall	0.0085	0.0016	0.0001	0.0289

Column 3 = mean of the uncertainty distribution of "mean number illnesses"; column 4 = median of the uncertainty distribution of "mean number illnesses"; column 5 = 5^{th} %-tile of uncertainty

Table A7-20. # of annual illnesses after 4.5 log reduction mitigation

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	2.23E-04	7.63E-05	3.87E-06	9.80E-04
	spring	1.65E-02	9.99E-03	1.09E-03	5.32E-02
	summer	4.61E-02	3.06E-02	3.49E-03	1.50E-01
	fall	4.24E-03	2.29E-03	1.98E-04	1.60E-02
Gulf non-LA	winter	8.79E-05	3.10E-05	1.43E-06	3.49E-04
	spring	6.22E-03	3.64E-03	4.11E-04	2.02E-02
	summer	9.71E-03	6.13E-03	7.00E-04	3.24E-02
	fall	1.63E-03	8.05E-04	6.58E-05	5.79E-03
Northeast Atlantic	winter	8.57E-07	0.00E+00	0.00E+00	4.92E-06
	spring	9.66E-05	3.61E-05	1.77E-06	3.90E-04
	summer	4.39E-04	2.20E-04	2.09E-05	1.64E-03
	fall	5.63E-05	1.94E-05	0.00E+00	2.34E-04
Mid-Atlantic	winter	3.40E-07	0.00E+00	0.00E+00	2.26E-06
	spring	1.42E-04	8.03E-05	8.54E-06	5.09E-04
	summer	2.23E-04	1.16E-04	1.17E-05	8.03E-04
	fall	1.16E-04	4.02E-05	1.45E-06	5.16E-04
PNW dredged	winter	1.49E-08	0.00E+00	0.00E+00	0.00E+00
	spring	1.49E-05	1.11E-06	0.00E+00	5.09E-05
	summer	1.40E-04	2.75E-05	1.50E-06	6.47E-04
	fall	6.71E-07	0.00E+00	0.00E+00	4.16E-06
PNW intertidal	winter	9.18E-08	0.00E+00	0.00E+00	0.00E+00
	spring	7.04E-04	5.40E-05	0.00E+00	3.47E-03
	summer	6.77E-03	2.04E-03	1.27E-04	3.01E-02
	fall	2.67E-05	3.37E-06	0.00E+00	1.09E-04
Total		0.093			

Column 3 = mean of the uncertainty distribution of "mean number illnesses"; column 4 = median of the uncertainty distribution of "mean number illnesses"; column 5 = 5^{th} %-tile of uncertainty

Table A7-21. Percent of harvest exceeding 10,000/g (1,000/g in PNW) at harvest

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	0.0284%	0.0000%	0.0000%	0.1500%
	spring	1.1798%	0.5650%	0.1795%	4.1100%
	summer	2.8700%	1.3850%	0.5895%	9.5050%
	fall	0.2293%	0.0900%	0.0100%	0.8900%
Gulf non-LA	winter	0.0297%	0.0000%	0.0000%	0.1600%
	spring	1.1838%	0.5700%	0.1700%	4.2010%
	summer	2.8658%	1.3950%	0.5800%	9.5640%
	fall	0.2279%	0.0900%	0.0100%	0.9200%
Northeast Atlantic	winter	0.0004%	0.0000%	0.0000%	0.0000%
	spring	0.0261%	0.0000%	0.0000%	0.1300%
	summer	0.2057%	0.0600%	0.0100%	0.8815%
	fall	0.0154%	0.0000%	0.0000%	0.0800%
Mid-Atlantic	winter	0.0004%	0.0000%	0.0000%	0.0000%
	spring	0.2021%	0.0800%	0.0100%	0.7905%
	summer	0.9556%	0.3950%	0.0895%	3.4910%
	fall	0.0391%	0.0100%	0.0000%	0.1805%
PNW dredged	winter	0.0000%	0.0000%	0.0000%	0.0000%
	spring	0.0023%	0.0000%	0.0000%	0.0100%
	summer	0.0235%	0.0000%	0.0000%	0.1200%
	fall	0.0001%	0.0000%	0.0000%	0.0000%
PNW intertidal	winter	0.0000%	0.0000%	0.0000%	0.0000%
	spring	0.4398%	0.1200%	0.0000%	2.0205%
	summer	4.0570%	3.2850%	0.7200%	10.9245%
	fall	0.0173%	0.0000%	0.0000%	0.0700%

Table A7-22. Percent of harvest exceeding 10,000/g (1,000/g in PNW) at cooldown

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	0.7187%	0.4900%	0.0700%	2.0805%
	spring	27.0453%	26.4400%	16.0095%	40.4635%
	summer	59.3192%	59.2150%	45.3275%	73.5945%
	fall	7.2283%	6.4100%	2.5395%	14.1435%
Gulf non-LA	winter	0.3857%	0.2300%	0.0200%	1.2805%
	spring	22.7366%	22.2350%	12.9260%	35.0890%
	summer	52.1745%	51.8150%	37.4160%	67.1665%
	fall	4.3686%	3.7850%	1.2295%	9.0805%
Northeast Atlantic	winter	0.0005%	0.0000%	0.0000%	0.0000%
	spring	1.0107%	0.8000%	0.1500%	2.5305%
	summer	7.0001%	6.1250%	2.0170%	14.9175%
	fall	0.1326%	0.0800%	0.0100%	0.4500%
Mid-Atlantic	winter	0.0006%	0.0000%	0.0000%	0.0000%
	spring	5.8520%	5.5350%	2.7985%	9.7605%
	summer	22.9236%	22.0350%	9.9590%	39.5220%
	fall	0.5801%	0.4300%	0.0600%	1.6200%
PNW dredged	winter	0.0000%	0.0000%	0.0000%	0.0000%
	spring	0.1645%	0.0200%	0.0000%	0.8110%
	summer	1.7676%	1.2100%	0.1600%	5.5115%
	fall	0.0043%	0.0000%	0.0000%	0.0200%
PNW intertidal	winter	0.0000%	0.0000%	0.0000%	0.0000%
	spring	1.4599%	0.6150%	0.0000%	5.9010%
	summer	11.8406%	10.8000%	3.2690%	25.2505%
	fall	0.0662%	0.0300%	0.0000%	0.2400%

Table A7-23. Percent of harvest exceeding 10,000/g (1,000/g in PNW) at retail

Region	Season	mean	median	5th %-tile	95th %-tile
Gulf LA	winter	0.3139%	0.1900%	0.0100%	1.0005%
	spring	17.5404%	16.8500%	9.0630%	28.5542%
	summer	42.9736%	42.5050%	27.9665%	59.0720%
	fall	4.4157%	3.7900%	1.2795%	9.2920%
Gulf non-LA	winter	0.1489%	0.0800%	0.0000%	0.5305%
	spring	13.9662%	13.3350%	6.7100%	23.4855%
	summer	35.3920%	34.6300%	21.5750%	51.4820%
	fall	2.3744%	1.9500%	0.5395%	5.4900%
Northeast Atlantic	winter	0.0001%	0.0000%	0.0000%	0.0000%
	spring	0.5058%	0.3700%	0.0500%	1.4620%
	summer	3.2025%	2.5700%	0.6785%	7.8675%
	fall	0.0506%	0.0300%	0.0000%	0.1805%
Mid-Atlantic	winter	0.0001%	0.0000%	0.0000%	0.0000%
	spring	3.4714%	3.1900%	1.3295%	6.4715%
	summer	13.0003%	11.8700%	4.2245%	25.9300%
	fall	0.2912%	0.1900%	0.0100%	0.9100%
PNW dredged	winter	0.0000%	0.0000%	0.0000%	0.0000%
	spring	0.0682%	0.0100%	0.0000%	0.3500%
	summer	0.7804%	0.4500%	0.0400%	2.7115%
	fall	0.0014%	0.0000%	0.0000%	0.0100%
PNW intertidal	winter	0.0000%	0.0000%	0.0000%	0.0000%
	spring	0.7493%	0.2300%	0.0000%	3.3505%
	summer	6.7454%	5.7250%	1.4190%	16.7305%
	fall	0.0275%	0.0100%	0.0000%	0.1100%

Table A7-24. Effect of Compliance Levels on the Effectiveness of Controlling Total *Vibrio parahaemolyticus* in Oysters at Cooldown for Gulf Coast Louisiana Summer

Total Vp/g At-Retail[a]	Compliance Level [b]	Reduction in Mean Risk per Serving (%)	Harvest Diverted (%)[c]	Illness Averted (%) [d]
100/g	50%	50.0%	49.0%	74.5%
	70%	70.1%	68.6%	90.6%
	90%	90.0%	88.2%	98.8%
	100%	~100%	98.0%	~100%
1000/g	50%	50.0%	43.5%	71.7%
	70%	70.0%	60.9%	88.3%
	90%	90.0%	78.3%	97.8%
	100%	~100%	87.0%	~100%
5000/g	50%	49.8%	34.5%	67.1%
	70%	69.9%	48.3%	84.4%
	90%	89.7%	62.1%	96.1%
	100%	99.6%	69.0%	99.9%
10,000/g	50%	49.5%	29.7%	64.6%
	70%	69.4%	41.5%	82.1%
	90%	89.2%	53.4%	95.0%
	100%	99.0%	59.3%	99.7%
100,000/g	50%	45.3%	13.9%	53.4%
	70%	63.4%	19.4%	71.2%
	90%	81.6%	25.0%	86.9%
	100%	90.6%	27.8%	94.1%

[a] Assumes that the level of *Vibrio parahaemolyticus* (Vp) is known in oysters at the time of harvest.

[b] The compliance level is the percentage oyster harvest, which is removed from the raw oyster consumption market or subjected to preventive controls; this percentage is assumed to have the same distribution of Vp/g as under the baseline (no mitigation) scenario.

[c] Refers to the harvest that would need to be diverted from the raw market or subjected to preventive controls.

[d] Assuming that the volume of product available for raw consumption is impacted (i.e., reduced) according to the estimate of the % of harvest lost from the raw market or subjected to preventive controls.

Appendix 8: Sensitivity Analysis

The tornado plots for the 24 region/season combination from Chapter V. Risk Characterization are provided in Figures A8-1 to A8-24.

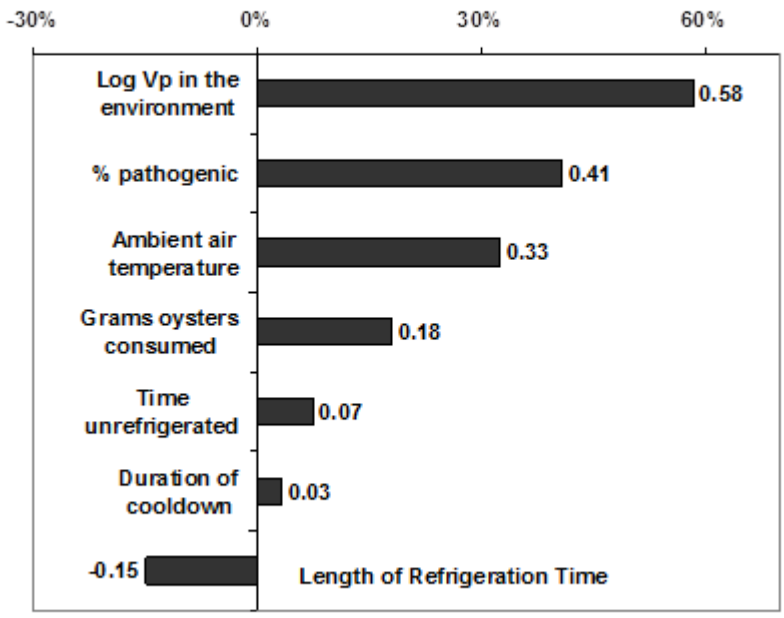


Figure A8-1. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (Louisiana) Winter Harvest

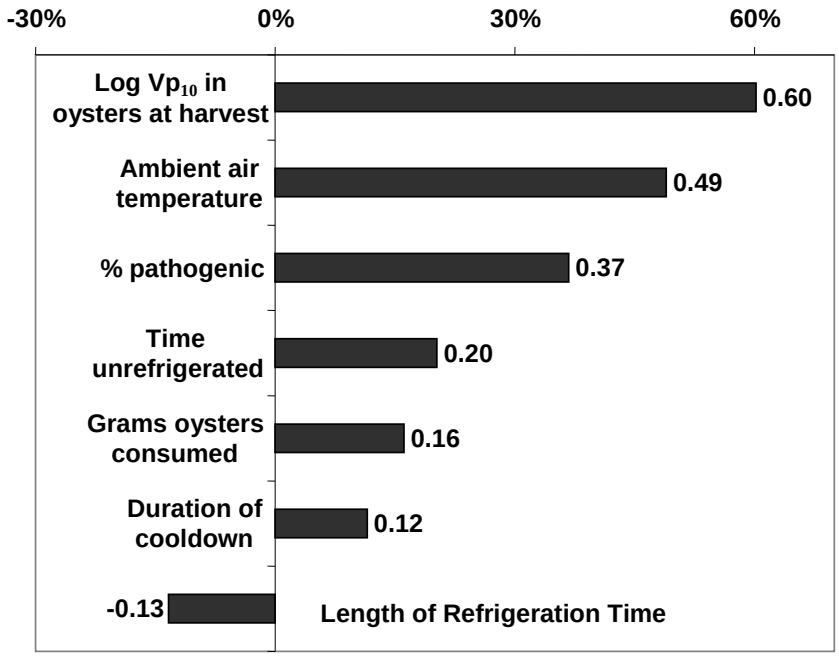


Figure A8-2. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (Louisiana) Spring Harvest

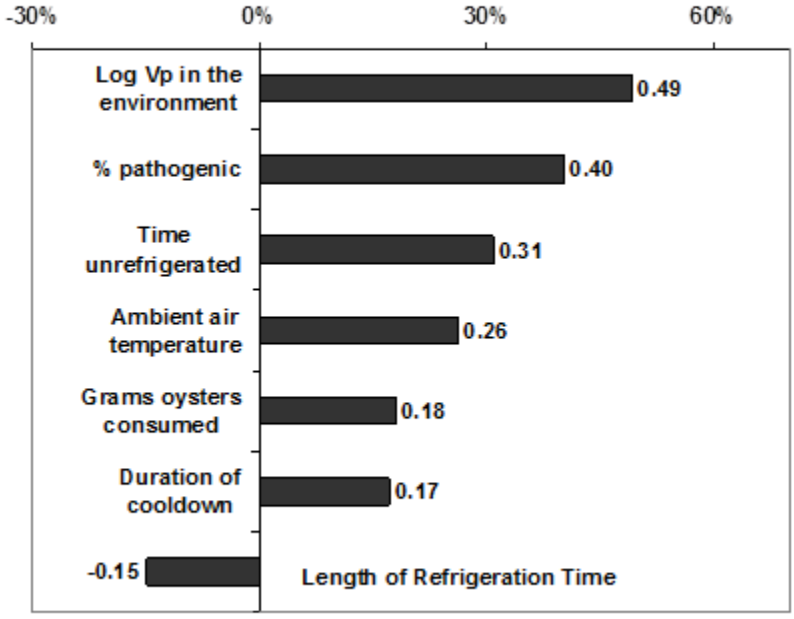


Figure A8-3. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (Louisiana) Summer Harvest

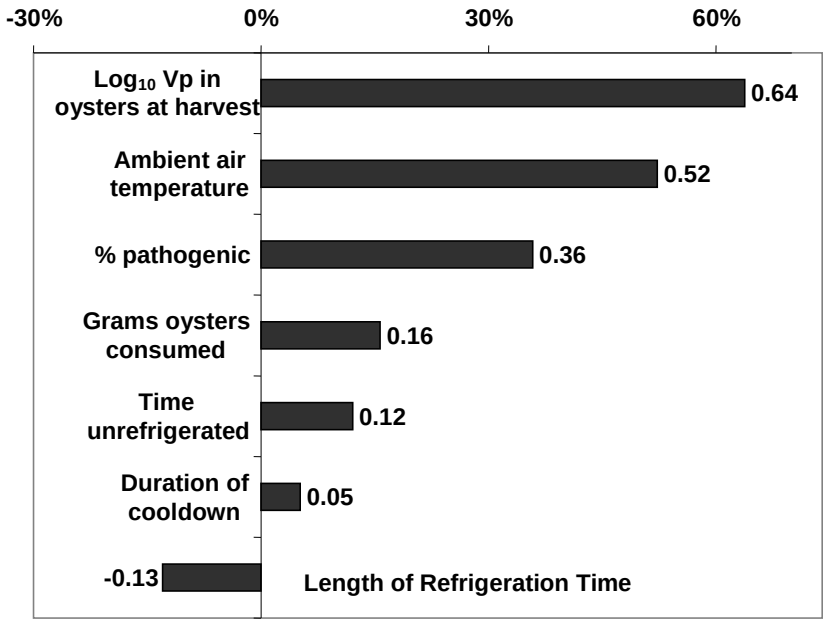


Figure A8-4. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (Louisiana) Fall Harvest

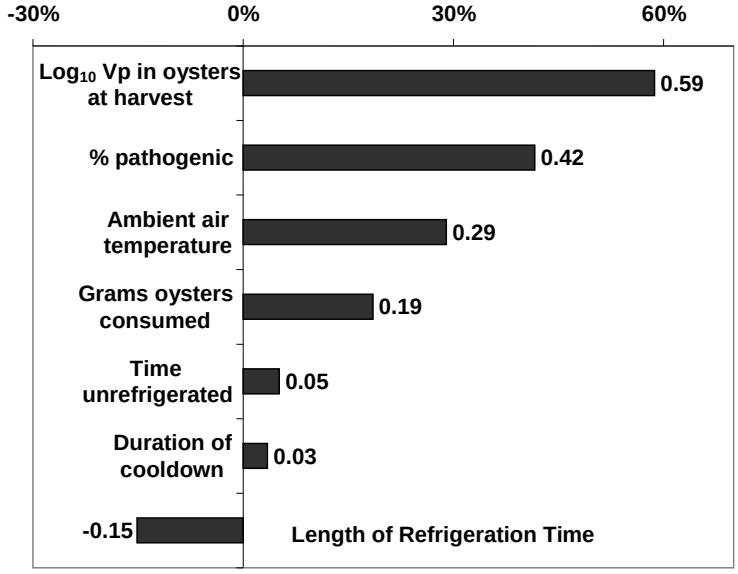


Figure A8-5. Tornado Plot of Influential Variability Parameters on log10 Risk of Vibrio parahaemolyticus Illness per Serving of Raw Oysters from the Gulf Coast (non-Louisiana) Winter Harvest

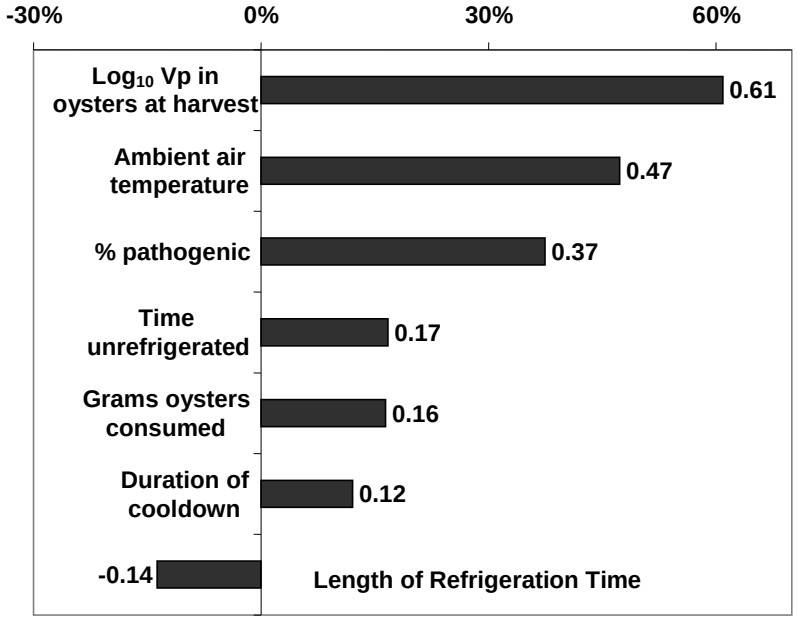


Figure A8-6. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (non-Louisiana) Spring Harvest

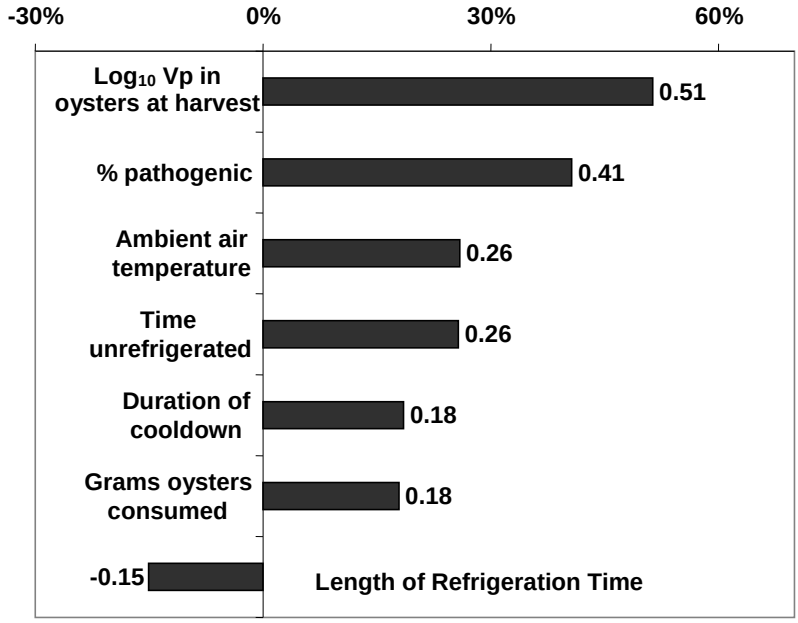


Figure A8-7. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (non-Louisiana) Summer Harvest

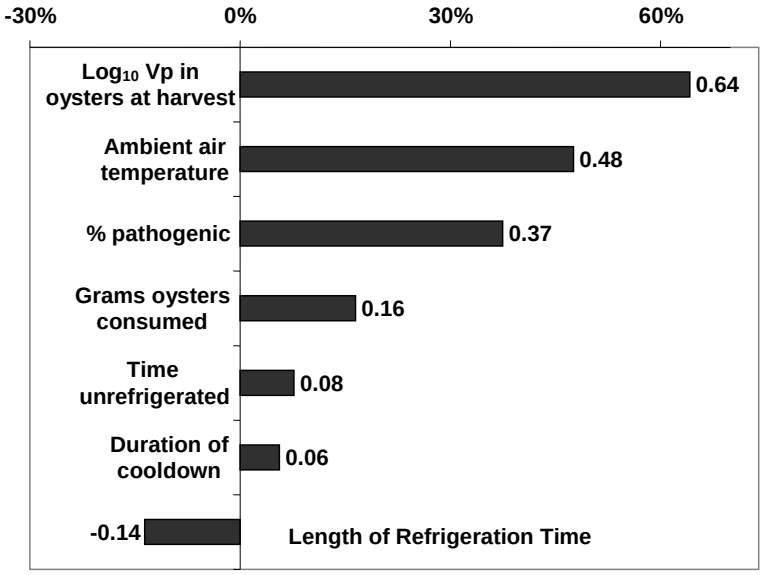


Figure A8-8. Tornado Plot of Influential Variability Parameters on log10 Risk of Vibrio parahaemolyticus Illness per Serving of Raw Oysters from the Gulf Coast (non-Louisiana) Fall Harvest

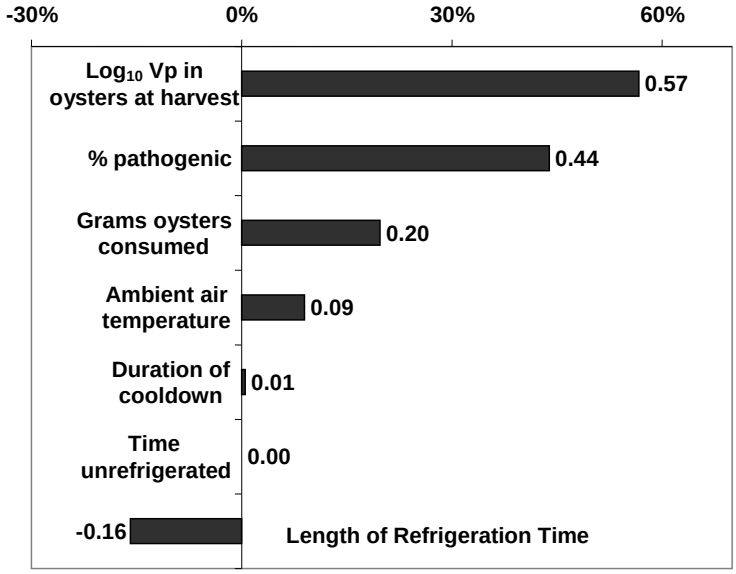


Figure A8-9. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Mid-Atlantic Winter Harvest

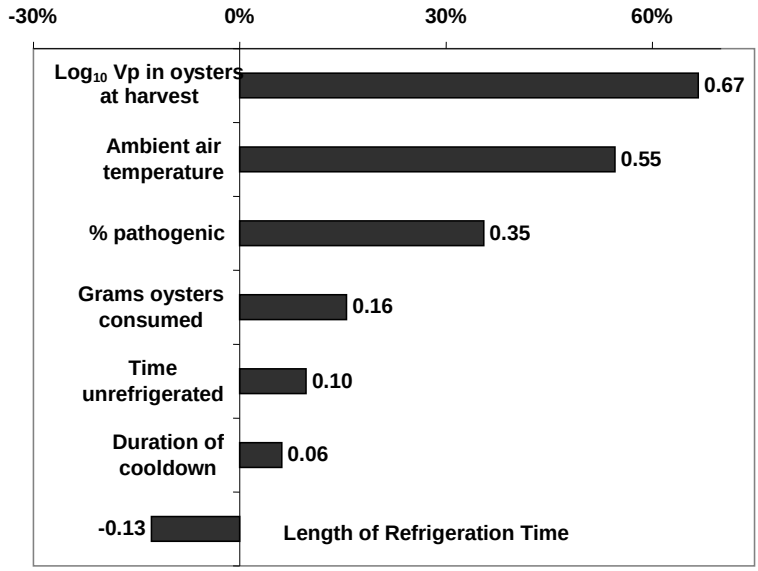


Figure A8-10. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Mid-Atlantic Spring Harvest

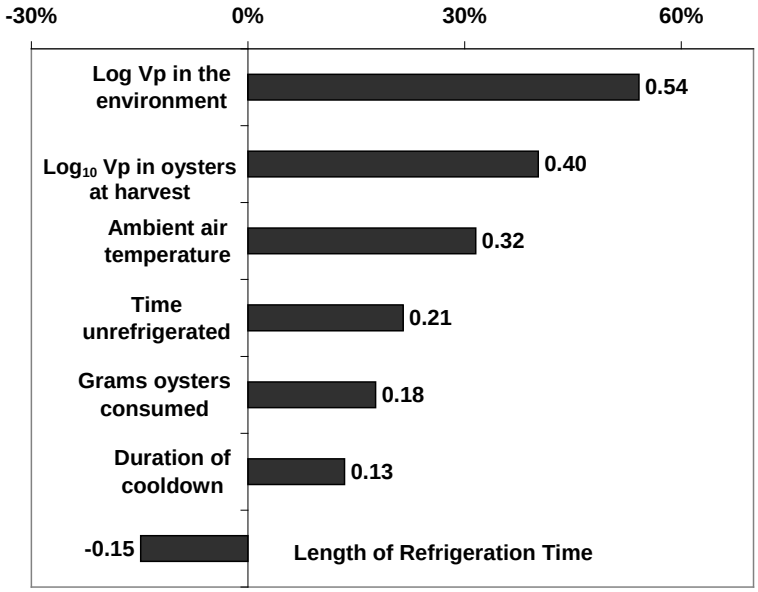


Figure A8-11. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Mid-Atlantic Summer Harvest

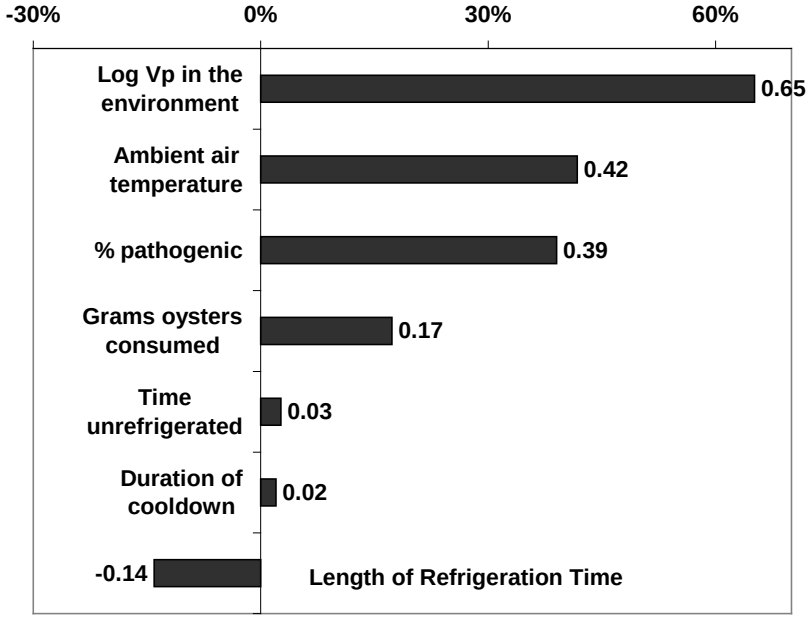


Figure A8-12. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Mid-Atlantic Fall Harvest

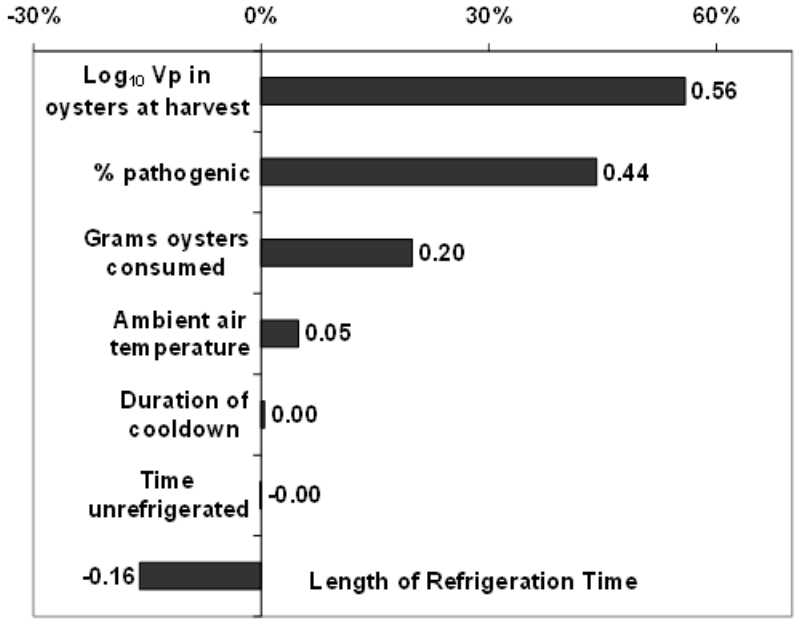


Figure A8-13. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Northeast Atlantic Winter Harvest

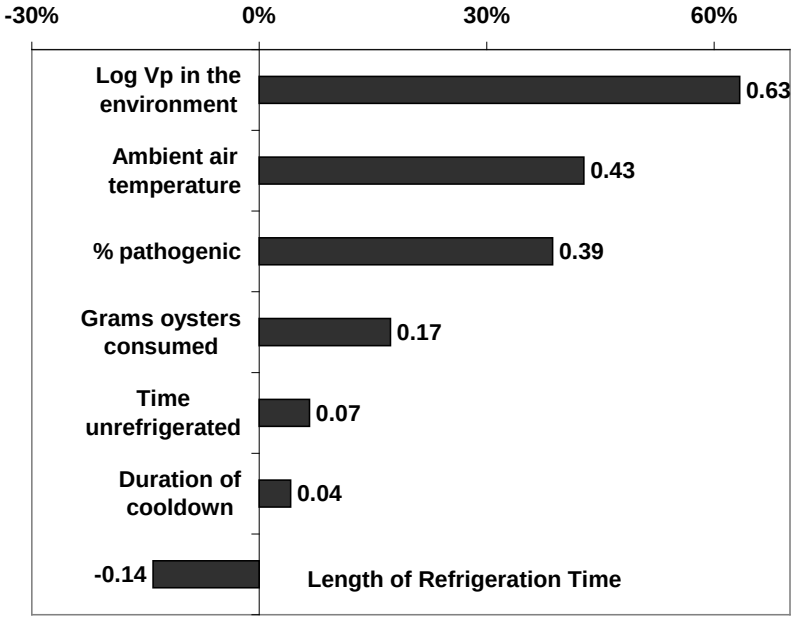


Figure A8-14. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Northeast Atlantic Spring Harvest

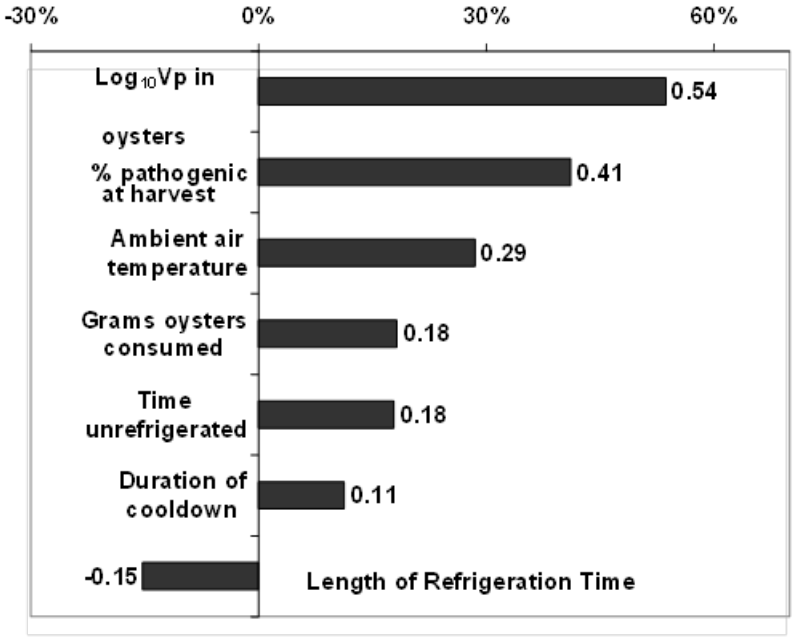


Figure A8-15. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Northeast Atlantic Summer Harvest

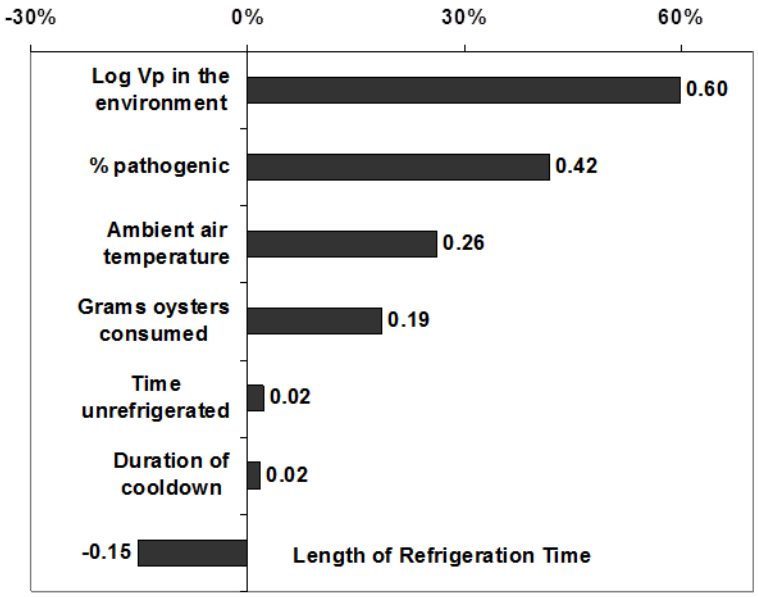


Figure A8-16. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Northeast Atlantic Fall Harvst

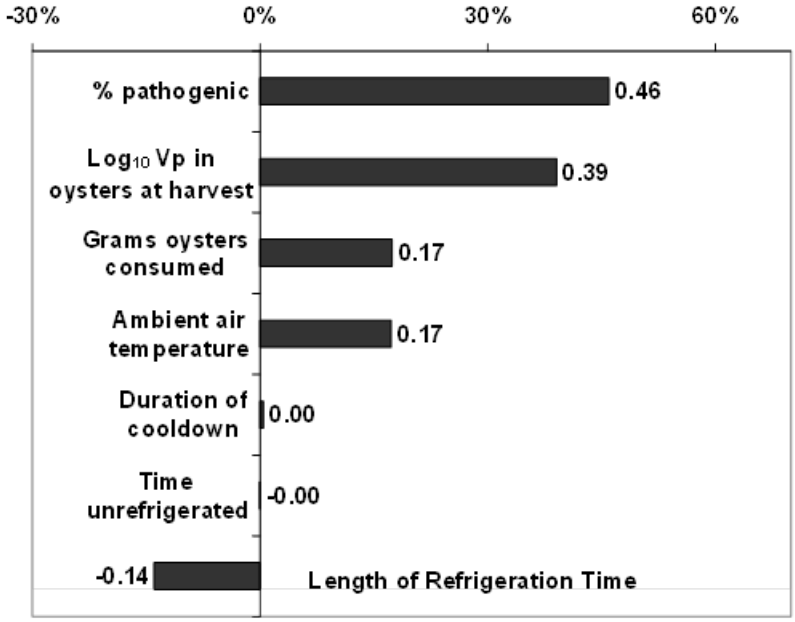


Figure A8-17. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Pacific Northwest (Dredged) Winter Harvest

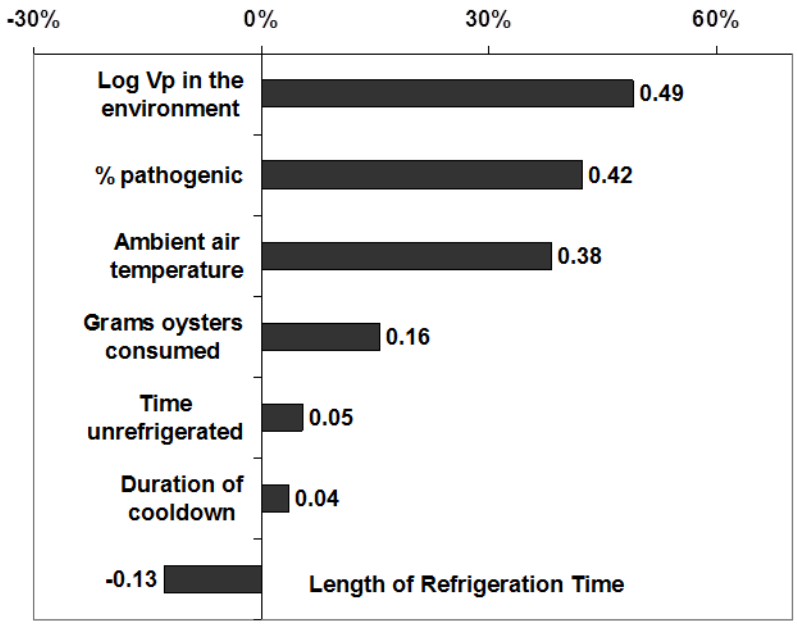


Figure A8-18. Tornado Plot of Influential Variability Parameters on log10 Risk of Vibrio parahaemolyticus Illness per Serving of Raw Oysters from the Pacific Northwest Coast (Dredged) Spring Harvest

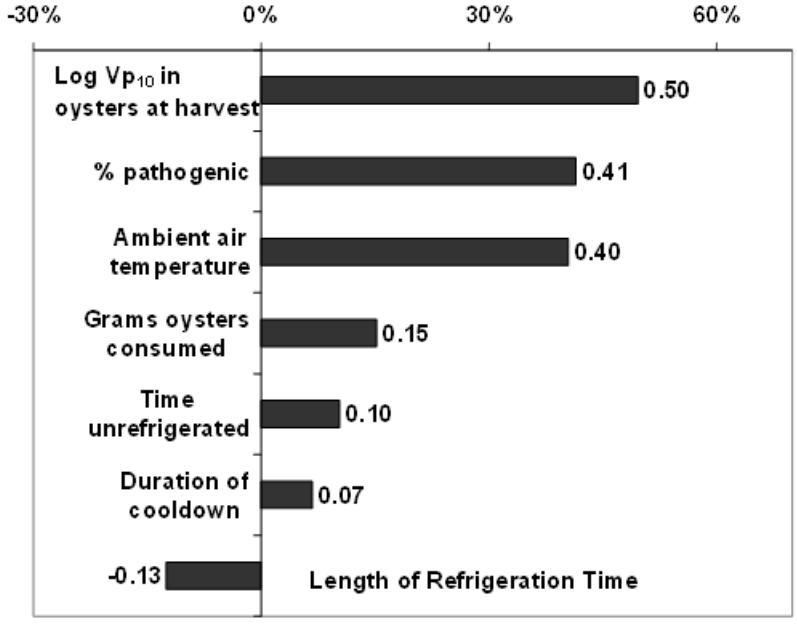


Figure A8-19. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Se (Dredged) Summer Harvest

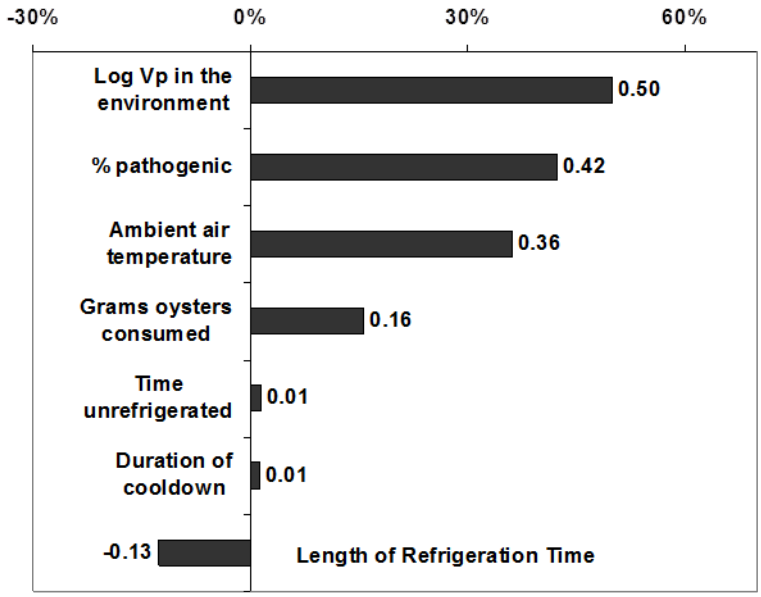


Figure A8-20. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Pacific Northwest (Dredged) Fall Harvest

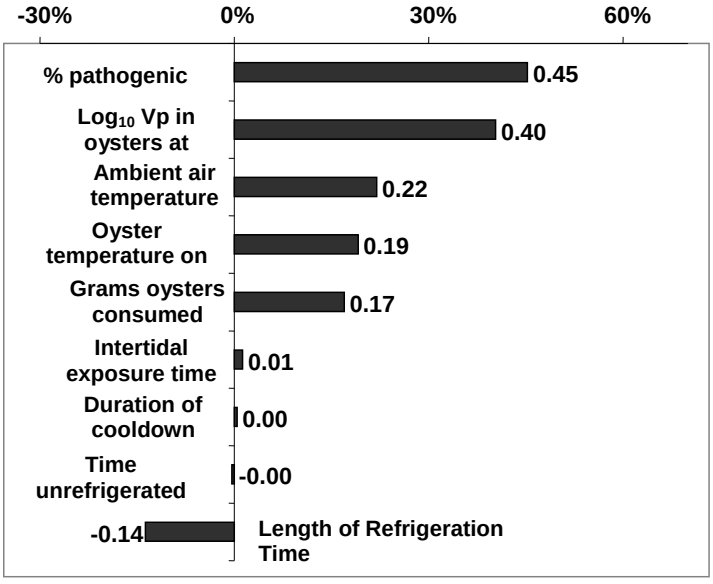


Figure A8-21. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Pacific Northwest (Intertidal) Winter Harvest

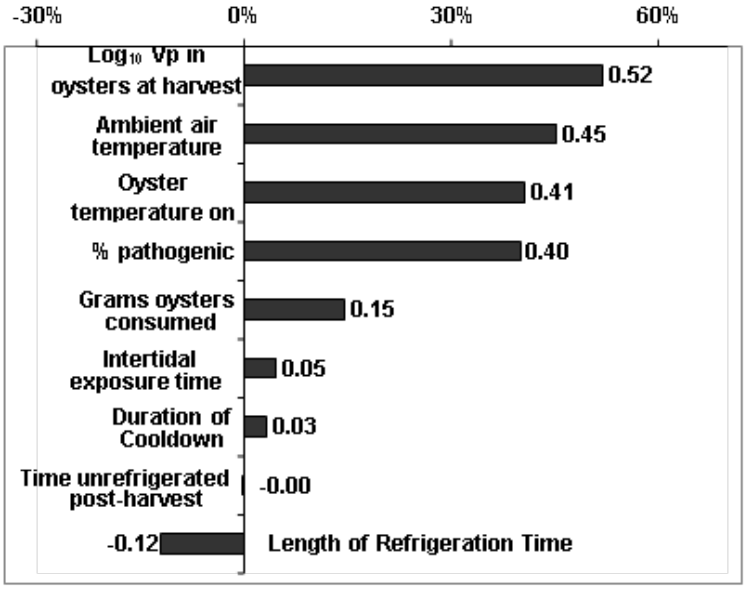


Figure A8-22. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Pacific Northwest (Intertidal) Spring Harvest

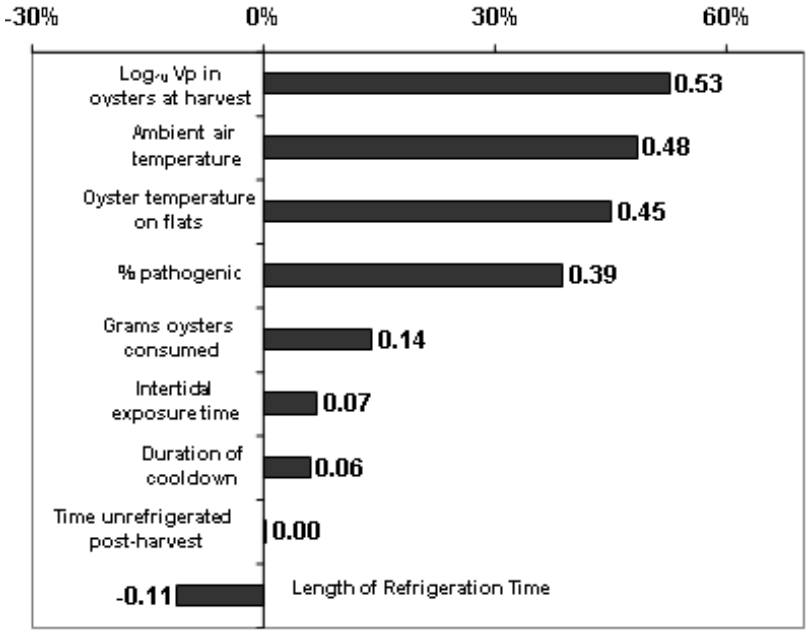


Figure A8-23. Tornado Plot of Influential Variability Parameters on log_{10} Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Pacific Northwest (Intertidal) Summer Harvest

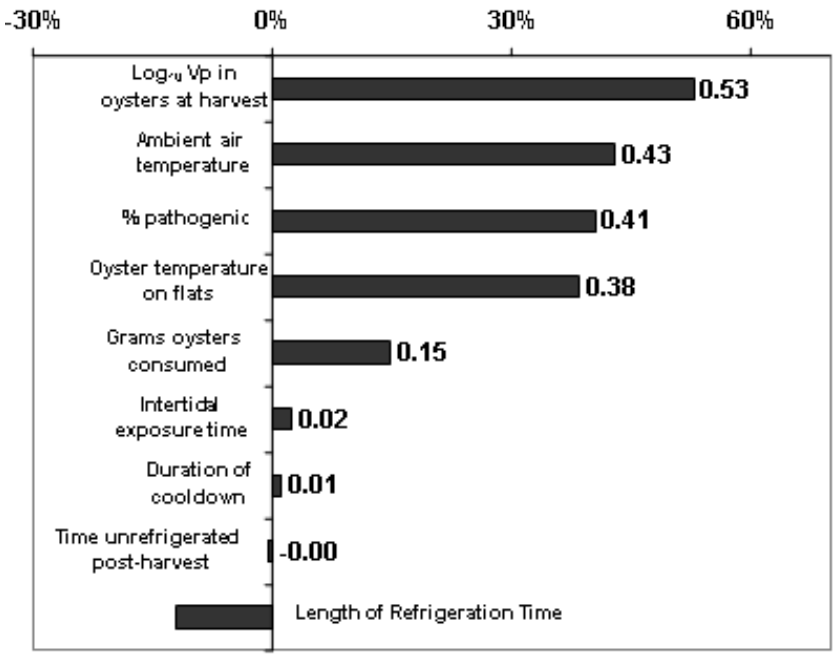


Figure A8-24. Tornado Plot of Influential Variability Parameters on log10 Risk of Vibrio parahaemolyticus Illness per Serving of Raw Oysters from the Pacific Northwest (Intertidal) Fall Harvest

Uncertainty Distributions of Predicted Illnesses

The uncertainty of the predicted number of annual *V. parahaemolyticus* illnesses was analyzed by creating uncertainty distributions 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. Figures A8-25 to A8-36 provide the uncertainty distribution graphs for each region/ season combination.

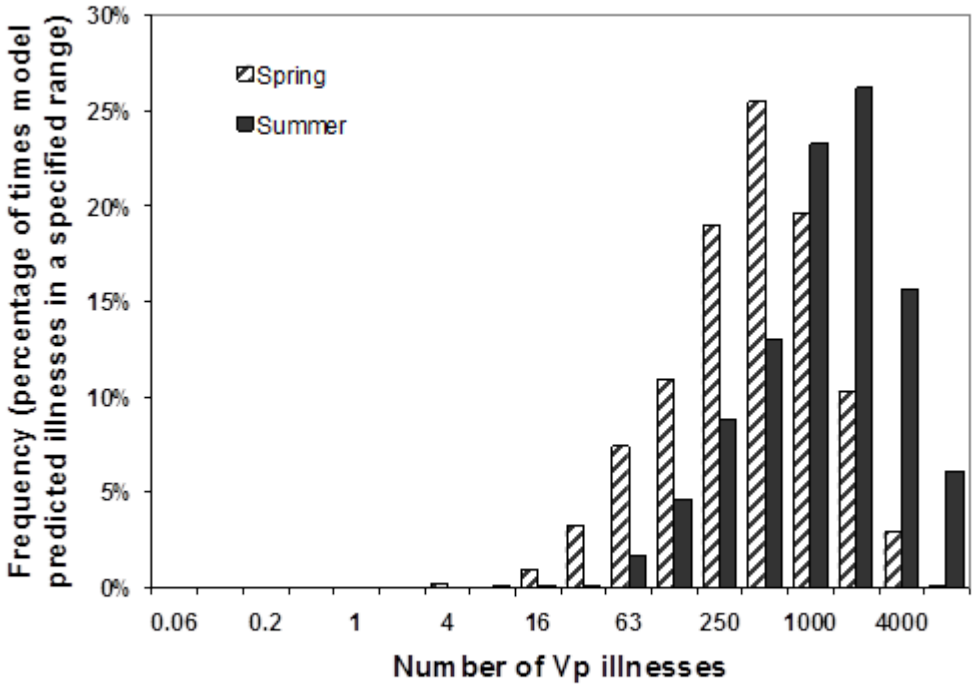


Figure A8-25. Uncertainty distributions of the annual number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Gulf Coast (Lousianna) Harvests.

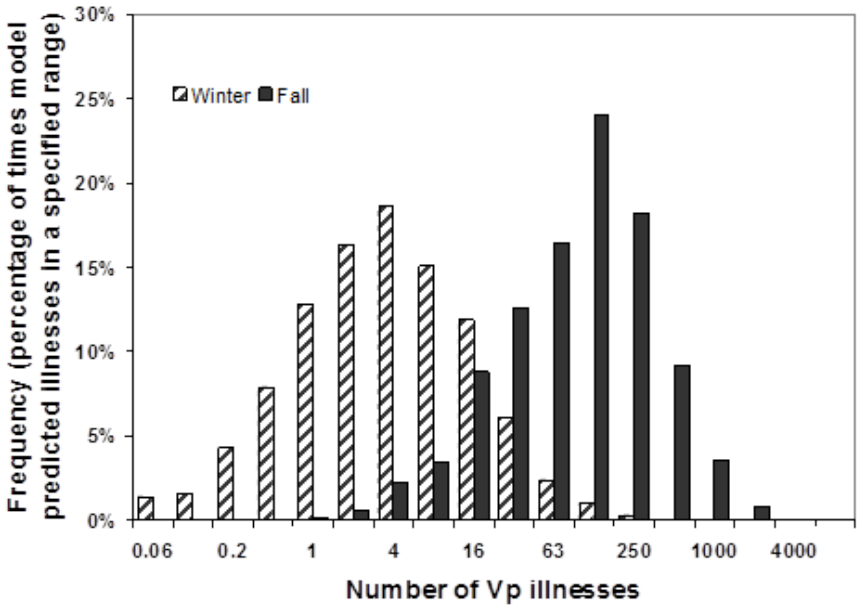


Figure A8-26. Uncertainty distributions of the annual number of Vibrio parahaemolyticus Illnesses Associated with Fall and Winter Gulf Coast (Lousianna) Harvests.

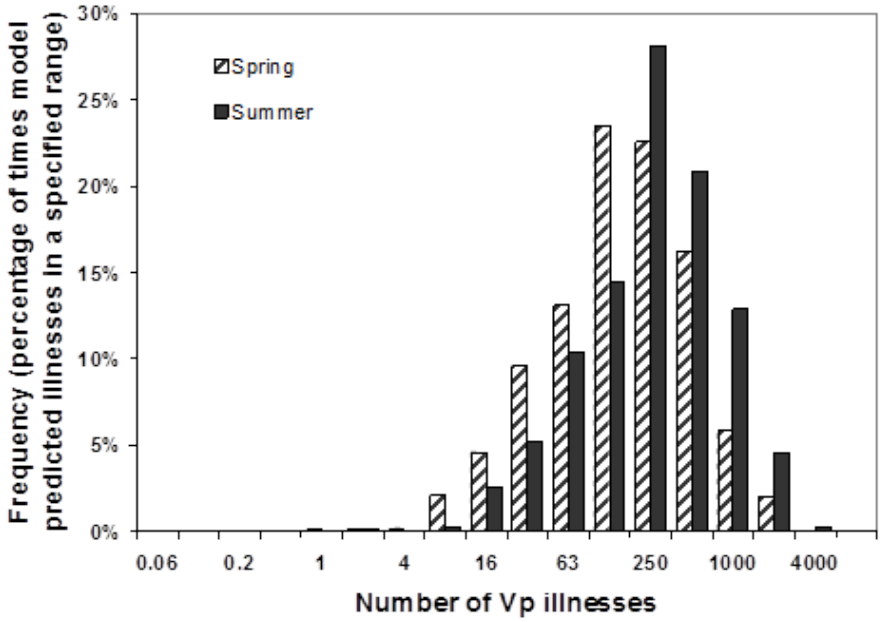


Figure A8-27. Uncertainty distributions of the annual number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Gulf Coast (Non-Lousianna) Harvests.

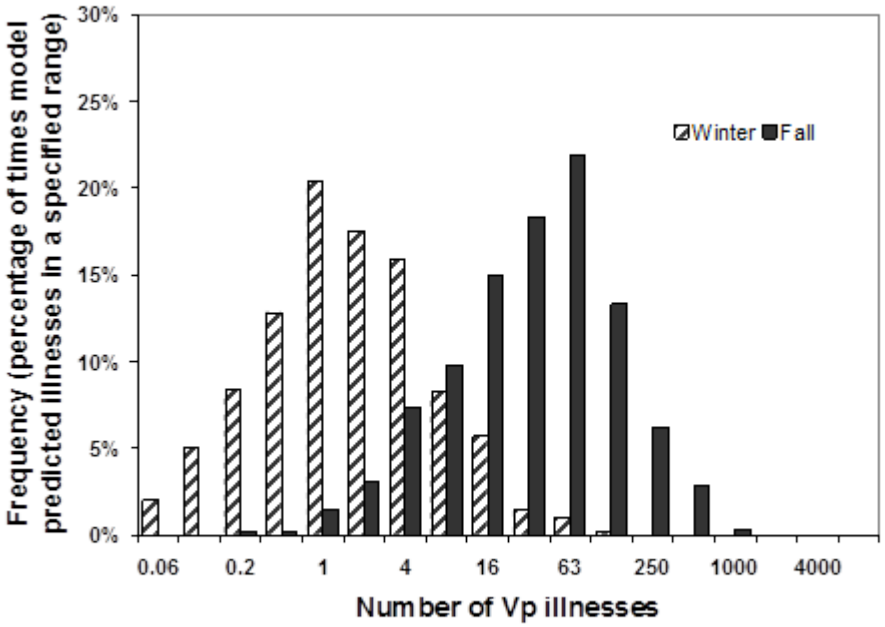


Figure A8-28. Uncertainty distributions of the annual number of *Vibrio parahaemolyticus* Illnesses Associated with Fall and Winter Gulf Coast (Non-Lousianna) Harvests.

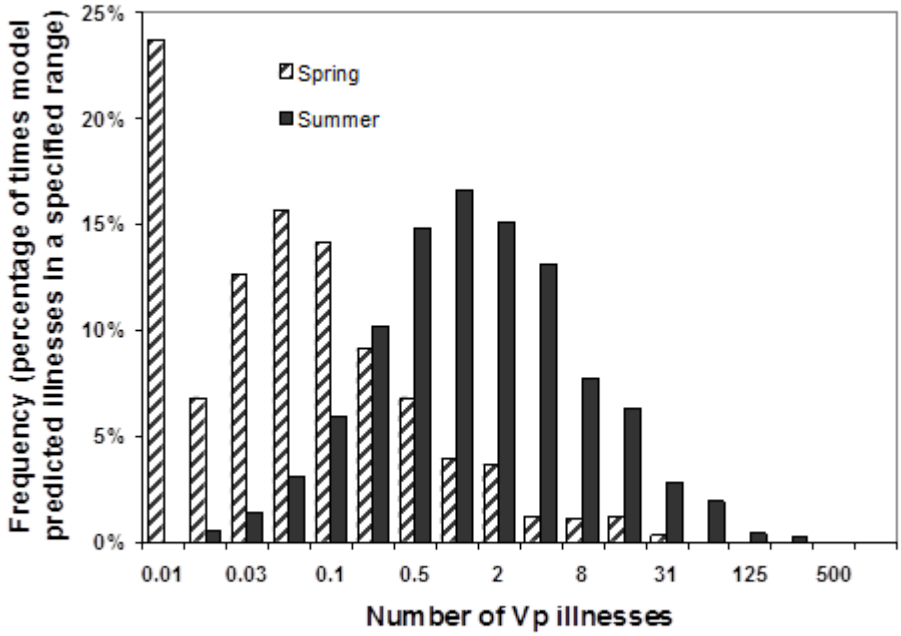


Figure A8-29. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Pacific Coast (dredged) Harvests.

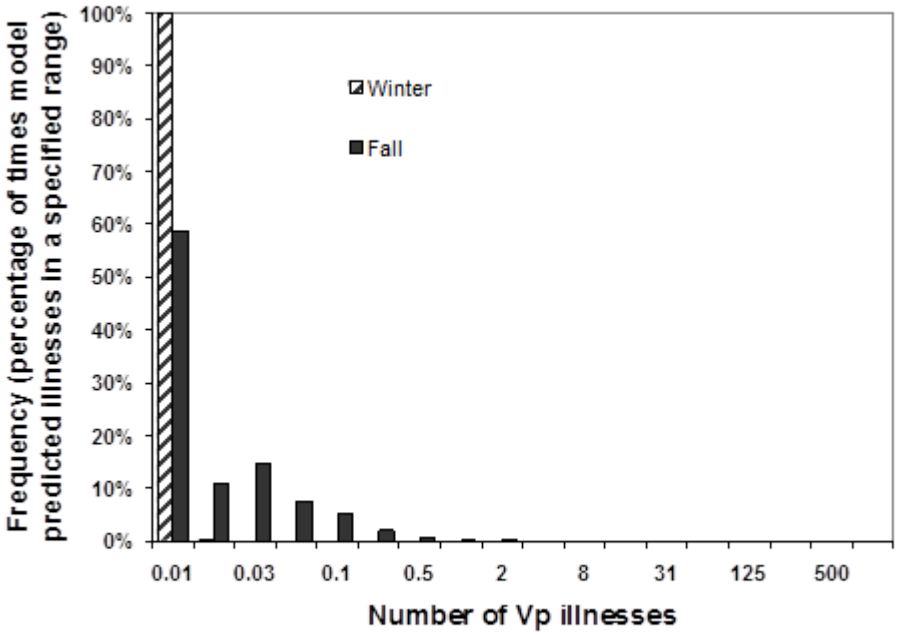


Figure A8-30. Uncertainty Distributions of the Annual Number of Vibrio parahaemolyticus Illnesses Associated with Fall and Winter Pacific Coast (dredged) Harvests.

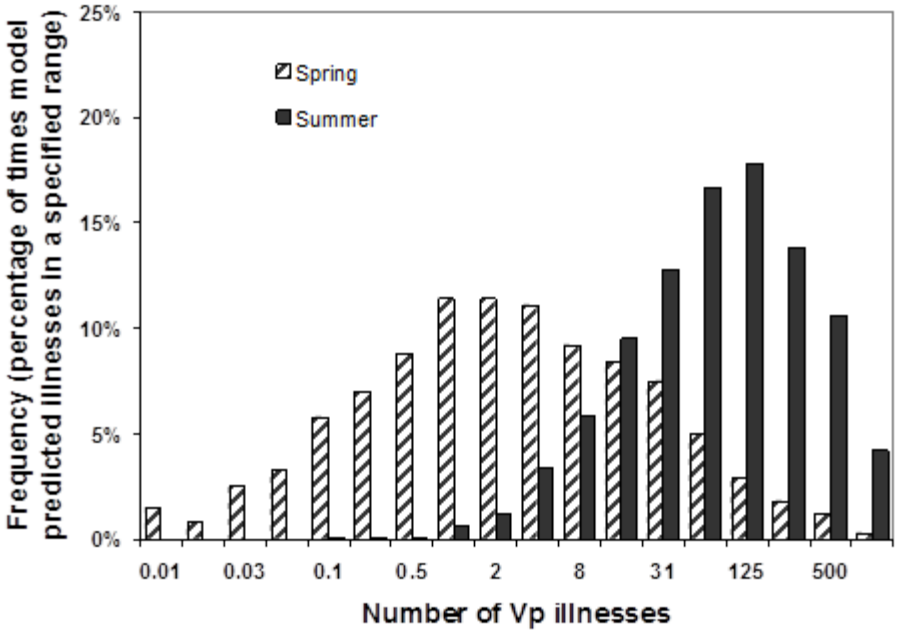


Figure A8-31. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Pacific Coast (intertidal) Harvests.

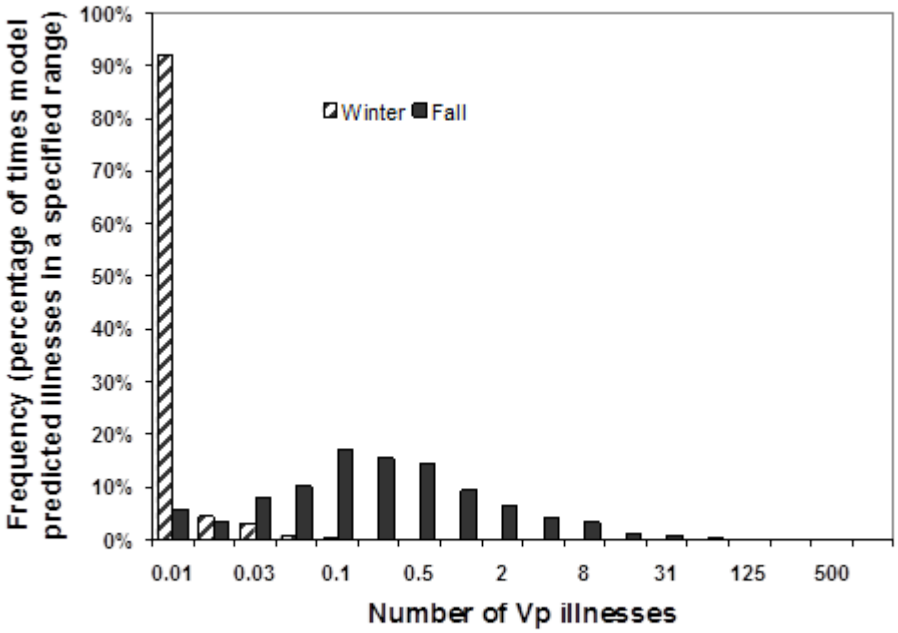


Figure A8-32. Uncertainty Distributions of the Annual Number of Vibrio parahaemolyticus Illnesses Associated with Fall and Winter Pacific Coast (intertidal) Harvests.

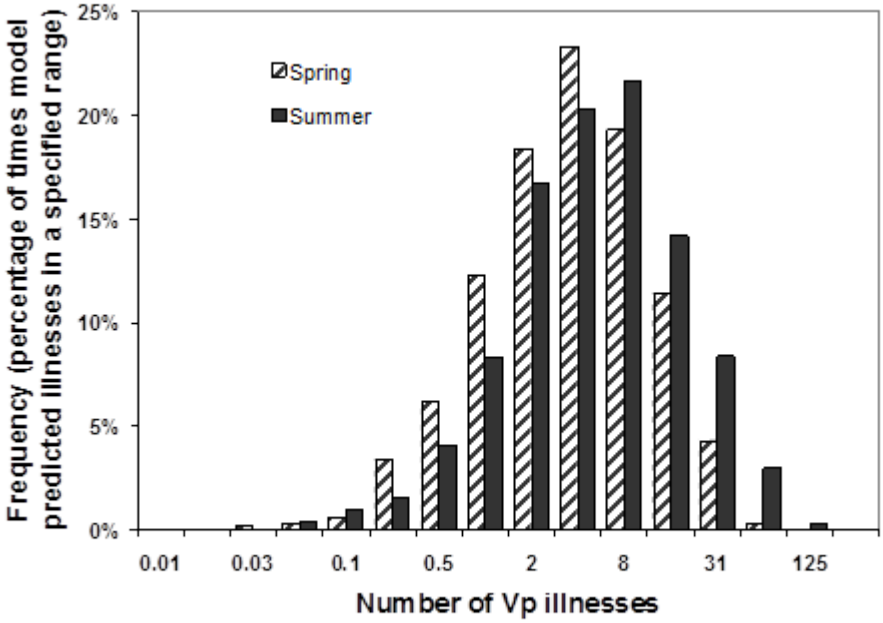


Figure A8-33. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Mid-Atlantic Harvests.

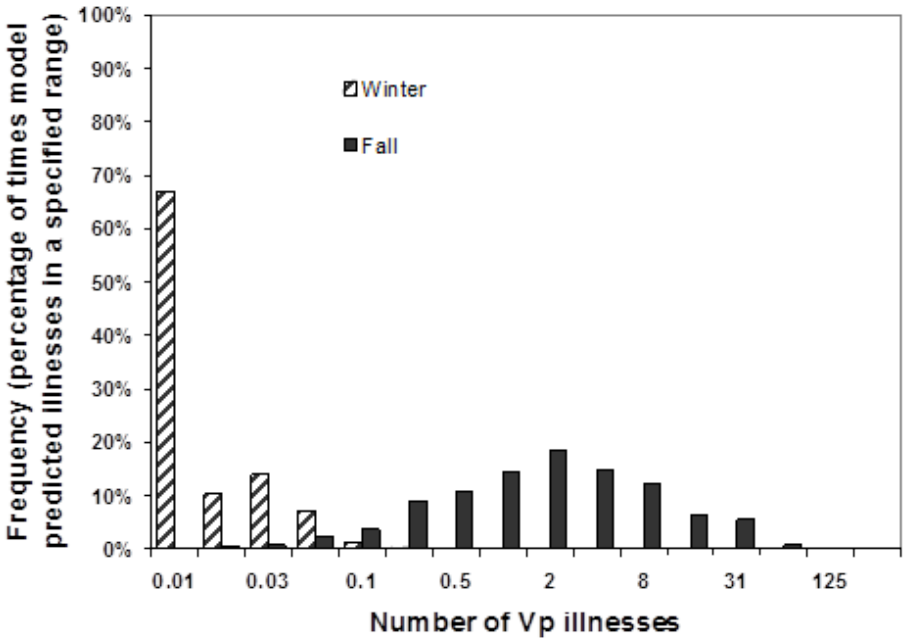


Figure A8-34. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Fall and Winter Mid-Atlantic Harvests.

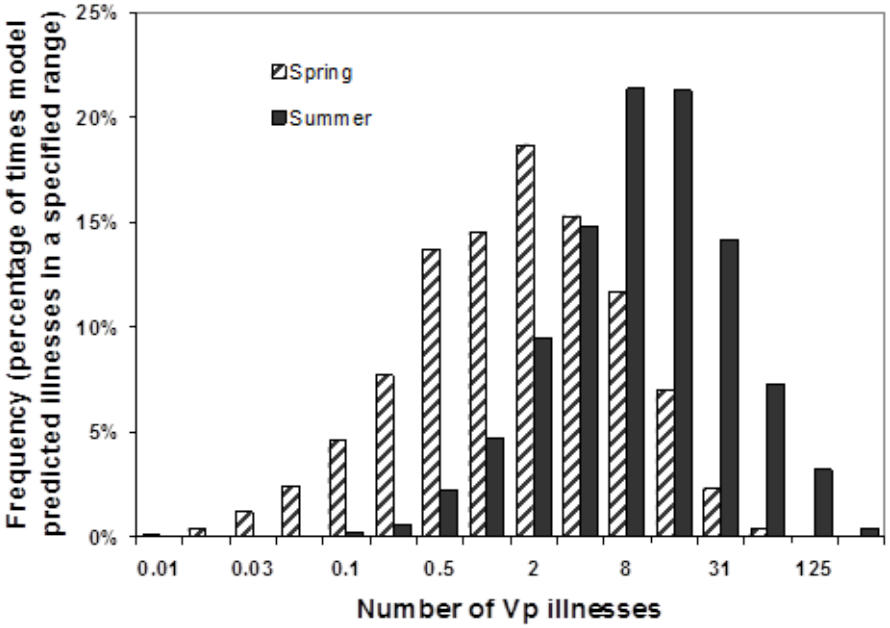


Figure A8-35. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Northeast Atlantic Harvests.

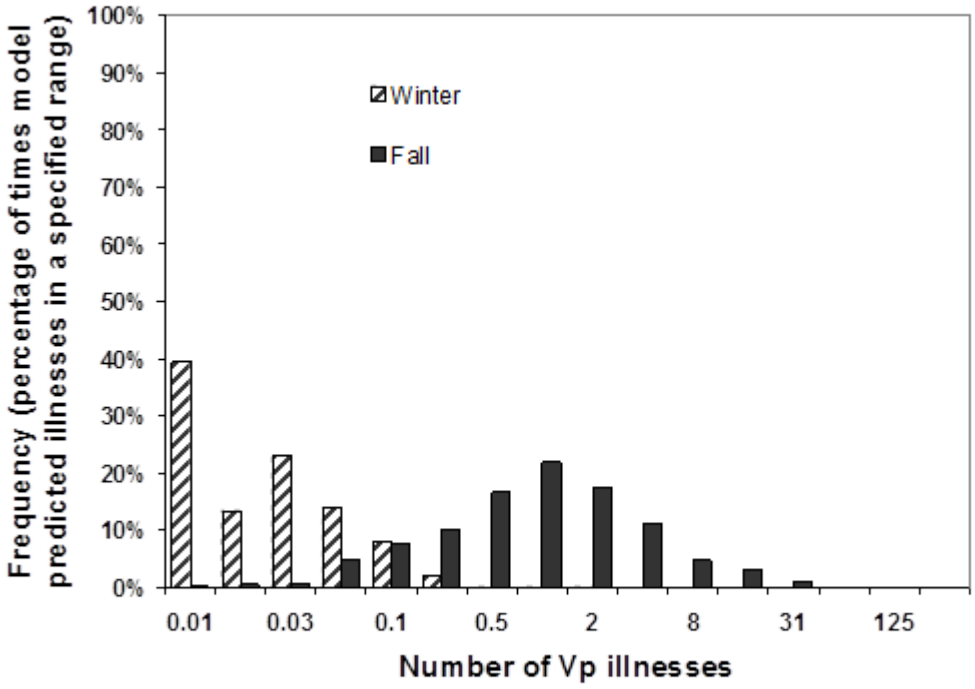


Figure A8-36. Uncertainty Distributions of the Annual Number of Vibrio parahaemolyticus Illnesses Associated with Fall and Winter Northeast Atlantic Harvests.

Appendix 9: Comparison of *Vibrio parahaemolyticus* Illnesses Predicted by Risk Assessment with Illness Reported through United States Surveillance Programs

Background

Surveillance data were compared to the model predictions as one of the approaches to validate the risk characterization portion of the model (i.e., the predicted illnesses attributed to oysters harvested from each region and season). Surveillance for *Vibrio* illness in the United States is conducted by the Centers for Disease Control and Prevention (CDC). State health departments submit reports of *Vibrio* illness to CDC's Cholera and Other *Vibrio* Illness Surveillance System (COVISS) (http://www.cdc.gov/foodborneoutbreaks/report_pub.htm).

Understanding the uncertainties associated with this approach to validating the risk assessment requires an understanding of how the data are acquired and interpreted. The difference can become important when a substantial portion of the oysters consumed in a region is not harvested from that region. The risk assessment model predicts illnesses associated with oysters **harvested** from a given region. Conversely, surveillance data are used to estimate the total number of cases based on the illnesses **reported** within a region. An illness caused by *V. parahaemolyticus* is reported to COVISS when the following occurs:

- A patient seeks medical attention;
- The patient's physician orders analysis of a clinical specimen;
- The clinical laboratory use appropriate materials and procedures to isolate *V. parahaemolyticus;*
- If there is a positive clinical sample, a report is submitted to the state health department;
- The state health department reports the positive finding to CDC.

Completeness of reporting varies among state health departments. Reporting clinical isolation of *V. parahaemolyticus* is mandatory in some states but not in others. Reporting to CDC is voluntary. FDA and state shellfish authorities attempt to gather traceback information on illnesses associated with bivalve molluscan shellfish. However, information on the source of illness may be incomplete. Consequently, there are limitations to be considered in comparing the results of model predictions to observational surveillance data. These limitations are discussed in detail below.

Total Annual Illnesses

As indicated in Chapter III: Hazard Characterization, the dose-response model is "anchored" using CDC's estimated average annual incidence of cases associated with raw oyster consumption (i.e., 2789 *V. parahaemolyticus* illnesses (Painter, 2003). This estimate is based on an analysis of *V. parahaemolyticus* illnesses reported in the National Notifiable Diseases Surveillance System (NNDSS) and the Cholera and Other *Vibrio* Illness Surveillance System (COVISS) from 1998 to 2002. Because some cases may be reported in both systems, a "capture-recapture" method was used to obtain an estimate of the number of *V. parahaemolyticus* cases for the five-year period. The reported cases

were adjusted to account for CDC's estimate of underreporting (a factor of 1:20) and the estimate that 62% of the cases are associated with oyster consumption. A complete description of the data and information that CDC used to estimate the annual illness burden in a manner appropriate to be considered in this risk assessment is provided in Chapter II. Hazard Identification, section titled "Annual Incidence." For the purposes of this specific comparison of predicted cases versus those estimated from surveillance data, COVISS surveillance data from 1998 to 2003 were used (Painter, 2004a and 2004b).

Seasonal Distribution

Table A9-1 provides a comparison of the seasonal distribution of *V. parahaemolyticus* illnesses within the United States predicted by the risk assessment model and the number of cases estimated by the CDC using reported illnesses. Between 1998 and 2003, COVISS received 1018 reports of *V. parahaemolyticus* illnesses in the United States (excluding Guam). Of those, 104 were associated with wounds and 914 were foodborne. Of the foodborne cases, 78% (713) are estimated to be oyster-associated. The observed seasonal frequency of illness occurrence for those 713 illnesses was then applied to the estimated total number of oyster associated cases per year (i.e., 2,789) and compared with number of illnesses predicted by the risk assessment model.

Table A9-1. Seasonal Distribution of Oyster-Associated Illness: Comparison of Reported Illness Estimates and those Predicted by the *V. parahaemolyticus* Risk Assessment

	Illnesses Estimated from			
	***V. parahaemolyticus* Risk Assessment [a]**		**Reported Illnesses[b]**	
Season	**Number**	**% of Annual**	**Number**	**% of Annual**
Winter (January-March)	10	0.3%	156	5.6%
Spring (April-June)	723	25.6%	841	30.1%
Summer (July-September)	1,903	67.3%	1,474	52.9%
Fall (October-December)	190	6.7%	318	11.4%
Total	**2,826**	**100%**	**2,789**	**100%**

[a]Model-predicted illnesses associated with consumption of oysters harvested from all regions.
[b]Values in the column "% of Annual" were calculated from illnesses reported to COVISS from 1998-2003, excluding patients with isolates from wound. Values in the column "Number" were calculated by multiplying the percent of annual for each season by the estimated total (2,789). Source: Painter, 2005.

As shown in Table A9-1, the risk assessment model and the surveillance data indicate similar trends in the seasonal distribution of *V. parahaemolyticus* illnesses. For spring, summer, and fall, estimated illnesses based on reported illness were similar to that predicted by the risk assessment model (Table A9-1). The percentage of illness reported

during winter months was substantially higher than the percentage of illnesses predicted by the risk assessment model. However, this difference accounts for a relatively small percentage (5%) of the total illnesses.

Preliminary data and observations provided by Canada (Buenaventura *et al*., 2002; Banerjee and Farber, 2005) suggest a significantly lower incidence of cases in the winter months in the British Columbia region. This observation is consistent with the model predictions. 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 in 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 model and surveillance estimates

Regional Distribution

V. parahaemolyticus illnesses were most frequently reported to CDC's Cholera and Other *Vibrio* Illness Surveillance (COVISSS) system from Pacific Coast states (Table A9-2). However, the reporting state typically indicates the state of residence of the patient, not the oyster harvest state.

Table A9-2. Reported *Vibrio parahaemolyticus* Foodborne Illnesses by Region

Region	Percentage Illnesses [a]
Atlantic[b]	21.3%
Gulf Coastc	26.4%
Pacific Coastd	45.9%
Non-coastal States	6.4%
Total	100%

[a]Percentages were calculated from the number of illnesses reported to COVISS from 1998-2003, excluding patients with isolates from wound.
Source: Painter, 2005
[b] Includes mid-Atlantic and Northeast Atlantic coast states.
[c] Florida is included in the Gulf Coast regions.
[d] The Pacific Coast includes Hawaii

In general, most oysters consumed in the Gulf Coast are harvested from that region. For other regions in the United States, the source of the oysters consumed is a mix of multiple harvest regions. As a means of comparing the model predictions with comparable surveillance data, illness cases reported to COVISS between 1998 and 2003 were sorted by reporting region and the source of the oysters, if known (Table A9-3). Of the 713 oyster-associated *V. parahaemolyticus* reported illnesses only 18.4% (131) were traced to

a specific harvest site. Of those 131 illnesses, the percent of illnesses from each reporting region that were traced to harvest regions are indicated in Table A9.3. This table illustrates the differences across regions. Of the illnesses reported in the Atlantic only 31% were traced to oysters harvested from that region. However, in the Gulf Coast, the vast majority of the illnesses were traced to that region (93%). In addition, the majority (57%) of the illnesses reported in the Pacific Northwest are associated with oysters from that same region.

Table A9-3. Percent of *Vibrio parahaemolyticus* Illnesses Traced to Commercially Harvested Oysters by Reporting Region

	Oyster Harvest Region[a]			
Patient Residence	**Atlantic[b]**	**Gulf Coast**	**Pacific Northwest[c]**	**Other Pacific States**
Atlantic[b]	31%	54%	15%	0%
Gulf Coastd	7%	93%	0%	0%
Pacific Coaste	10%	12%	57%	21%
Non-coastal States	40%	40%	20%	0%

[a]Source: Painter, 2005.
[b] Includes mid-Atlantic and Northeast Atlantic coast states.
[c]Includes the states of Oregon and Washington.
[d] Florida is included in the Gulf Coast region.
[e]The Pacific Coast includes Hawaii.

The percentage of illnesses attributable to each harvest region was estimated by combining the data from Tables A9-2 and A9-3. The total attributable illness for each region was calculated as a weighted average of the percent of cases attributed to each harvest region, weighted by the percentage of cases reported from each region (Table A9-4). For example, the following calculations were performed to determine the percentage of illnesses attributable to Atlantic region oysters:

- Cases due to oysters harvested from the Atlantic and reported in the Atlantic states: 31% X 21.3% = 6.6%.
- Cases due to oysters harvested from the Atlantic and reported in the Gulf Coast states: 7% X 26.4% = 1.8%
- Cases due to oysters harvested from the Atlantic and reported in the Pacific Coast states: 10% X 45.9% = 4.6%
- Cases due to oysters harvested from the Atlantic and reported in non-coastal states: 40% X 6.4% = 2.6%

Thus, a total of 15.6% (6.6% + 1.8% + 4.6% +2.6%) of all oyster-associated *V. parahaemolyticus* cases were attributed to oysters harvested from the Atlantic region.

Table A9-4. Percentage of *Vibrio parahaemolyticus* Illnesses Attributed to Each Harvest Region

	Oyster Harvest Region[a]			
Patient Residence	**Atlantic[b]**	**Gulf Coast**	**Pacific Northwest[c]**	**Other Pacific**
Atlantic[b]	6.6%	11.5%	3.2%	0%
Gulf Coastd	1.8%	24.6%	0%	0%
Pacific Coaste	4.6%	5.5%	26.2%	9.6%
Non-coastal States	2.6%	2.5%	1.3%	0%
Total Attributed Illnesses	15.6%	44.1%	30.7%	9.6%

[a] Source: Painter, 2005.
[b] Includes mid-Atlantic and Northeast Atlantic coast states.
[c] Includes states of Oregon and Washington.
[d] Florida is included in the Gulf Coast region.
[e] Hawaii is included in the Pacific Coast region.

Differences between the illnesses estimated based on COVISS data and the number of illnesses predicted by the risk assessment is evidence that there are as yet unaccounted for factor(s) in the either the model or the surveillance data, or both. Surveillance data are limited by variation in reporting rates between states, incomplete food history, and incomplete traceback information. Risk assessment models may be limited by unrecognized factors in post-retail handling or in consumption patterns of raw oysters during the winter months. Nonetheless, the above information provides the best available description of the data patterns that are observed.

Although the magnitude of the numbers are different, information from reported illness and the risk assessment model predictions indicate that most oyster associated *V. parahaemolyticus* illnesses are associated with the Gulf Coast oysters, followed by Pacific Northwest oysters. Thus, the predictions of the risk assessment model is consistent, both in terms of seasonal and regional differences, are consistent with the surveillance data. Because of the intrinsic difference in what the two systems measure (location of illness occurrence vs. harvest region of oysters that cause illness), full validation of the regional model predictions of illness based on regional surveillance data would benefit from additional research and targeted surveillance initiatives to acquire more thorough traceback data.

Appendix 10: Additional Information: What-if Scenarios

Table A10-1. Predicted Mean Annual Illnesses with and without Mitigation

Region	Season	Predicted Mean Number of Illnesses per Annum[a]			
		Baseline	**Immediate Refrigeration (~1 log_{10} Reduction)**	**2-log_{10} Reduction**	**4.5-log_{10} Reduction**
Gulf Coast (Louisiana	Spring	505 (36, $1.6x10^{3}$)	54 (3.0, 180)	5.2 (0.35, 17)	0.017 ($1.1x10^{-3}$, 0.053)
	Summer	1,406 (109, $4.4x10^{3}$)	139 (7.6, 490)	15 (1.1, 47)	0.046 ($3.5x10^{-3}$, 0.15)
	Fall	132 (6.4, 470)	8.8 (0.34, 34)	1.3 (0.060, 5.0)	$4.2x10^{-3}$ ($2.0x10^{-4}$, 0.016)
	Winter	6.7 (0.16, 26)	0.80 (0.04, 2.5)	0.070 ($1.7x10^{-3}$, 0.30)	$2.2x10^{-4}$ ($3.9x10^{-6}$, $9.8x10^{-4}$)
Gulf Coast (Non-Louisiana)	Spring	193 (13, 630)	29 (1.5, 98)	2.0 (0.13, 6.3)	$6.2x10^{-3}$ ($4.1x10^{-4}$, 0.020)
	Summer	299 (22, 980)	42 (2.6, 140)	3.1 (0.22, 10)	$9.7x10^{-3}$, ($7.0x10^{-4}$, 0.032)
	Fall	51 (2.0, 180)	7.7 (0.32, 28)	0.51 (0.021, 1.8)	$1.6x10^{-3}$ ($6.6x10^{-5}$, $5.8x10^{-3}$)
	Winter	2.9 (0.08, 11)	0.72 (0.04, 2.3)	0.028 ($9.0x10^{-4}$, 0.11)	$8.8x10^{-5}$ ($1.4x10^{-6}$, $3.5x10^{-4}$)
Mid-Atlantic	Spring	4.4 (0.25, 15)	0.53 (0.024, 2.0)	0.045 ($2.7x10^{-3}$, 0.16)	$1.4x10^{-4}$ ($8.5x10^{-6}$, $5.1x10^{-4}$)
	Summer	6.9 (0.36, 25)	0.83 (0.040, 3.2)	0.070 ($3.8x10^{-3}$, 0.26)	$2.2x10^{-4}$ ($1.2x10^{-5}$, $8.0x10^{-4}$)
	Fall	3.8 (0.08, 17)	0.64 (0.025, 2.4)	0.037 ($8.0x10^{-4}$, 0.16)	$1.2x10^{-4}$ ($1.5x10^{-6}$, $5.2x10^{-4}$)
	Winter	0.012 ($1.0x10^{-3}$, 0.041)	0.01 ($5.0x10^{-4}$, 0.037)	$1.1 x 10^{-4}$ ($5.4x10^{-6}$, $4.1x10^{-4}$)	$3.4x10^{-7}$ (0.0, $2.3x10^{-6}$)
Northeast Atlantic	Spring	3.0 (0.07, 12)	0.33 (0.013, 1.2)	0.031 ($8.0x10^{-4}$, 0.13)	$9.7x10^{-5}$ ($1.8x10^{-6}$, $3.9x10^{-4}$)
	Summer	14 (0.64, 53)	1.7 (0.099, 6.2)	0.14 ($7.0x10^{-3}$, 0.53)	$4.4x10^{-4}$ ($2.1x10^{-5}$, $1.6x10^{-3}$)
	Fall	1.7 (0.05, 6.8)	0.55 (0.029, 1.8)	0.018 ($5.0x10^{-4}$, 0.073)	$5.6x10^{-5}$ (0.0, $2.3x10^{-4}$)
	Winter	0.027 ($1.0x10^{-3}$, 0.083)	0.024 ($1.1x10^{-3}$, 0.081	$2.5 x 10^{-4}$ ($1.1x10^{-5}$, $8.7x10^{-4}$)	$8.6x10^{-7}$ (0.0, $4.9x10^{-6}$)
Pacific Northwest (Dredged)	Spring	0.42 ($1.9x10^{-3}$, 1.5)	0.051 ($9.0x10^{-4}$, 0.16)	$4.7x10^{-3}$ ($1.7x10^{-5}$, $1.7x10^{-2}$)	$1.5x10^{-5}$ (0.0, $5.1x10^{-5}$)
	Summer	3.9 (0.06, 16)	0.37 (0.010, 1.5)	0.044 ($6.0x10^{-4}$, 0.20)	$1.4x10^{-4}$ ($1.5x10^{-6}$, $6.5x10^{-4}$)
	Fall	0.024 ($6.0x10^{-4}$, 0.085)	$8.1 x 10^{-3}$ ($4.0x10^{-4}$, 0.031)	$2.1 x 10^{-4}$ ($6.6x10^{-6}$, $7.4x1^{4}$)	$6.7x10^{-7}$ (0.0, $4.2x10^{-6}$)
	Winter	$6.0 x 10^{-4}$ (0.0, $2.2x 10^{-3}$)	$5.0 x 10^{-4}$ ($1.9x10^{-5}$, $2.0x10^{3}$	$5.5 x 10^{-6}$ (0.0, $2.2x10^{-5}$)	$1.5x10^{-8}$ (0.0, 0.0)
Pacific Northwest (Intertidal)[b]	Spring	18 (0.03, 82)	10 (0.02, 50)	0.22 ($3.0x10^{-4}$, 1.1)	$7.0x10^{-4}$ (0.0, $3.5x10^{-3}$)
	Summer	173 (3.8, 750)	96 (1.9, 420)	2.1 (0.039, 9.4)	$6.8x10^{-3}$ ($1.3x10^{-4}$, 0.03)
	Fall	1.0 (0.01, 4.3)	0.49 (0.01, 1.7)	$8.5x10^{-3}$ ($1.0x10^{-4}$, 0.029)	$2.7x10^{-5}$ (0.0, $1.1x10^{-4}$)
	Winter	$3.3 x 10^{-3}$ ($1.0x10^{-4}$, 0.013)	$3.2 x 10^{-3}$ ($1.0x10^{-4}$, 0.013)	$3.4 x 10^{-5}$ (0.0, $1.4x10^{-4}$)	$9.2x10^{-8}$ (0.0, 0.0)

[a]Values in parentheses are the 5th percentile and 95th percentile of the uncertainty distribution. Values rounded to 2 significant digits. See Appendix 7 for actual illness numbers

[b] After intertidal exposure

Table A10-2. Predicted Mean Levels of Pathogenic *Vibrio parahaemolyticus* per gram in Oysters at Retail after Mitigation Treatments that Reduce Pathogen Levels

		Predicted Mean Levels of Pathogenic *Vibrio parahaemolyticus* per gram[a]			
Region	**Season**	**No Mitigation**	**Immediate Refrigeration (~1 $\log_{10}$ Reduction)**	**2 $\log_{10}$ Reduction**	**4.5 $\log_{10}$ Reduction**
Gulf Coast (Louisiana)	Spring	39 (12, 88)	4.2 (0.84, 12)	0.39 (0.11, 0.89)	1.2×10^{-3} (3.6×10^{-4}, 2.8×10^{-3})
	Summer	100 (37, 220)	10 (2.3, 29)	1.0 (0.36, 2.2)	3.3×10^{-3} (1.2×10^{-3}, 6.8×10^{-3})
	Fall	10 (1.8, 25)	0.65 (0.09, 2.1)	0.10 (0.016, 0.24)	3.1×10^{-4} (5.0×10^{-5}, 7.7×10^{-4})
	Winter	0.48 (0.04, 1.6)	0.059 (0.013, 0.16)	5.0×10^{-3} (3.9×10^{-4}, 0.018)	1.6×10^{-5} (9.9×10^{-7}, 5.7×10^{-5})
Gulf Coast (Non-Louisiana)	Spring	28 (7.6, 65)	4.2 (0.82, 12)	0.28 (0.075, 0.65)	8.8×10^{-4} (2.4×10^{-4}, 2.0×10^{-3})
	Summer	73 (24, 160)	10 (2.4, 28)	0.73 (0.24, 1.6)	2.3×10^{-3} (7.5×10^{-4}, 5.0×10^{-3})
	Fall	4.4 (0.64, 12)	0.65 (0.09, 2.1)	0.043 (5.6×10^{-3}, 0.12)	1.4×10^{-4} (1.8×10^{-5}, 4.0×10^{-4})
	Winter	0.23 (0.026, 0.80)	0.060 (0.014, 0.17)	2.3×10^{-3} (2.7×10^{-4}, 7.5×10^{-3})	7.2×10^{-6} (5.0×10^{-7}, 2.4×10^{-5})
Mid-Atlantic	Spring	7.3 (1.7, 18)	0.88 (0.14, 2.7)	0.073 (0.015, 0.17)	2.3×10^{-4} (5.1×10^{-5}, 5.4×10^{-4})
	Summer	21 (3.8, 54)	2.6 (0.46, 7.6)	0.21 (0.036, 0.54)	6.7×10^{-4} (1.1×10^{-4}, 1.7×10^{-3})
	Fall	0.54 (0.035, 2.0)	0.09 (0.014, 0.32)	5.1×10^{-3} (3.3×10^{-4}, 0.019)	1.6×10^{-5} (9.7×10^{-7}, 6.0×10^{-5})
	Winter	2.4×10^{-3} (4.0×10^{-4}, 5.8×10^{-3})	2.3×10^{-3} (4.0×10^{-4}, 5.4×10^{-3})	2.4×10^{-5} (3.5×10^{-6}, 6.1×10^{-5})	7.5×10^{-8} (0.0, 5.0×10^{-7})
Northeast Atlantic	Spring	0.88 (0.064, 3.0)	0.097 (0.015, 0.29)	8.9×10^{-3} (6.2×10^{-4}, 0.032)	2.8×10^{-5} (1.5×10^{-6}, 1.0×10^{-4})
	Summer	4.3 (0.68, 12)	0.52 (0.11, 1.5)	0.042 (6.8×10^{-3}, 0.11)	1.3×10^{-4} (2.1×10^{-5}, 3.7×10^{-4})
	Fall	0.088 (0.012, 0.29)	0.030 (7.1×10^{-3}, 0.08)	9.9×10^{-4} (1.2×10^{-4}, 3.4×10^{-3})	3.2×10^{-6} (0.0, 1.2×10^{-5})
	Winter	2.5×10^{-3} (4.0×10^{-4}, 6.3×10^{-3})	2.3×10^{-3} (4.2×10^{-4}, 5.9×10^{-3})	2.4×10^{-5} (3.5×10^{-6}, 6.1×10^{-5})	8.3×10^{-8} (0.0, 5.0×10^{-7})
Pacific Northwest (Dredged)[b]	Spring	0.22 (0.002, 0.87)	0.022 (1.1×10^{-3}, 0.076)	2.1×10^{-3} (2.0×10^{-5}, 9.2×10^{-3})	6.9×10^{-6} (0.0, 3.0×10^{-5})
	Summer	2.3 (0.10, 11)	0.20 (0.02, 0.68)	0.023 (9.9×10^{-4}, 0.097)	7.4×10^{-5} (3.0×10^{-6}, 3.1×10^{-4}
	Fall	5.8×10^{-3} (6.0×10^{-4}, 0.018)	1.9×10^{-3} (4.0×10^{-4}, 5.0×10^{-3})	4.9×10^{-5} (5.9×10^{-6}, 1.4×10^{-7})	1.7×10^{-7} (0.0, 9.9×10^{-7})
	Winter	1.9×10^{-4} (2×10^{-5}, 6.1×10^{-4})	1.7×10^{-4} (1.9×10^{-5}, 5.6×10^{-4})	1.9×10^{-6} (0.00, 6.4×10^{-6})	5.5×10^{-9} (0.0, 0.0)
Pacific Northwest (Intertidal)[c]	Spring	3.7 (0.014, 19)	1.9 (9.2×10^{-3}, 9.7)	0.035 (1.2×10^{-4}, 0.20)	1.1×10^{-4} (3.9×10^{-7}, 6.3×10^{-4})
	Summer	38 (2.0, 140)	20 (0.95, 84)	0.38 (0.018, 1.5)	1.2×10^{-3} (5.6×10^{-5}, 4.9×10^{-3})
	Fall	0.086 (3.0×10^{-3}, 0.30)	0.038 (2.2×10^{-3}, 0.13)	6.9×10^{-4} (3.0×10^{-5}, 2.3×10^{-3})	2.2×10^{-6} (8.7×10^{-8}, 7.3×10^{-6})
	Winter	4.0×10^{-4} (3.0×10^{-5}, 1.4×10^{-3})	3.7×10^{-4} (3.0×10^{-5}, 1.3×10^{-3})	4.0×10^{-6} (3.4×10^{-7}, 1.4×10^{-5})	1.3×10^{-8} (1.1×10^{-9}, 4.3×10^{-8})

[a]Values in parentheses are the 5th percentile and 95th percentile of the uncertainty distribution. Values rounded to 2 significant digits. See Appendix 7 for actual predicted levels.

Table A10-3. Predicted Mean Numbers of Pathogenic *Vibrio parahaemolyticus* per Serving of Oysters after Mitigation Treatments that Reduce Pathogen Levels

Region	Season	At Harvest[a]	No Mitigation[b]	Immediate Refrigeration	2 log_{10} reduction[b]	4.5 log_{10} reduction[b]
Gulf Coast (Louisiana)	Spring	320	$7.9x10^3$ ($2.3x10^3$, 1.8×10^4)	840 (170, $2.4x10^3$)	78 (22, 180)	0.25 (0.072, 0.57)
	Summer	720	$2.1x10^4$ ($7.5x10^3$, 4.4×10^4)	$2.0x10^3$ (470, $5.8x10^3$)	210 (73, 440)	0.66 (0.23, 1.4)
	Fall	80	$2.0x10^3$ (320, $5.1x10^3$)	130 (18, 420)	20 (3.2, 49)	0.06 (0.01, 0.16)
	Winter	18	98 (8.1, 330)	12 (2.6, 33)	1.0 (0.078, 3.6)	$3.2x10^{-3}$ (2.0×10^{-4}, 0.012)
Gulf Coast (Non-Louisiana)	Spring	320	$5.6x10^3$ ($1.5x10^3$, 1.3×10^4)	850 (170, $2.4x10^3$)	56 (15, 130)	0.18 (0.048, 0.41)
	Summer	720	1.5×10^4 ($4.9x10^3$, 3.2×10^4)	$2.0x10^3$ (480, $5.7x10^3$)	150 (49, 320)	0.47 (0.15, 1.0)
	Fall	80	880 (110, $2.5x10^3$)	130 (19, 430)	8.6 (1.1, 25)	0.027 ($3.7x10^{-3}$, 0.08)
	Winter	18	47 (5.1, 160)	12 (2.7, 35)	0.46 (0.054, 1.5)	$1.5x10^{-3}$ ($1.0x10^{-4}$, $4.9x10^{-3}$)
Mid-Atlantic	Spring	66	$1.5x10^3$ (330, $3.5x10^3$)	180 (27, 550)	15 (3.1, 35)	0.047 (0.01, 0.11)
	Summer	260	$4.3x10^3$ (750, 1.1×10^4)	520 (92, $1.5x10^3$)	43 (7.3, 110)	0.14 (0.023, 0.34)
	Fall	18	110 (7.1, 410)	18 (2.8, 64)	1.0 (0.07, 3.9)	$3.2x10^{-3}$ ($2.0x10^{-4}$, 0.012)
	Winter	1.2	0.48 (0.09, 1.2)	0.46 (0.08, 1.1)	$4.9x10^{-3}$ (7.0×10^{-4}, 0.012)	$1.5x10^{-5}$ (0.0, $1.0x10^{-4}$
Northeast Atlantic	Spring	14	180 (12, 620)	20 (2.9, 59)	1.8 (0.13, 6.5)	$5.7x10^{-3}$ ($3.0x10^{-4}$, 0.02)
	Summer	78	860 (130, $2.5x10^3$)	100 (22, 300)	8.5 (1.4, 23)	0.027 ($4.2x10^{-3}$, 0.074)
	Fall	12	17 (2.4, 57)	6.1 (1.4, 16)	0.20 (0.024, 0.68)	$6.4x10^{-4}$ (0.0, $2.4x10^{-3}$)
	Winter	1.2	0.5 (0.09, 1.2)	0.47 (0.085, 1.2)	$4.9x10^{-3}$ (7.0×10^{-4}, 0.012)	1.7×10^{-5} (0.0, 1.0×10^{-4})
Pacific Northwest (Dredged)	Spring	4	43 (0. 4, 160)	4.5 (0.23, 15)	0.43 (4.1×10^{-3}, 1.9)	1.4×10^{-3} (0.0, 6.0×10^{-3})
	Summer	24	460 (21, $2.1x10^3$)	40 (4.7, 140)	4.7 (0. 2, 19)	0.015 (6.0×10^{-4}, 0.062)
	Fall	0.68	1.2 (0.12, 3.6)	0.39 (0.081, 1.0)	$9.9x10^{-3}$ (1.2×10^{-3}, 0.03)	3.3×10^{-5} (0.0, 2.0×10^{-4})
	Winter	0.08	0.04 (0.00, 0.12)	0.034 ($3.9x10^{-3}$, 0.11)	3.8×10^{-4} (0.0, 1.3×10^{-3})	1.1×10^{-6} (0.0, 0.0)
Pacific Northwest (Intertidal)	Spring	280	740 (2.6, $3.7x10^4$)	380 (1.9, $2.0x10^3$)	7.1 (0.025, 40)	0.022 (8.0×10^{-5}, 0.13)
	Summer	$3.0x10^3$	$7.5x10^3$ (370, 3.0×10^4)	$4.1x10^3$ (190, 1.7×10^4)	77 (3.6, 310)	0.24 (0.011, 0.98)
	Fall	10	17 (0.50, 74)	7.7 (0.45, 27)	0.14 ($5.6x10^{-3}$, 0.47)	4.4×10^{-4} (2.0×10^{-5}, $1.5x10^{-3}$)
	Winter	0.18	0.08 (0.01, 0.28)	0.075 ($6.6x10^{-3}$, 0.26)	8.0×10^{-4} (7.0×10^{-5}, $2.8x10^{-3}$)	2.5×10^{-6} (2.2×10^{-7}, 8.7×10^{-6})

[a] Mean number of pathogenic *V. parahaemolyticus* consumed per serving (average over variabilities and uncertainties)

[b] Values in parentheses are the 5th percentile and 95th percentile of the uncertainty distribution. Values rounded to 2 significant digits. See Appendix 7 for actual predicted levels.

Impact of overnight submersion of oysters during intertidal harvesting on the predicted risk of illness

Table A10-4. Effect of Overnight Submersion of Oysters during Intertidal Harvest on Predicted Risk in the Pacific Northwest Harvest Region

Type of Harvest	Season	Mean Risk per Serving
Baseline Intertidal Harvest	Winter	$1.7x10^{-9}$
	Spring	$1.3x10^{-5}$
	Summer	$1.4x10^{-4}$
	Fall	$3.9x10^{-7}$
Overnight Submersion of Intertidal Harvest[a]	Winter	$8.1x10^{-10}$
	Spring	$8.7x10^{-7}$
	Summer	$1.0x10^{-5}$
	Fall	$2.7x10^{-8}$

[a]This assumes levels of *V. parahaemolyticus* in oysters after submersion overnight are similar to dredged.

Predicted Effects of Maximum Time-to-refrigeration on Illness Using Ice (Rapid Refrigeration) or Conventional Refrigeration (Air- Circulated)

Tables A10-5 to A10-8 show the impact of rapid cooling with ice on predicted reduction in levels of total *V. parahaemolyticus* at-retail compared with the baseline levels. Figures A10-1 to A10-6 show predicted effects on illness of maximum time-to-refrigeration of oyster shellstock with conventional refrigeration (i.e., up to 10 hours to reach no-growth temperatures) for each season and region. Figures A10-7 –A10-12 show predicted effects on illness of maximum time-to-refrigeration of oyster shellstock with rapid cooling on ice (i.e., 1 hour to reach no-growth temperatures) for each season and region. Figures A10-13 to A10-18 compare the predicted effects between conventional refrigeration and rapid cooling for the summer harvest of all 6 harvesting regions. As mentioned in Chapter VII of the technical document, predicted reductions for regions and seasons with lower air temperatures are less dramatic than those with higher air temperatures as shown in the figures below.

Effect of Limiting Time to Refrigeration followed by rapid cooling (icing) on the mean and 90th %-tile of total Vp/g at retail (point of consumption)

Table A10-5. Best estimate of the Mean total Vp/g at retail for all region/seasons

Region	Season	Maximum Time-to-Refrigeration				
		1 hr	2 hr	3 hr	4 hr	baseline
Gulf Louisiana	winter	25[a]	31	37	44	290
	spring	970	$1.6x10^3$	$2.5x10^3$	$3.8x10^3$	$2.3x10^4$
	summer	$2.3x10^3$	$3.8x10^3$	$6.1x10^3$	$9.1x10^3$	$6.0x10^4$
	fall	170	270	400	610	$5.7x10^3$
Gulf non-Louisiana	winter	26	31	36	42	140
	spring	970	$1.6x10^3$	$2.4x10^3$	$3.4x10^3$	$1.6x10^4$
	summer	$2.3x10^3$	$3.8x10^3$	$5.8x10^3$	$8.3x10^3$	$4.2x10^4$
	fall	180	270	380	530	$2.5x10^3$
Northeast Atlantic	winter	1.3	1.4	1.4	1.4	1.5
	spring	28	40	56.0	77	510
	summer	165	230	310	410	$2.5x10^3$
	fall	14	16	18	20	52
Mid-Atlantic	winter	1.3	1.3	1.3	1.3	1.4
	spring	190	320	500	750	$4.2x10^3$
	summer	680	$1.0x10^3$	$1.5x10^3$	$2.1x10^3$	$1.2x10^4$
	fall	32	43	567	73	310
Pacific Northwest (dredged)	winter	0.007	0.007	0.007	0.007	0.008
	spring	0.54	0.74	1.0	1.3	9.1
	summer	4.1	6.1	8.7	12	100
	fall	0.070	0.080	0.091	0.102	0.230
Pacific Northwest (intertidal)	winter	0.015	0.015	0.015	0.015	0.017
	spring	47	54	60	63	150
	summer	520	600	660	700	$1.7x10^3$
	fall	1.6	1.6	1.8	1.9	3.9

[a]Levels of *V. parahaemolyticus* at-retail after cooling at various time intervals after harvest; values are rounded to 2 significant digits

Table A10-6. Best estimate of the 90th percentile of the distribution of total Vp/g at retail for all region/seasons

Region	Season	Maximum Time-to-Refrigeration				
		1 hr	2 hr	3 hr	4 hr	Baseline
Gulf Louisiana	winter	35[a]	40	45	51	120
	spring	$1.1x10^3$	$1.9x10^3$	$2.9x10^3$	$4.4x10^3$	$4.6x10^4$
	summer	$3.8x10^3$	$6.8x10^3$	$1.1x10^4$	$1.8x10^4$	$2x10^5$
	fall	160	210	280	370	$2.8x10^3$
Gulf non-Louisiana	winter	35	39	44	48	84
	spring	$1.2x10^3$	$1.9x10^3$	$2.8x10^3$	$3.9x10^3$	$2.6x10^4$
	summer	$3.8x10^3$	$6.7x10^3$	$1.1x10^4$	$1.6x10^4$	$1.2x10^5$
	fall	160	210	270	330	$1.0x10^3$
Northeast Atlantic	winter	2.3	2.3	2.3	2.3	2.3
	spring	27	33	39	45	100
	summer	240	330	440	560	$2.5x10^3$
	fall	18	19	21	22	28
Mid-Atlantic	winter	2.1	2.1	2.1	2.1	2.2
	spring	140	190	260	330	$1.3x10^3$
	summer	990	$1.5x10^3$	$2.2x10^3$	$3.1x10^3$	$2.2x10^4$
	fall	23	27	29	31	48
Pacific Northwest (dredged)	winter	0.015	0.015	0.016	0.016	0.017
	spring	0.70	0.86	1.0	1.2	2.6
	summer	5.7	7.6	9.8	12	40
	fall	0.098	0.10	0.11	0.12	0.15
Pacific Northwest (intertidal)	winter	0.028	0.028	0.028	0.028	0.030
	spring	11	13	14	15	27
	summer	240	280	310	330	800
	fall	0.40	0.40	0.41	0.42	0.51

[a]Levels of *V. parahaemolyticus* at-retail after cooling at various time intervals after harvest; values are rounded to 2 significant digits

Effect of Limiting Time to Refrigeration followed by conventional cooling on the mean and 90th %-tile of total Vp/g at retail (point of consumption)

Table A10-7. Best estimate of the Mean total Vp/g at retail for all region/seasons

Region	Season	Maximum Time-to-Refrigeration				
		1 hr	2 hr	3 hr	4 hr	Baseline
Gulf Louisiana	winter	43[a]	55	70	89	290
	spring	$4.0x10^3$	$6.2x10^3$	$8.9x10^3$	$1.2x10^4$	$2.2x10^4$
	summer	9.8x103	$1.5x10^4$	$2.3x10^4$	$3.1x10^4$	$6.0x10^4$
	fall	620	950	$1.4x10^3$	$1.9x10^3$	$5.7x10^3$
Gulf non-Louisiana	winter	43	55	68	82	140
	spring	$4.0x10^3$	$6.1x10^3$	$8.6x10^3$	$1.1x10^4$	$1.6x10^4$
	summer	$9.8x10^3$	$1.5x10^4$	$2.2x10^4$	$2.8x10^4$	$4.2x10^4$
	fall	620	930	$1.3x10^3$	$1.7x10^3$	$2.5x10^3$
Northeast Atlantic	winter	1.4	1.4	1.4	1.4	1.5
	spring	90	140	200	270	510
	summer	460	670	930	$1.2x10^3$	$2.5x10^3$
	fall	21	25	30	35	52
Mid-Atlantic	winter	1.3	1.3	1.4	1.4	1.4
	spring	860	$1.3x10^3$	$1.9x10^3$	$2.5x10^3$	$4.2x10^3$
	summer	$2.4x10^3$	$3.7x10^3$	$5.2x10^3$	$6.9x10^3$	$1.2x10^4$
	fall	78	110	150	190	310
Pacific Northwest (dredged)	winter	0.007	0.008	0.008	0.008	0.008
	spring	1.5	2.2	3.1	4.3	9.1
	summer	14	21	32	44	100
	fall	0.10	0.12	0.15	0.17	0.23
Pacific Northwest (intertidal)	winter	0.016	0.016	0.017	0.017	0.017
	spring	110	130	140	150	150
	summer	$1.2x10^3$	$1.4x10^3$	$1.5x10^3$	$1.6x10^3$	$1.7x10^3$
	fall	3.6	3.6	4.0	4.2	3.9

[a]Levels of *V. parahaemolyticus* at-retail after cooling at various time intervals after harvest; values are rounded to 2 significant digits

Table A10-8. Best estimate of the 90th percentile of the distribution of total Vp/g at retail for all region/seasons

Region	Season	Maximum Time-to-Refrigeration				
		1 hr	2 hr	3 hr	4 hr	baseline
Gulf Louisiana	winter	48[a]	56	65	73	120
	spring	$4.3x10^3$	$7.3x10^3$	$1.2x10^4$	$1.8x10^4$	$4.7x10^4$
	summer	$1.9x10^4$	$3.4x10^4$	$5.5x10^4$	$8.3x10^4$	$2.0x10^5$
	fall	340	470	650	880	$2.8x10^3$
Gulf non-Louisiana	winter	48	56	63	70	84
	spring	$4.3x10^3$	$7.2x10^3$	$1.1x10^4$	$1.6x10^4$	$2.6x10^4$
	summer	$1.9x10^4$	$3.3x10^4$	$5.3x10^4$	$7.5x10^4$	$1.3x10^5$
	fall	340	470	610	760	$1.0x10^3$
Northeast Atlantic	winter	2.3	2.3	2.3	2.3	2.3
	spring	45	57	68	80	100
	summer	590	840	$1.1x10^3$	$1.5x10^3$	$2.5x10^3$
	fall	22	23	25	26	28
Mid-Atlantic	winter	2.1	2.2	2.2	2.2	2.2
	spring	330	480	650	830	$1.3x10^3$
	summer	$3.3x10^3$	$5.3x10^3$	$7.9x10^3$	$1.1x10^4$	$2.2x10^4$
	fall	31	35	39	42	48
Pacific Northwest (dredged)	winter	0.016	0.016	0.016	0.016	0.017
	spring	1.2	1.4	1.7	2.0	2.6
	summer	12	17	22	27	40
	fall	0.12	0.13	0.13	0.14	0.15
Pacific Northwest (intertidal)	winter	0.029	0.029	0.029	0.029	0.030
	spring	21	24	26	27	27
	summer	550	650	730	780	800
	fall	0.49	0.49	0.50	0.51	0.51

[a]Levels of *V. parahaemolyticus* at-retail after cooling at various time intervals after harvest; values are rounded to 2 significant digits

Effect of Limiting Time to Refrigeration (Conventional Cooling and Rapid Cooling on Ice) on Average Levels of Total Vp/g at Retail (Point of Consumption)

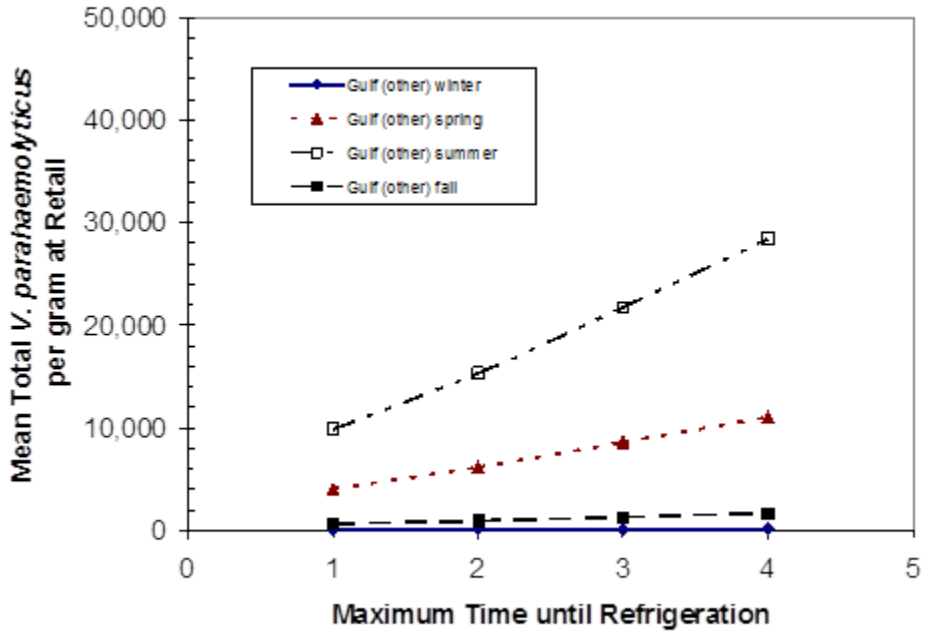


Figure A10-1. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Non- Louisiana Harvest).

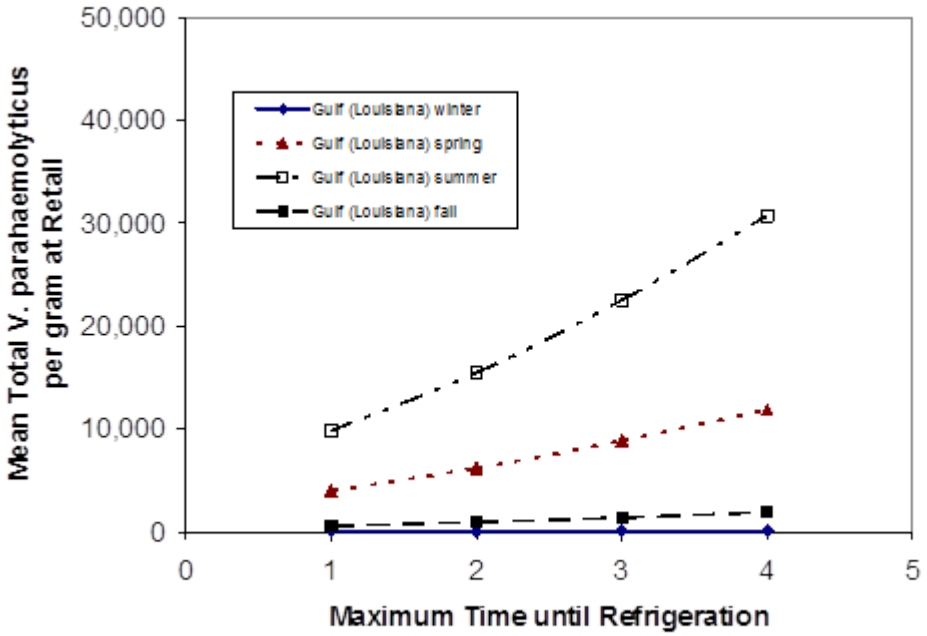


Figure A10-2. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest).

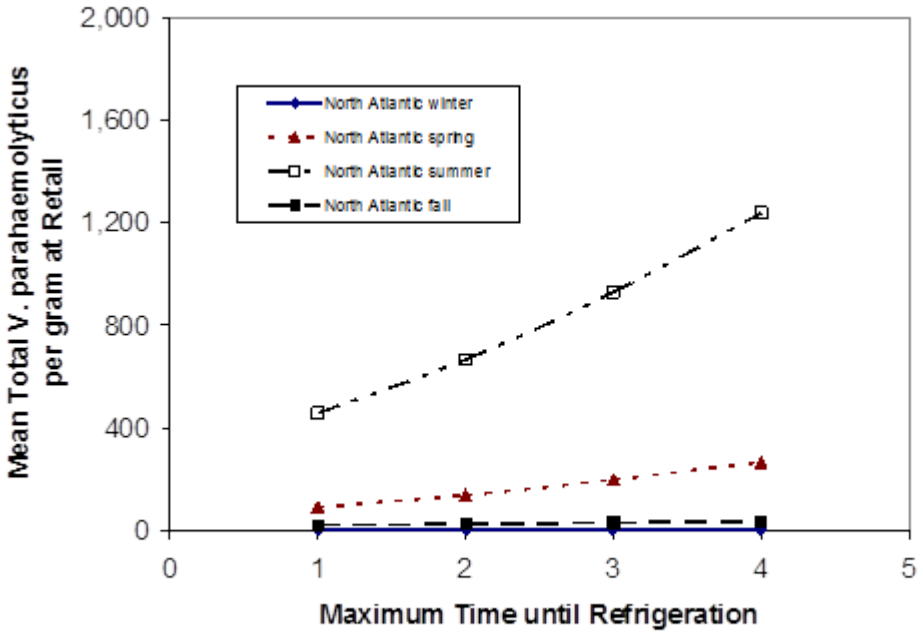


Figure A10-3. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).

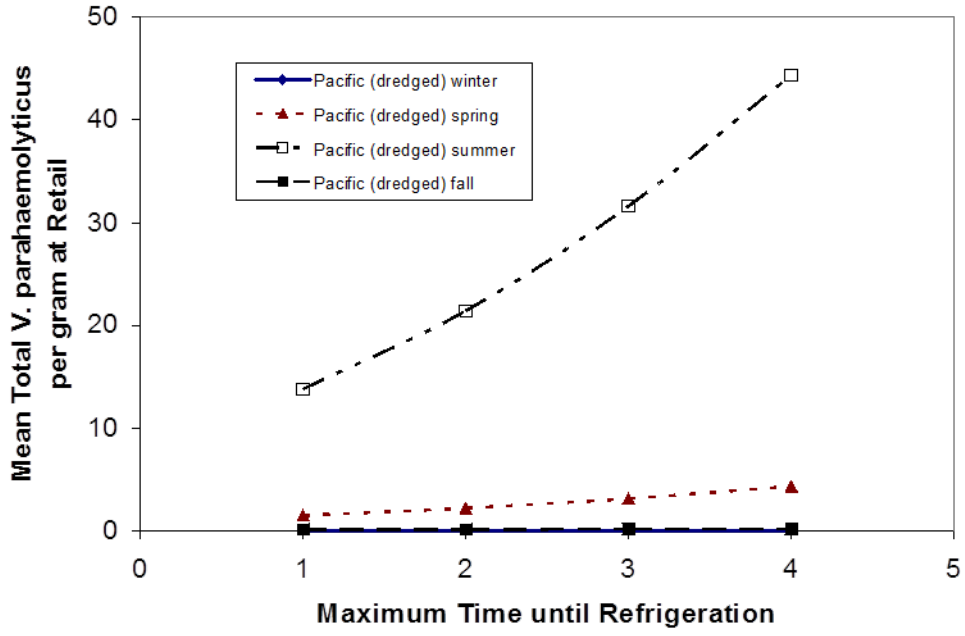


Figure A10-4. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Mid-Atlantic Harvest)

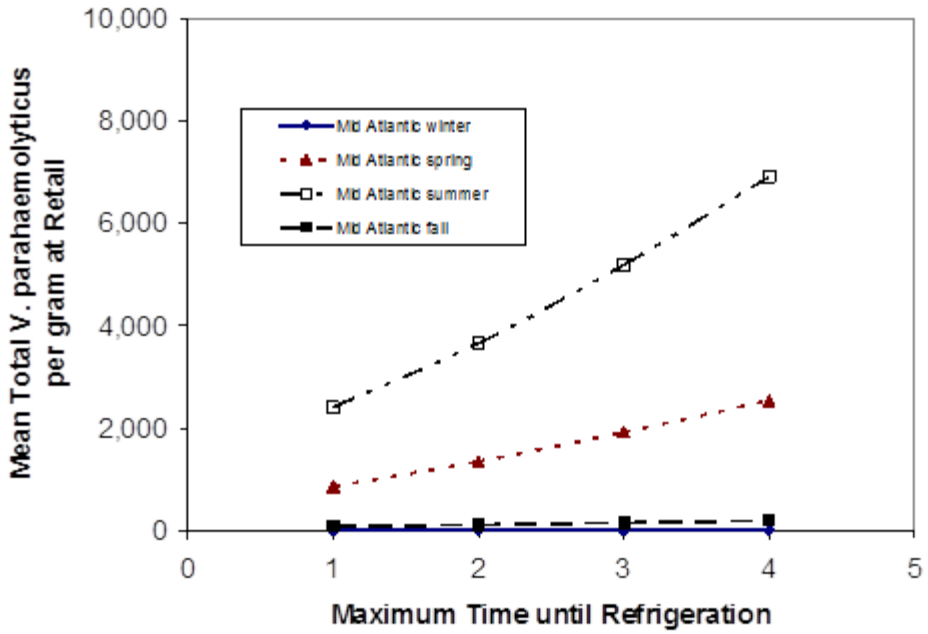


Figure A10-5. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Dredged Harvest).

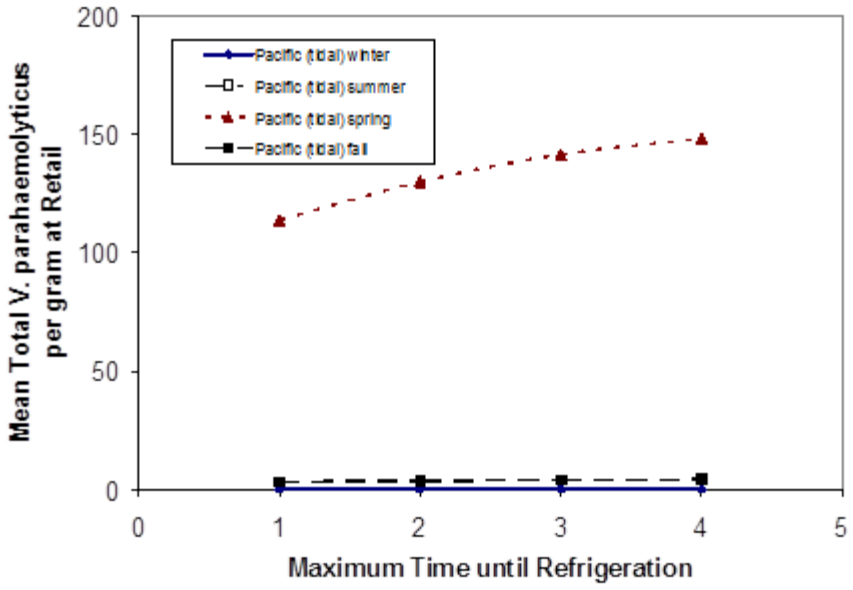


Figure A10-6. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Intertidal Harvest).

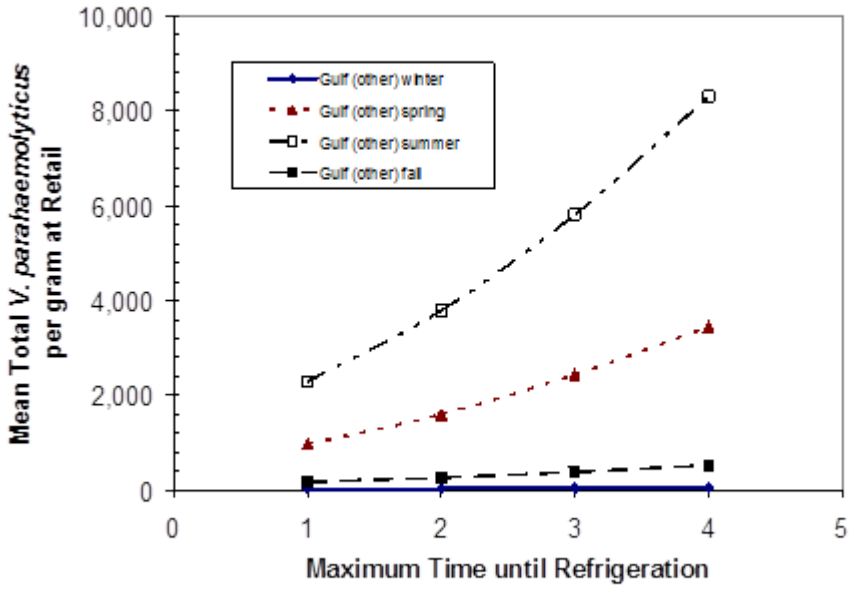


Figure A10-7. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Non-Louisiana Harvest).

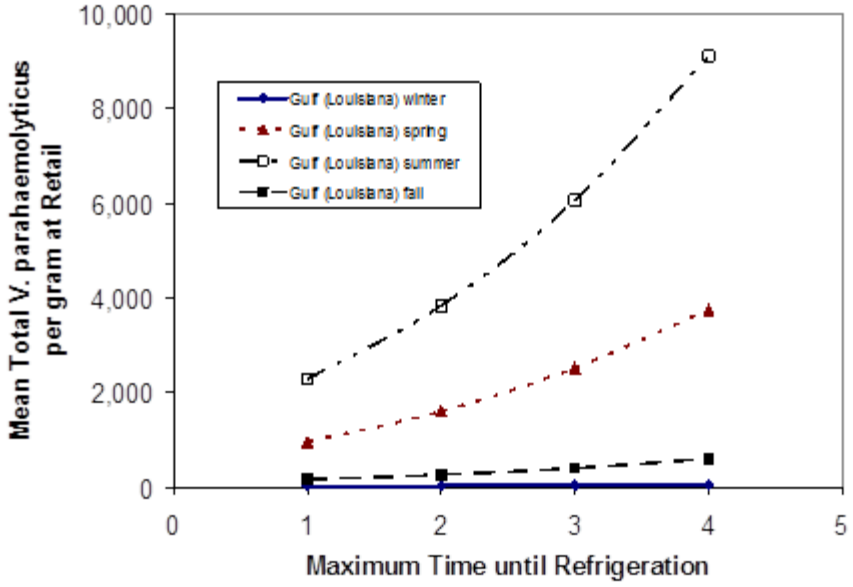


Figure A10-8. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest).

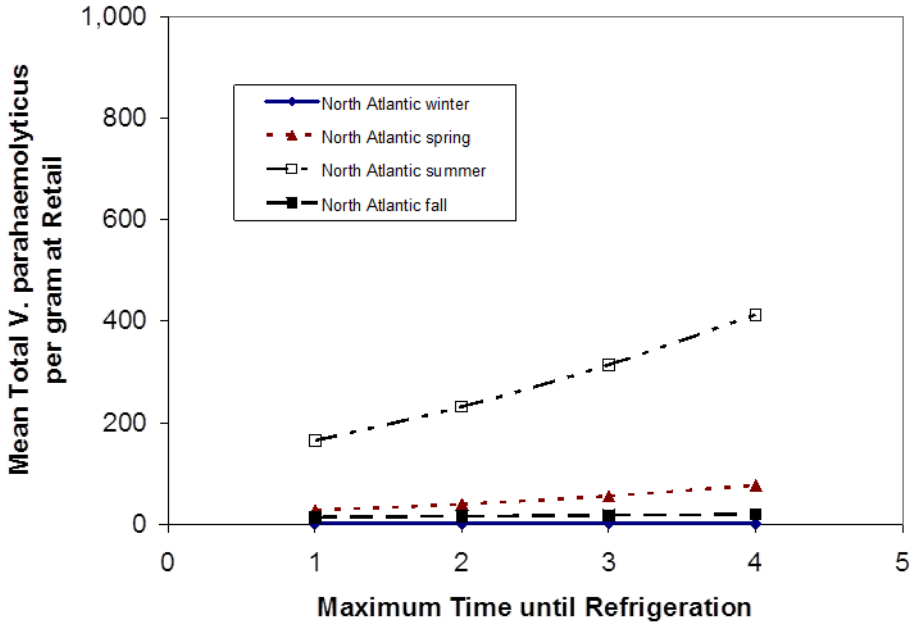


Figure A10-9. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).

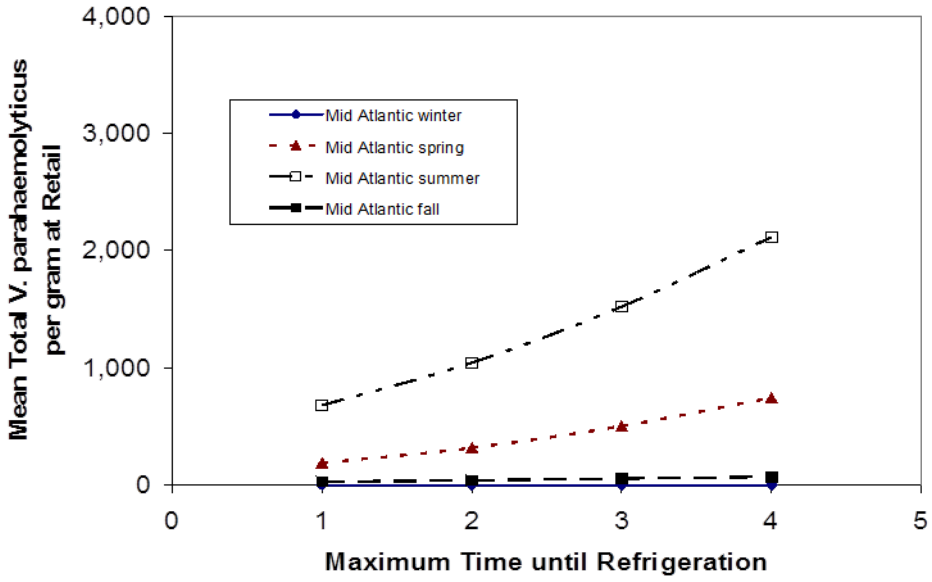


Figure A10-10. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Mid-Atlantic Harvest).

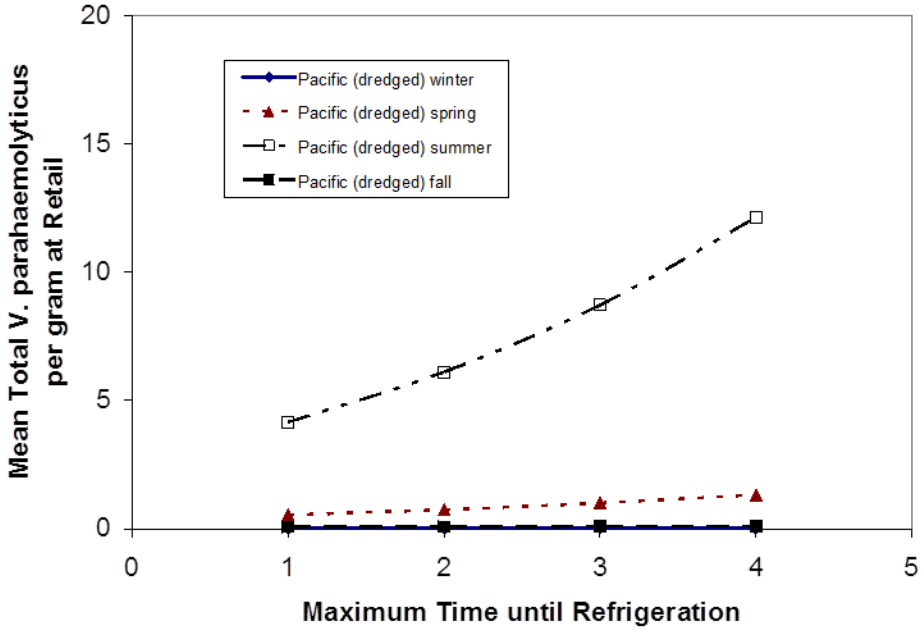


Figure A10-11. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Pacific Northwest Dredged Harvest).

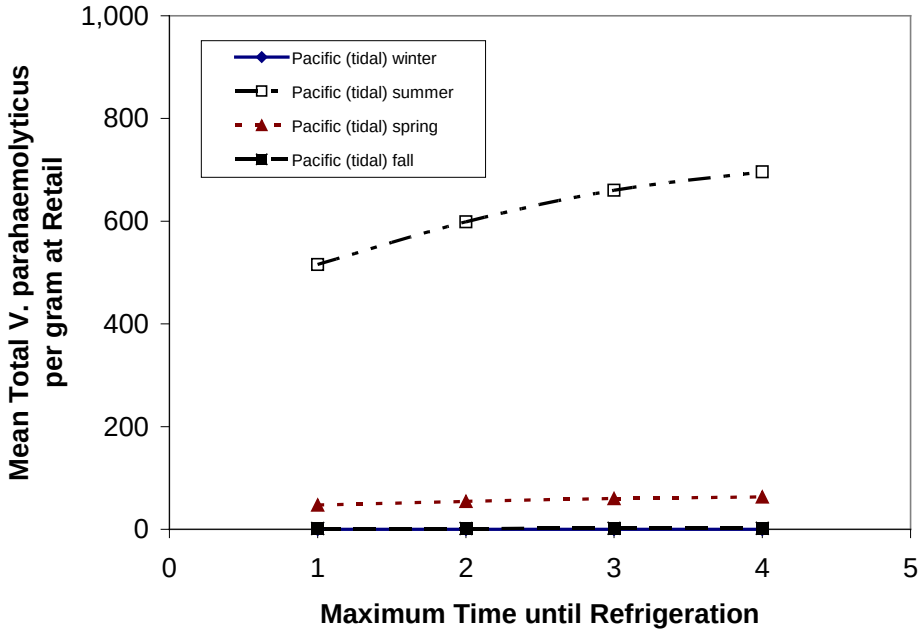


Figure A10-12. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Pacific Northwest Intertidal Harvest).

Figures on Effect of Limiting Time to Refrigeration (conventional cooling and rapid cooling) on the 90th percentile of the distribution of total *V. parahaemolyticus*/g at retail (point of consumption)

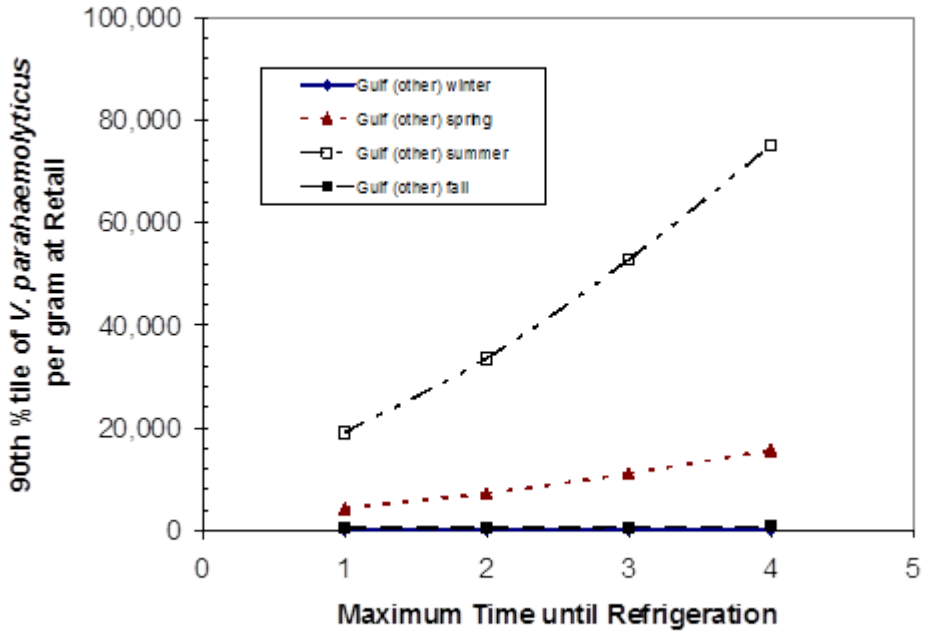


Figure A10-13. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Non- Louisiana Harvest).

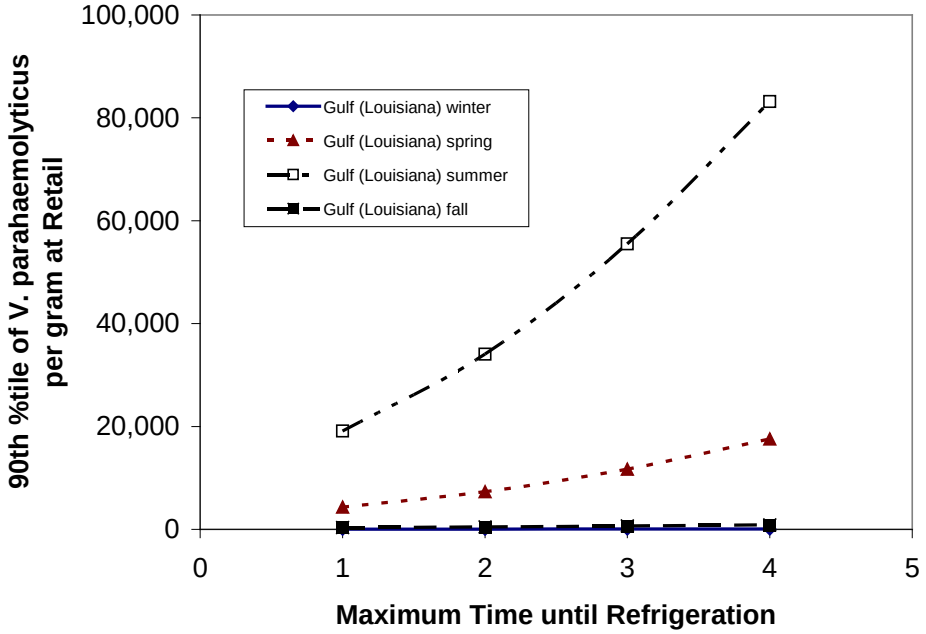


Figure A10-14. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest).

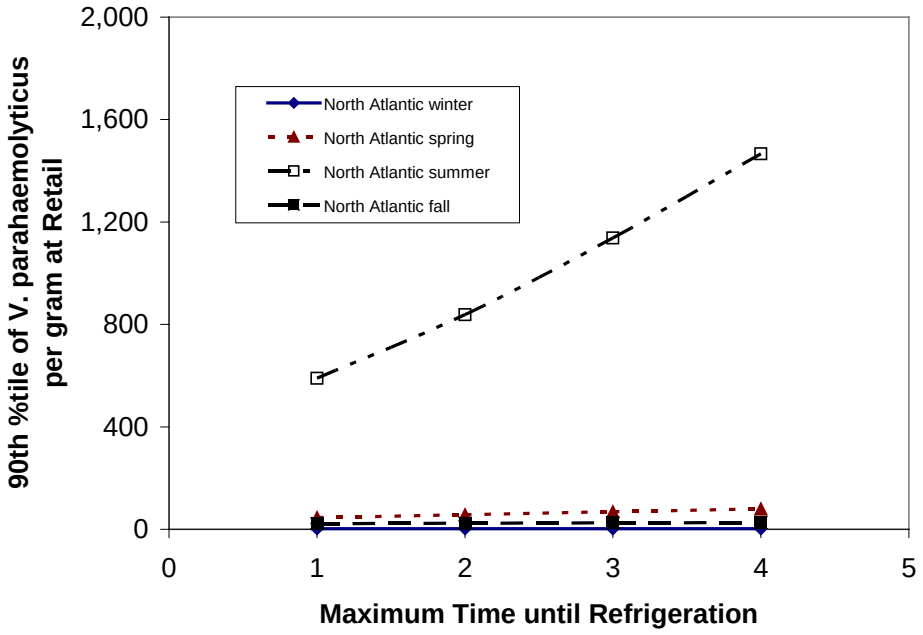


Figure A10-15. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).

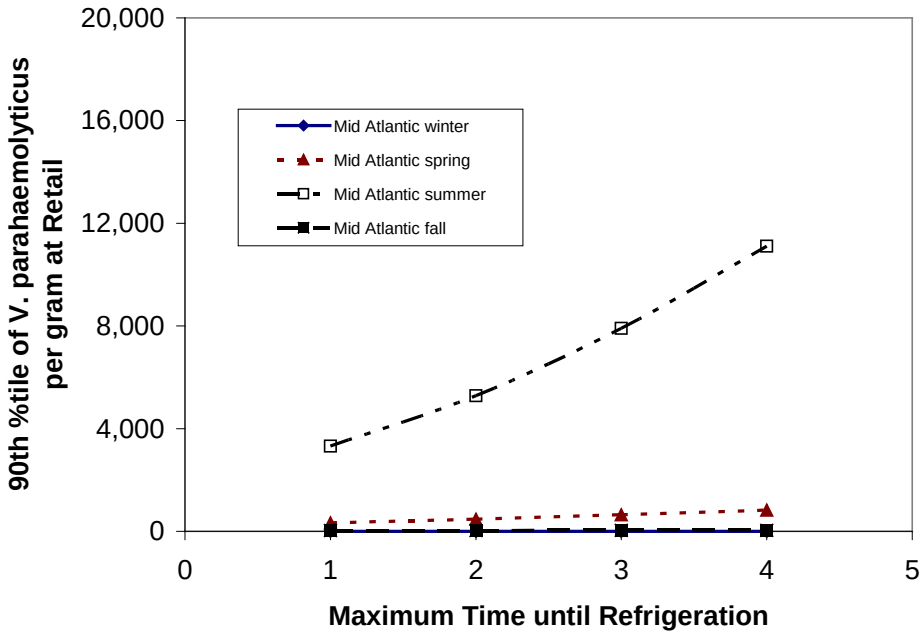


Figure A10-16. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Mid-Atlantic Harvest).

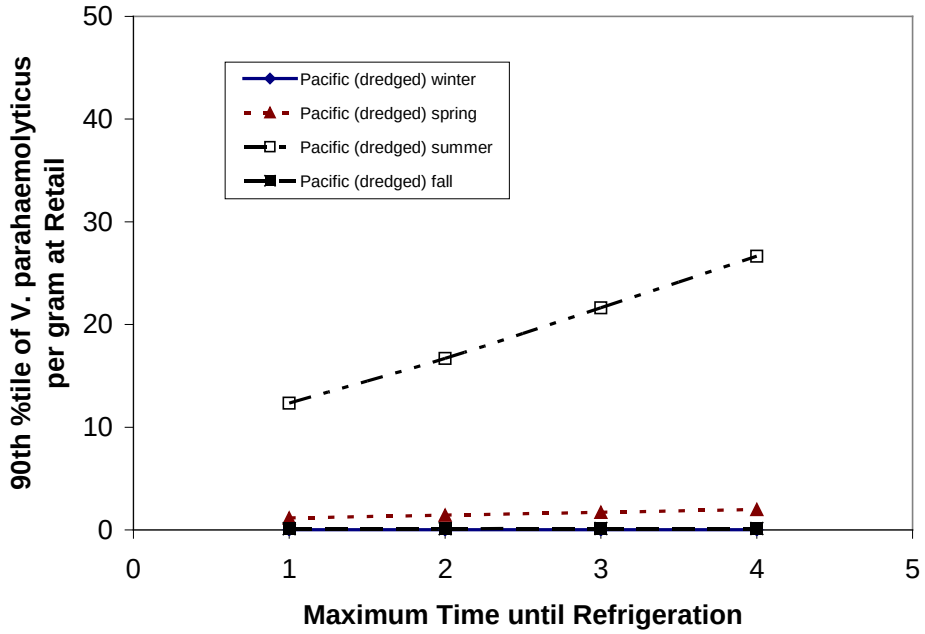


Figure A10-17. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Dredged Harvest).

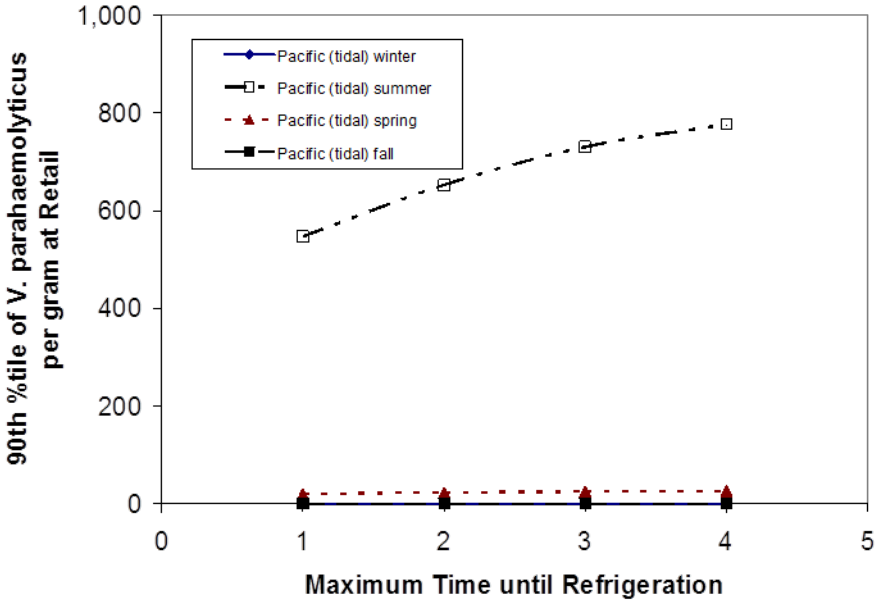


Figure A10-18. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Intertidal Harvest).

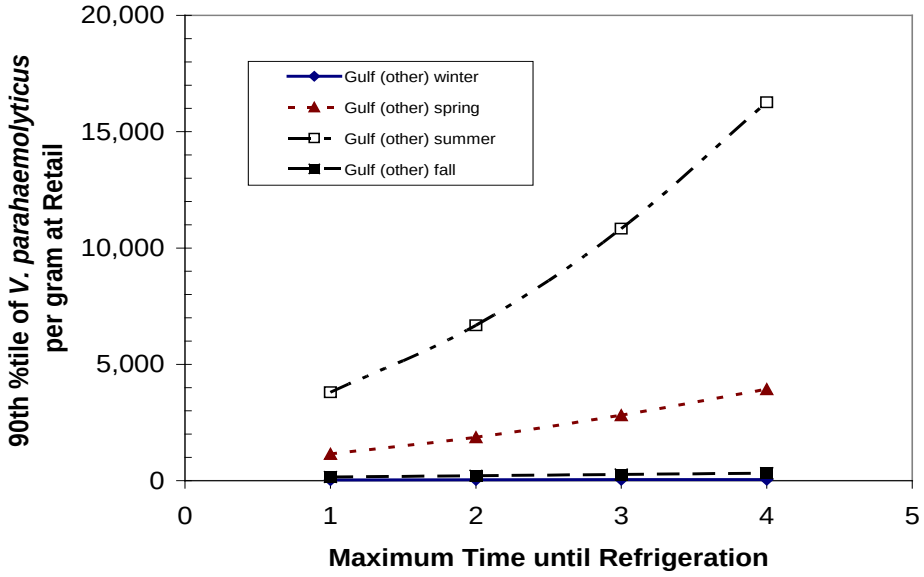


Figure A10-19. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Non-Louisiana Harvest).

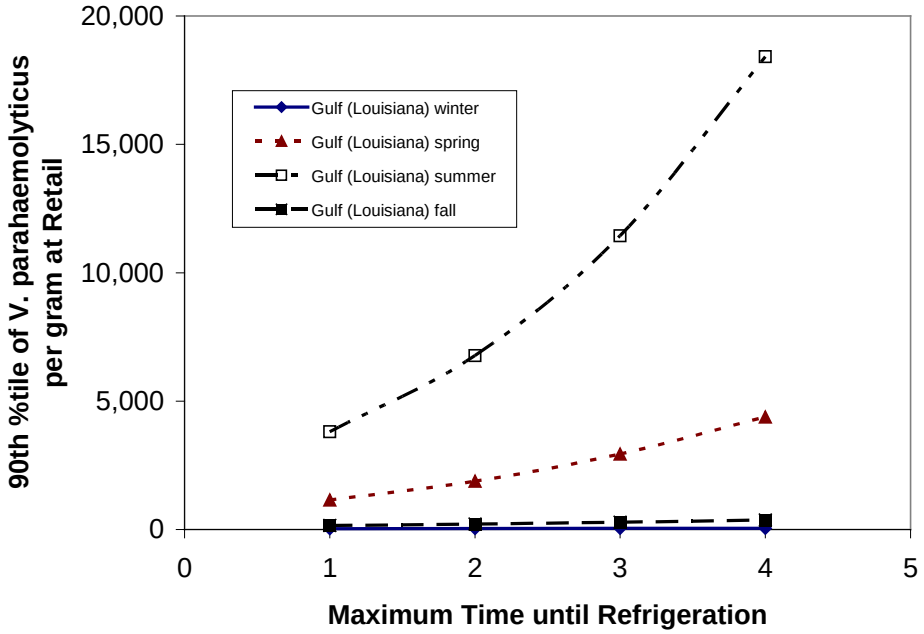


Figure A10-20. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest).

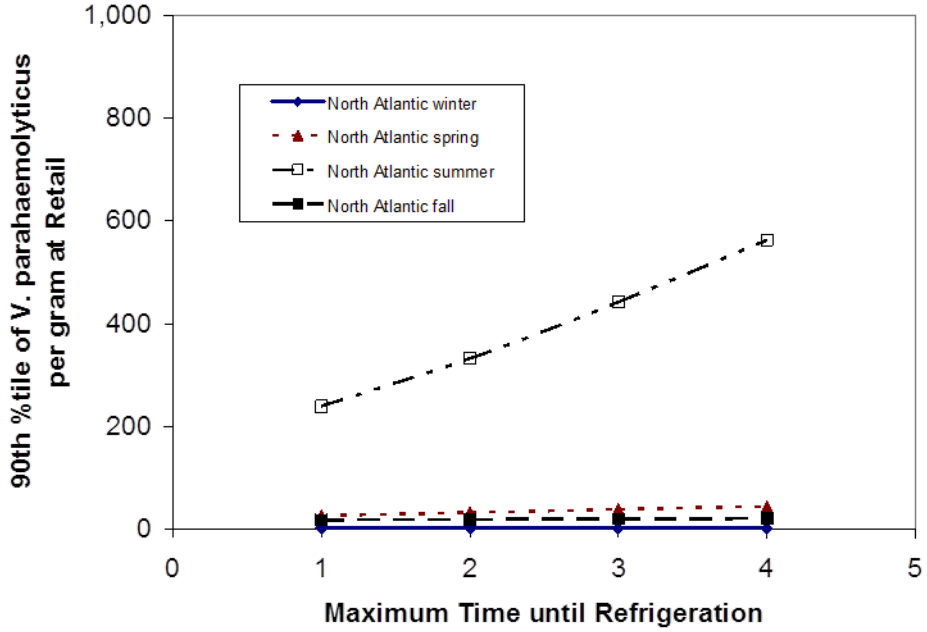


Figure A10-21. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).

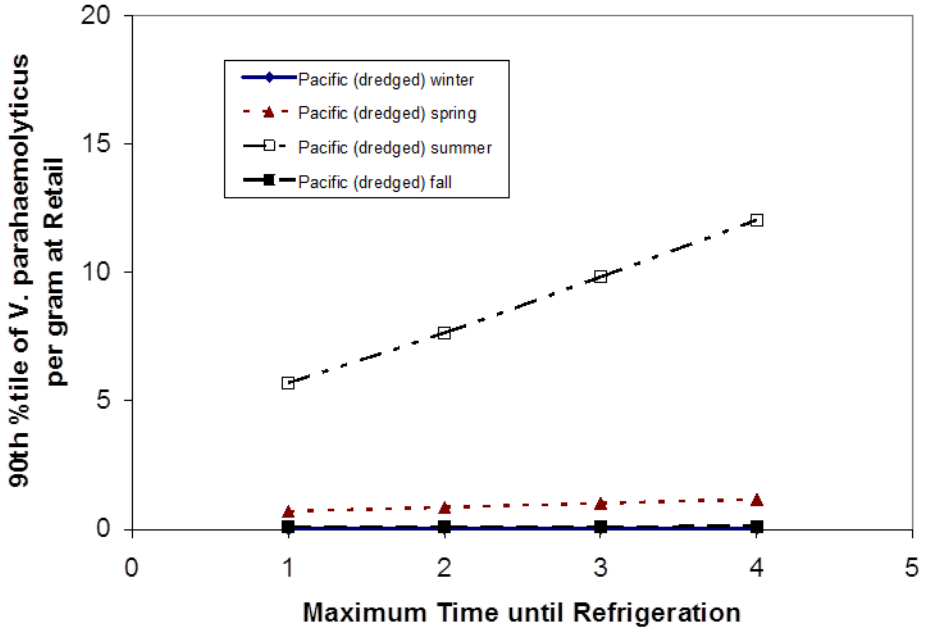


Figure A10-22. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Mid-Atlantic Harvest).

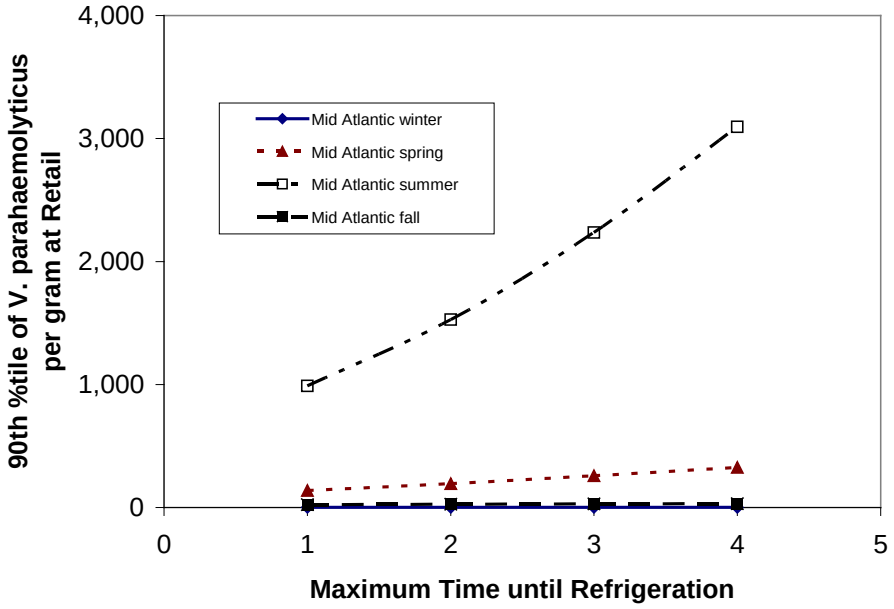


Figure A10-23. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Pacific Northwest Dredged Harvest).

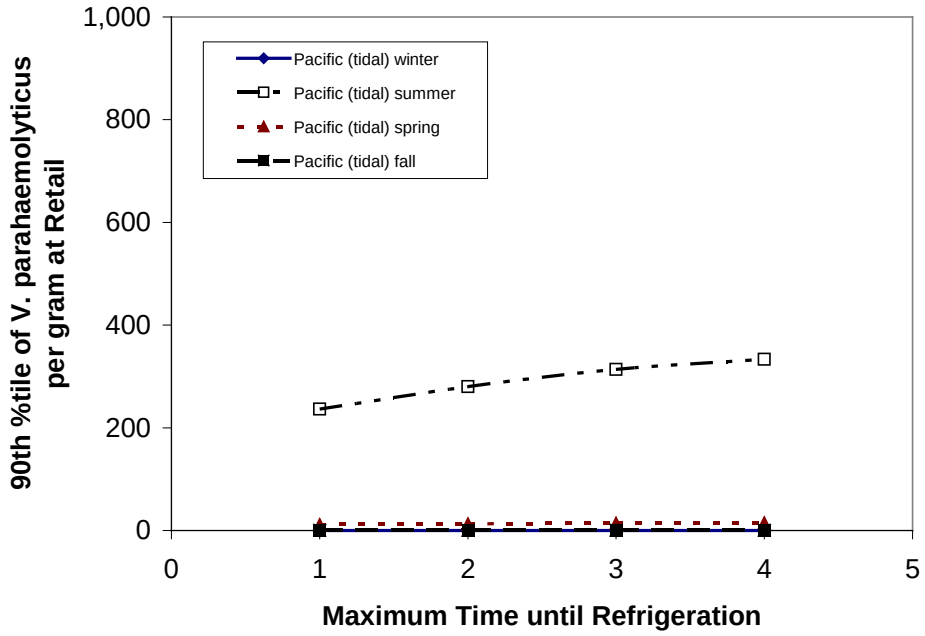


Figure A10-24. Predicted Effect of Maximum Time to Refrigeration with Rapid (on (on ice) Cooling of Oyster Shellstock (Pacific Northwest Intertidal Harvest).

Table A10-9 shows the impact of rapid cooling on ice on reducing the levels of *V. parahaemolyticus* with the corresponding decrease in risk per serving.

Table A10-9. Percentage Reduction of *Vibrio parahaemolyticus* /g versus Risk after Immediate Refrigeration with Icing for the Gulf Coast (Louisiana) Summer Harvest

Time-to-Refrigeration (h)	% reduction of total Vp/g	% reduction of risk per serving
1	96.2%	96.5%
2	93.6%	94.1%
3	89.9%	90.7%
4	84.8%	85.9%

Figures on Effect of Limiting Time to Refrigeration (conventional cooling and rapid cooling) on the Reduction of Risk per Serving

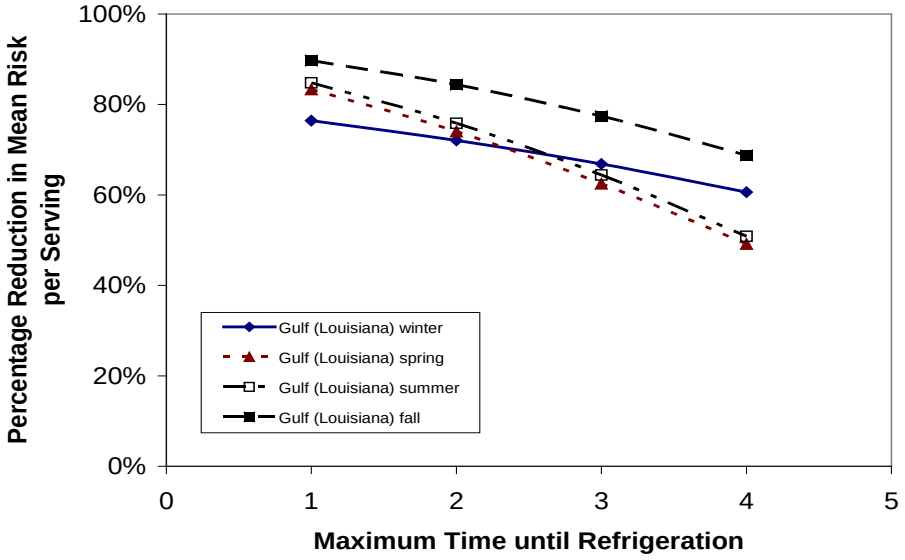


Figure A10-25. Predicted Effect of Maximum Time-to-refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest.

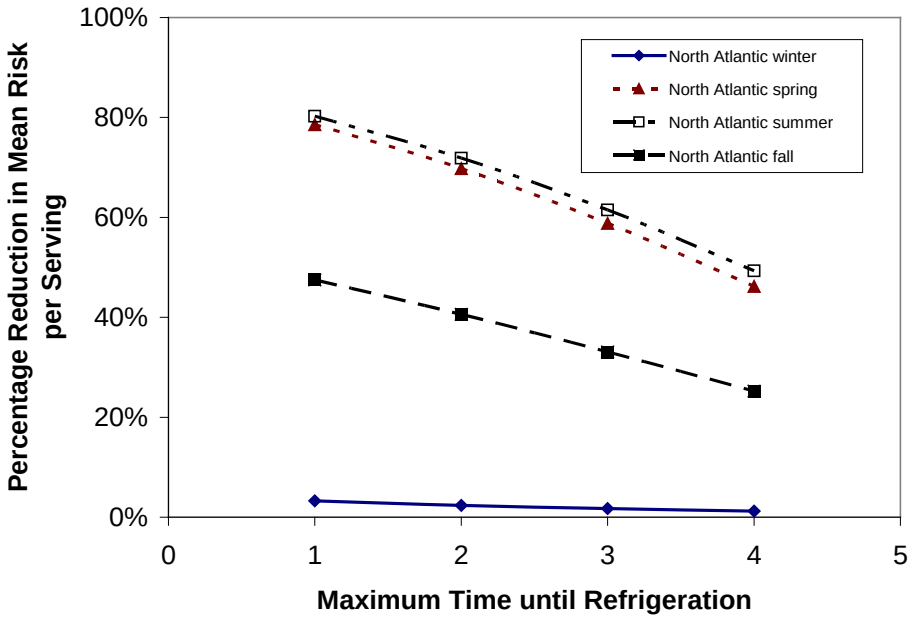


Figure A10-26. Predicted Effect of Maximum Time-to-refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).

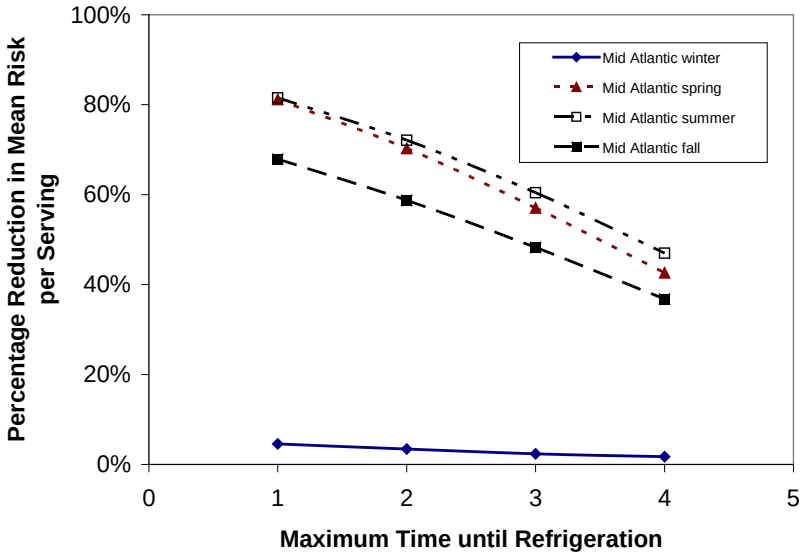


Figure A10-27. Predicted Effect of Maximum Time-to-refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Mid-Atlantic Harvest).

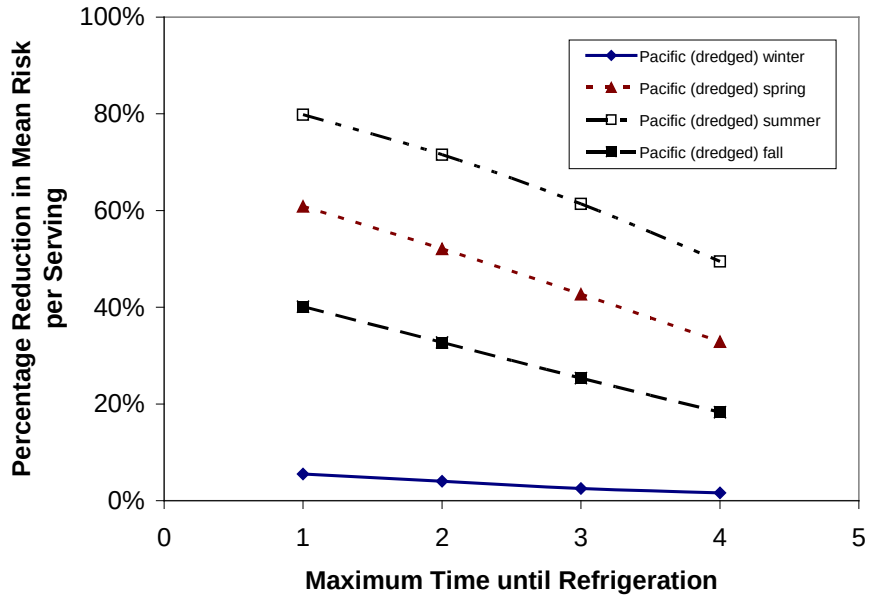


Figure A10-28. Predicted Effect of Maximum Time-to-refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Dredged Harvest).

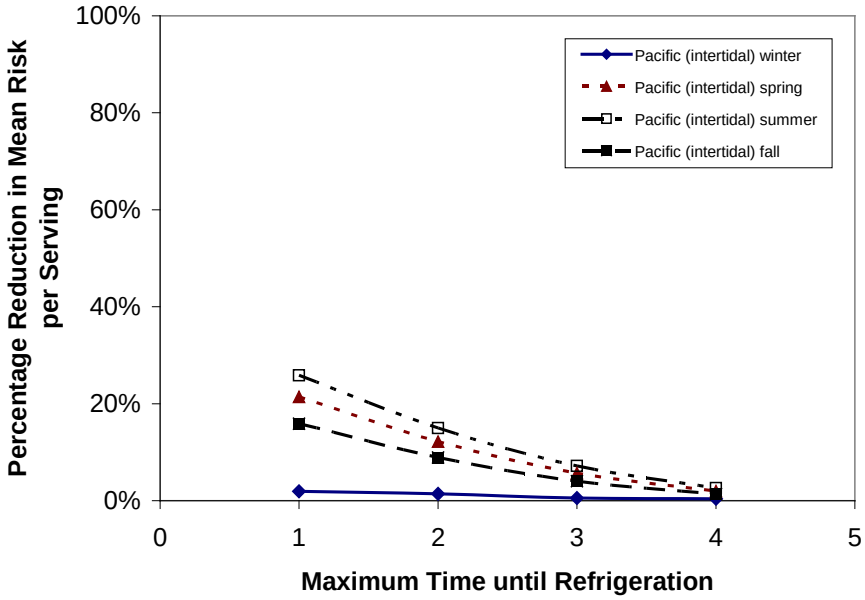


Figure A10-29. Predicted Effect of Maximum Time-to-refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Intertidal Harvest).

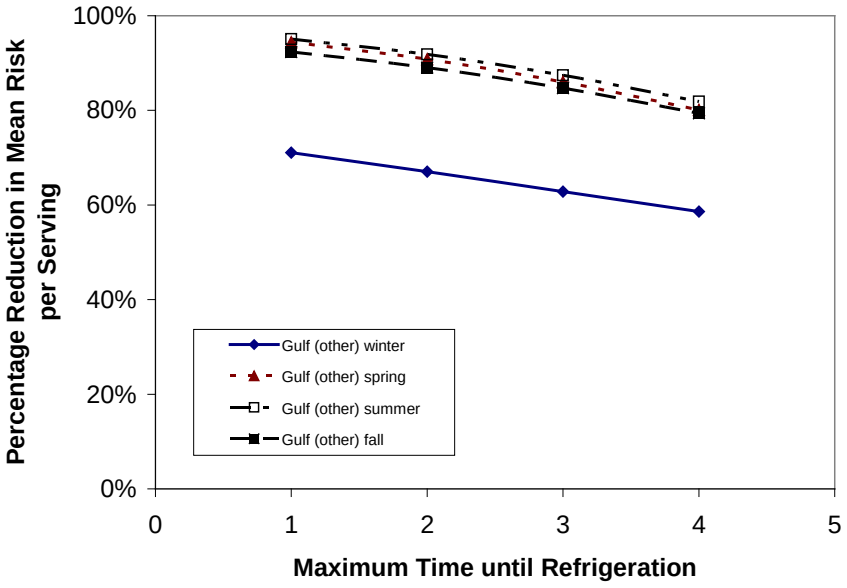


Figure A10-30. Predicted Effect of Maximum Time-to-refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Non-Louisiana Harvest).

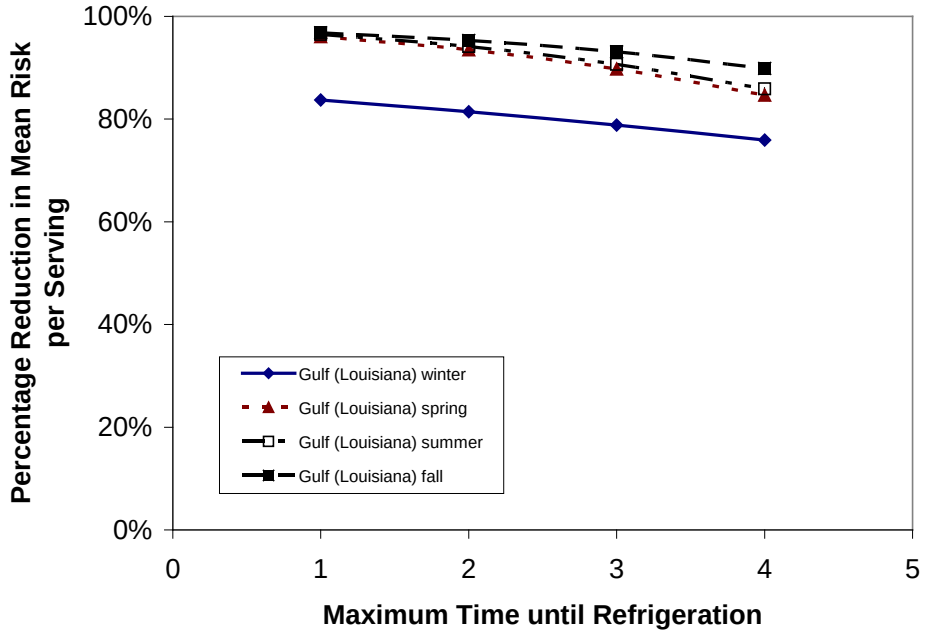


Figure A10-31. Predicted Effect of Maximum Time-to-refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest).

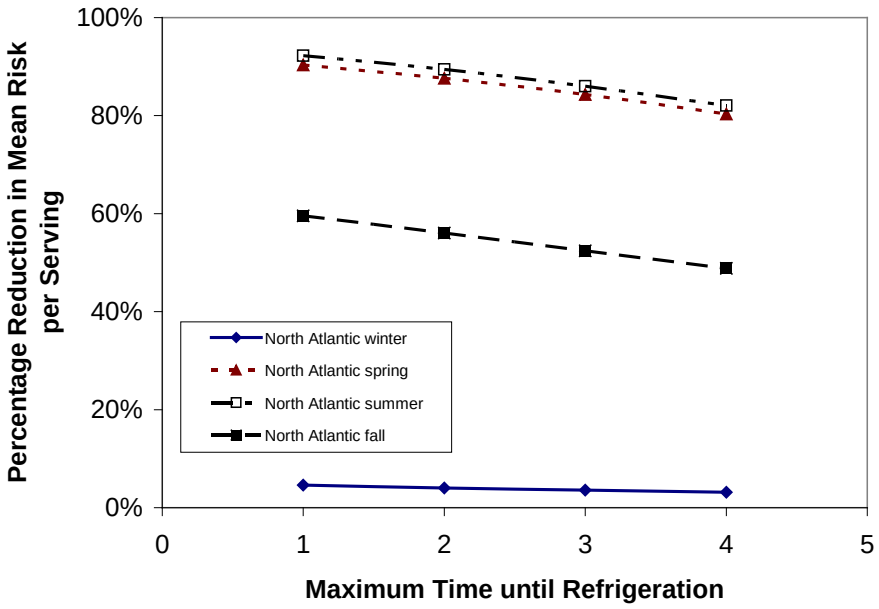


Figure A10-32. Predicted Effect of Maximum Time-to-refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).

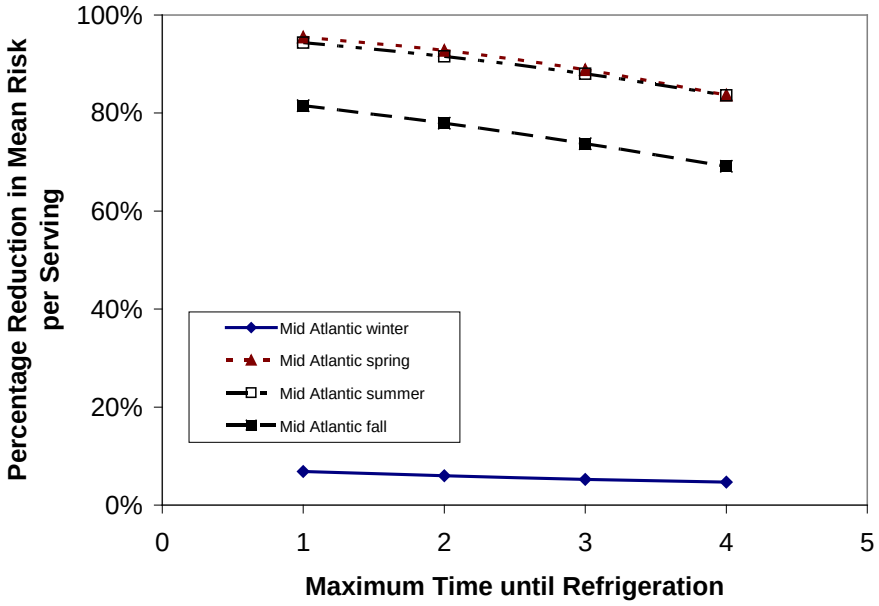


Figure A10-33. Predicted Effect of Maximum Time-to-refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Mid-Atlantic Harvest).

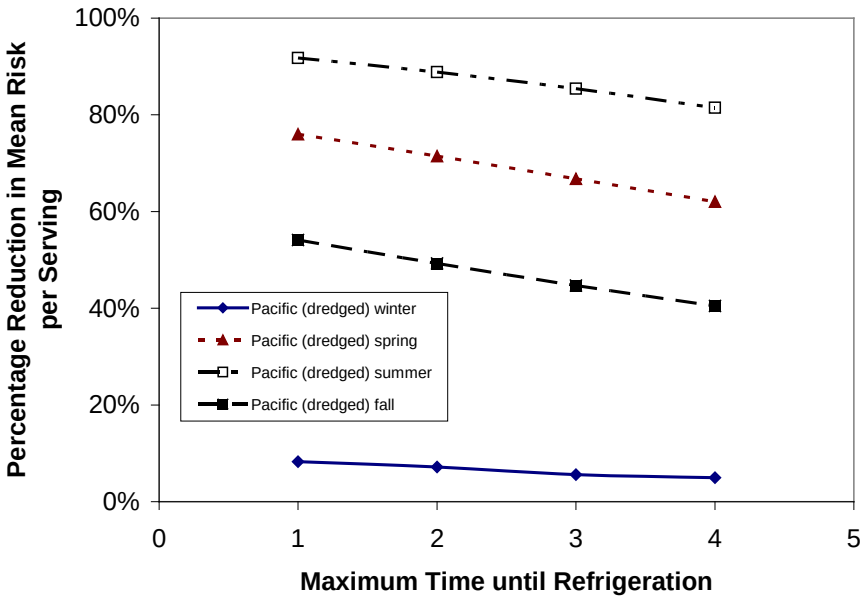


Figure A10-34. Predicted Effect of Maximum Time-to-refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Pacific Northwest Dredged Harvest).

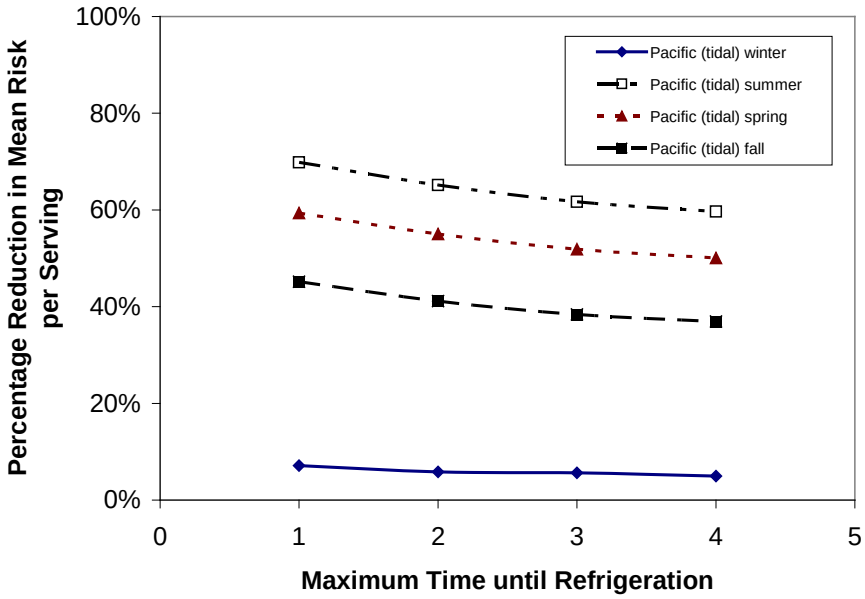


Figure A10-35. Predicted Effect of Maximum Time-to-refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Pacific Northwest Intertidal Harvest).

Comparison on Impact of Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock on Reduction of Mean Risk Per Serving

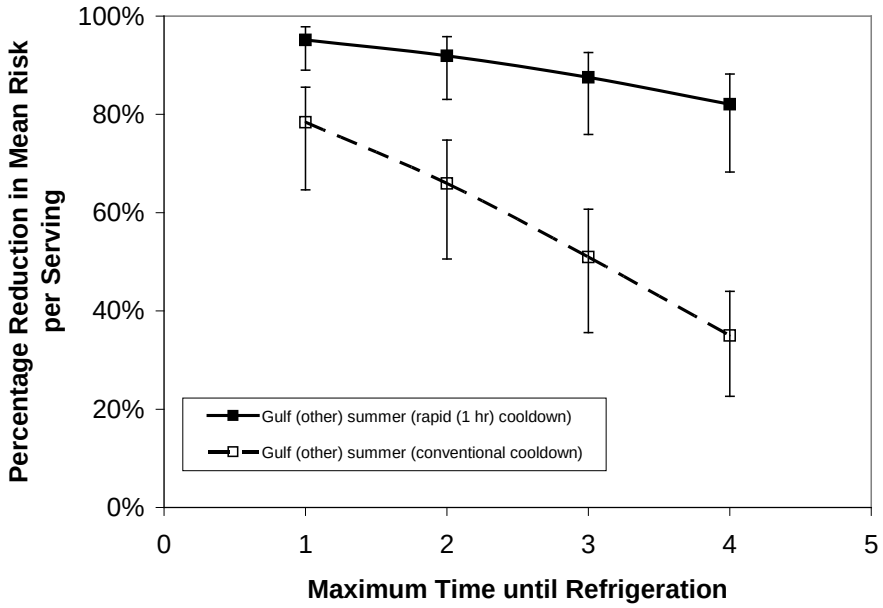


Figure A10-36. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Non-Louisiana Summer Harvest).

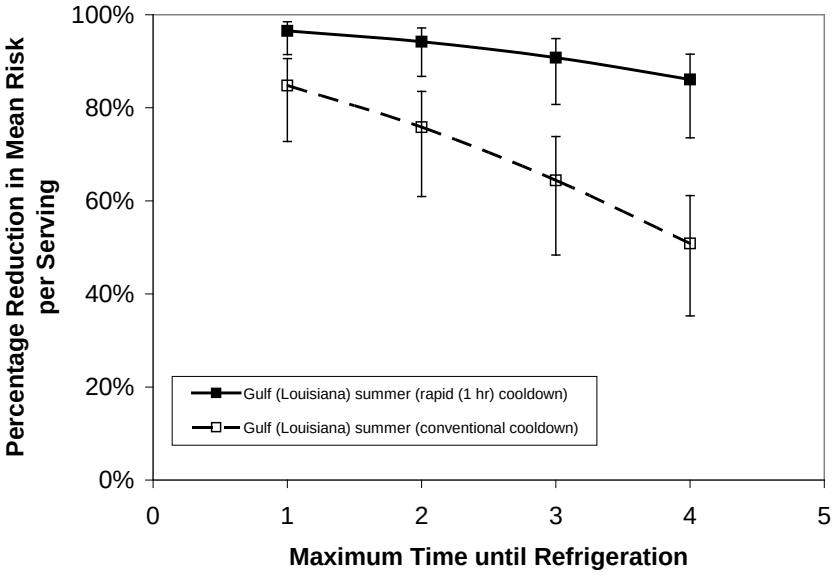


Figure A10-37. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Suumer Harvest).

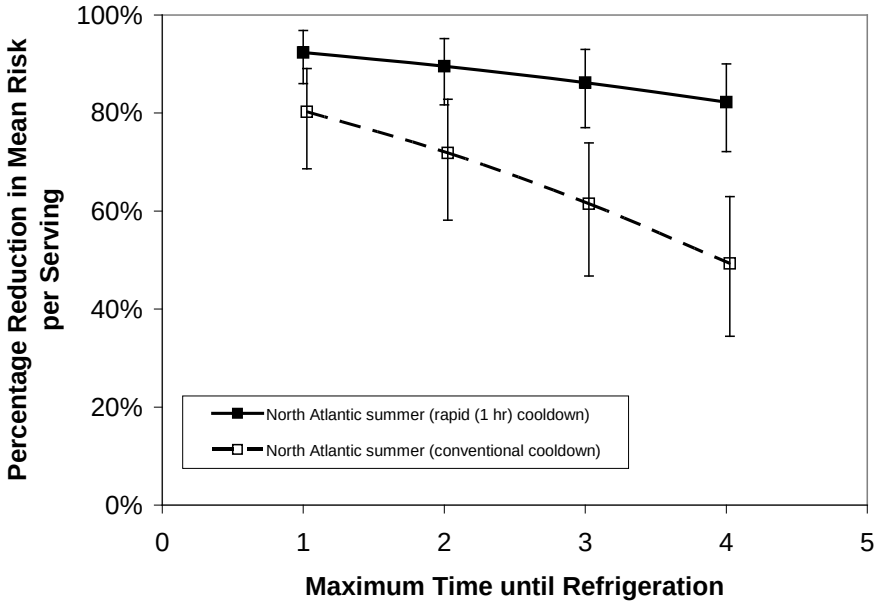


Figure A10-38. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Northeast Atlantic Summer Harvest).

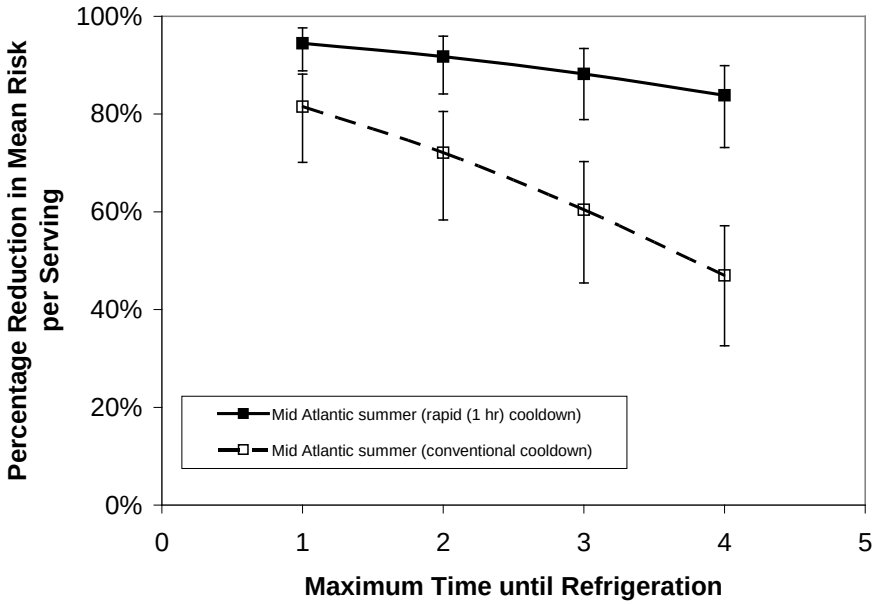


Figure A10-39. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Mid-Atlantic Summer Harvest).

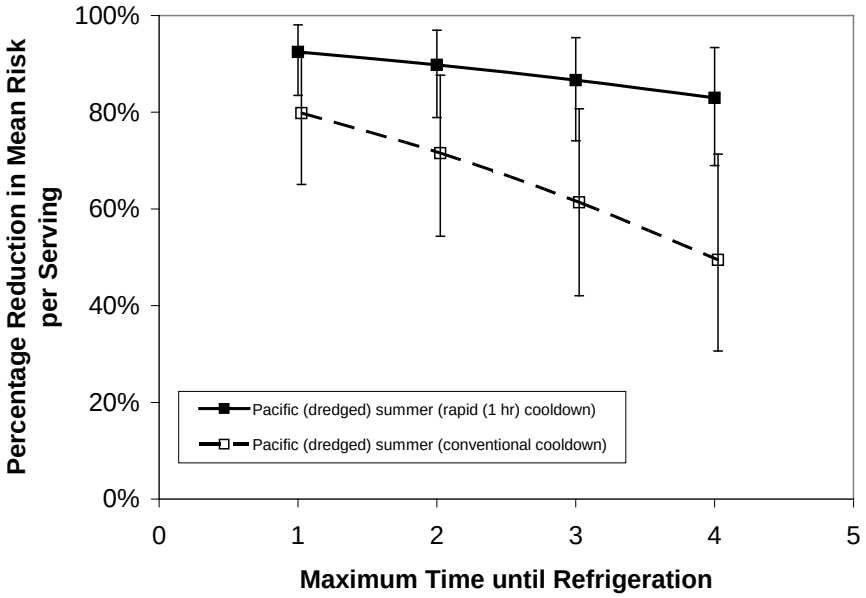


Figure A10-40. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Summer Dredged Harvest).

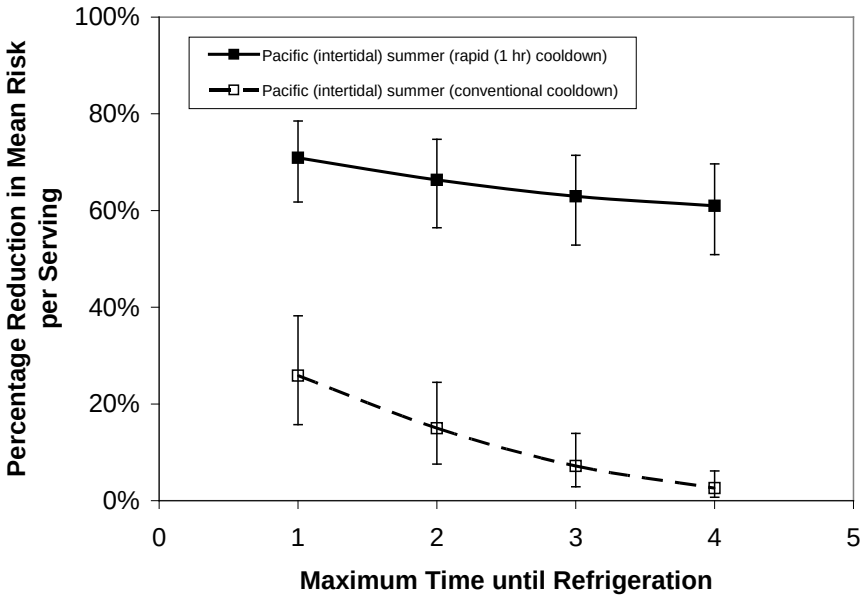


Figure A10-41. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Summer Intertidal Harvest).

Effect of Deviation from Compliance on "At-Harvest" Guidance Levels Scenarios

The impact on illness and effect on harvest at different *V. parahaemolyticus* guidance levels for "at harvest" control was evaluated in Chapter VI of the technical document. It was recognized that deviation from compliance with these harvest guidance levels can occur in any region and season. The Louisiana Gulf Coast Summer harvest was selected as the region/season combination for illustrative example because the Gulf has the highest summer temperatures and Louisiana has the longest potential time for having oysters out of the water.

Selected levels of deviation from compliance (ranging from 0 to 50%) with different guidance levels (ranging from 100 to 100,000/g) were evaluated. The analyses were accomplished by altering the baseline model to represent the potential effect of the different levels of deviation from compliance. In other words, the impact of the different guidance levels determined in the above evaluation of the 10,000 *V. parahaemolyticus*/g was used as the 100% compliance (or 0% deviation from compliance) control and the outcome when 0, 10, 30, or 50% of the oysters containing more *V. parahaemolyticus*/g than the guidance level in question were allowed to reach the consumer. As seen in Table A10-10, the lower the standard level in question, the greater the impact of deviation from compliance on both percentage illnesses averted and loss of oyster harvest. At an "at-harvest" guidance level of 100 *V. parahaemolyticus*/g, a 30% deviation from compliance only reduces illness by 82% as compared to the 98% reduction predicted if 100% compliance were met.

At 10,000 and 100,000 *V. parahaemolyticus*/g the differences in illness reduction between 100% compliance and 70% compliance are not large. Therefore, as demonstrated in Figures A10-42 to A10-46, as the level of the microbiological criterion increases, the impact of compliance is less important. Conversely, strict microbiological criteria must be matched with a high level of compliance if they are to be effective.

Table A10-10. Effect of Compliance Levels on the Effectiveness of Controlling Total *Vibrio parahaemolyticus* in Oysters at the Time of Harvest for Gulf Coast Louisiana Summer

Total Vp/g At Time of Harvest[a]	Compliance Level [b]	Reduction in Mean Risk per Serving (%)	Harvest Diverted (%)[c]	Illness Averted (%) [d]
100/g	50%	47.7%	33.0%	64.9%
	70%	66.7%	46.2%	82.1%
	90%	85.7%	59.4%	94.2%
	100%	95.3%	66.0%	98.4%
1000/g	50%	29.6%	10.6%	37.3%
	70%	41.3%	14.9%	50.4%
	90%	53.0%	19.1%	62.6%
	100%	58.9%	21.3%	68.2%
5000/g	50%	11.4%	2.8%	14.4%
	70%	15.9%	3.9%	19.9%
	90%	20.4%	5.1%	25.4%

Total Vp/g At Time of Harvest[a]	Compliance Level [b]	Reduction in Mean Risk per Serving (%)	Harvest Diverted (%)[c]	Illness Averted (%) [d]
	100%	22.7%	5.6%	28.1%
10,000/g	50%	6.4%	1.4%	8.2%
	70%	8.9%	2.0%	11.4%
	90%	11.4%	2.6%	14.6%
	100%	12.7%	2.9%	16.2%
100,000/g	50%	0.57%	0.12%	0.79%
	70%	0.77%	0.17%	1.11%
	90%	0.99%	0.22%	1.43%
	100%	1.10%	0.25%	1.58%

[a] Assumes that the level of *Vibrio parahaemolyticus* (Vp) is known in oysters at the time of harvest.
[b] The compliance level is the percentage oyster harvest, which is removed form the raw oyster consumption market; this percentage is assumed to have the same distribution of Vp/g as under the baseline (no mitigation) scenario.
[c] Refers to the harvest that would need to be diverted from the "raw market."
[d] Assuming that the volume of product available for raw consumption is impacted (i.e., reduced) according to the estimate of the % of harvest lost from the raw market.

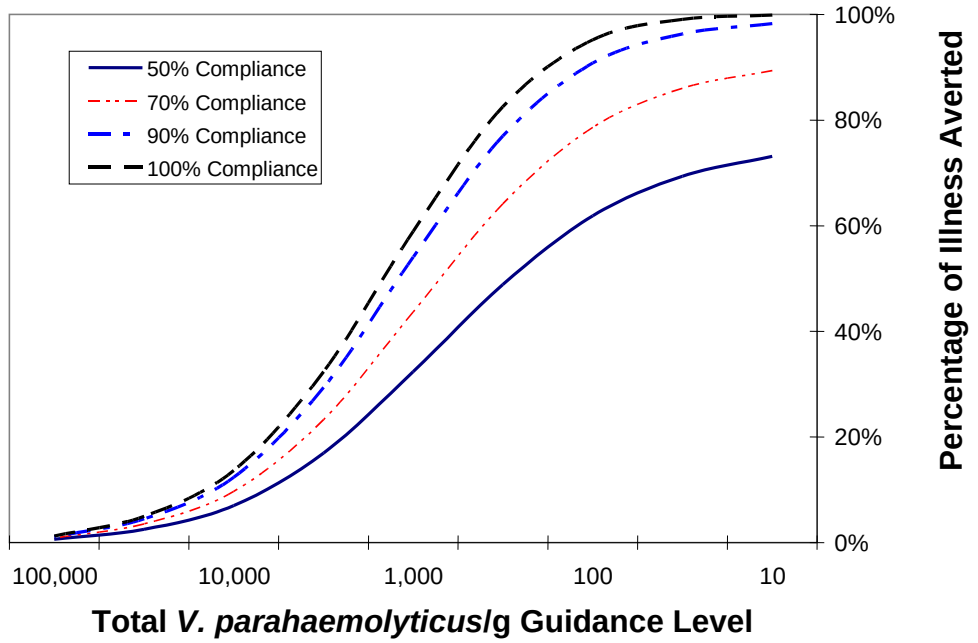


Figure A10-42. Percentage of Illnesses Averted

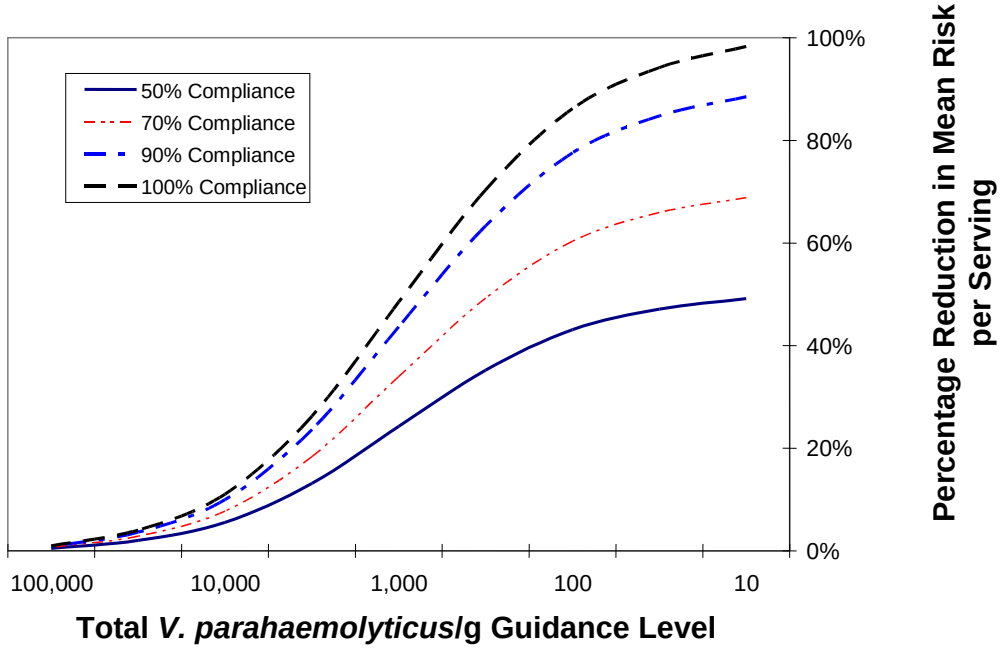


Figure A10-43. Percentage Reduction in Mean Risk per Serving

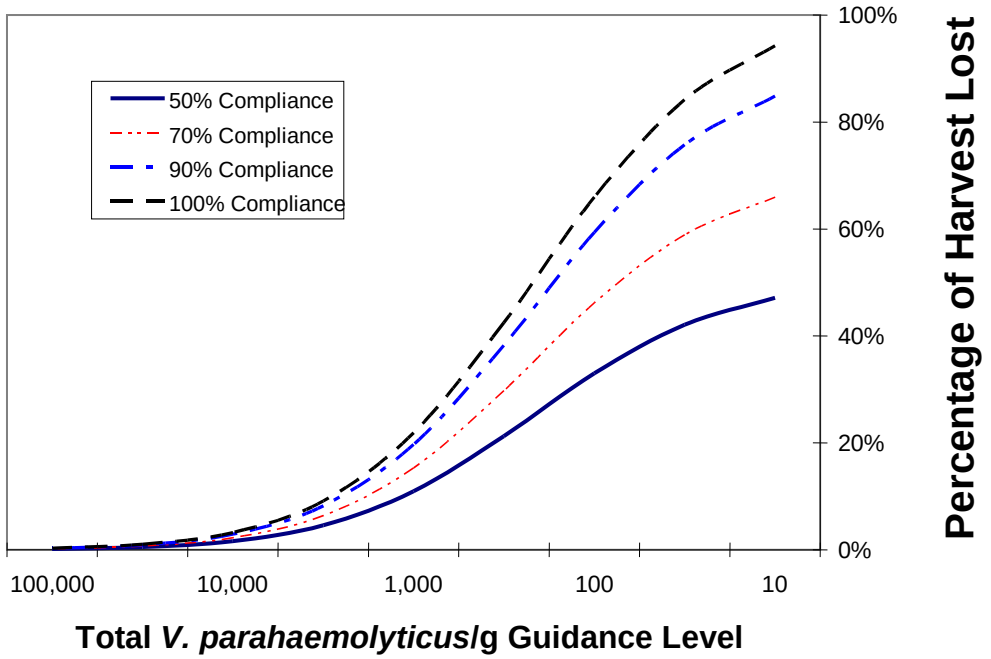


Figure A10-44. Percentage of Oyster Harvest Diverted from the "Raw" Market or Subjected to Preventive Controls.

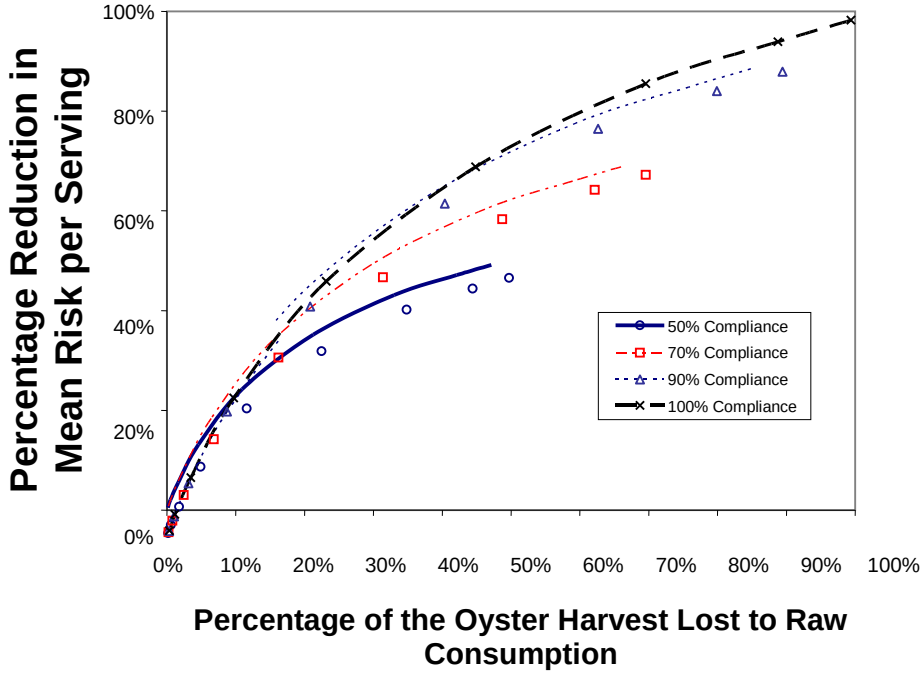


Figure A10-45. Percentage Reduction in Mean Risk per Serving versus Percentage of Harvest Diverted from the "Raw Market or Subjected to Preventive Controls

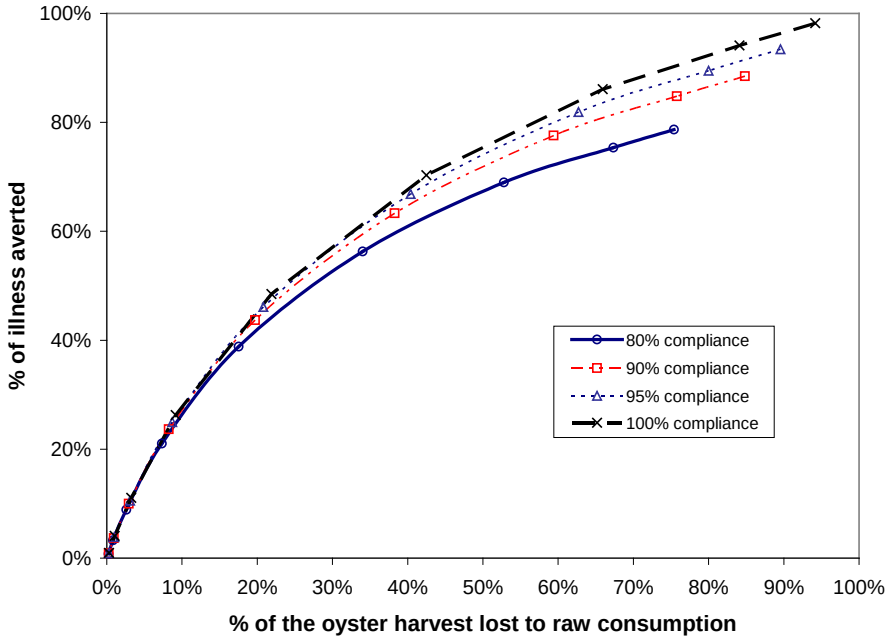


Figure A10-46. Percentage of Illnesses Averted versus Percentage of Harvest Diverted From the "Raw Market" or Subjected to Preventive Controls.

Effect of Deviation from Compliance on "At-Retail" Guidance Levels Scenarios

The impact of deviation from compliance at retail was evaluated in a similar manner to that at harvest. Selected levels of deviation from compliance (ranging from 0 to 50%) with different guidance levels (ranging from 100 to 100,000/g) was evaluated. Impact of deviation from compliance at retail is much higher at the higher standard levels at retail compared to that of at-harvest deviation from compliance (compare Tables A10-4 and A10-5). As seen in Table A10-5, like deviation from compliance at harvest, the lower the standard level in question, the greater the impact of deviation from compliance on loss of oyster harvest to the raw market. However, in the case of illness, deviation from compliance at retail appears to have a greater impact when the guidance level is high, even though a compliance rate of 100% does not result in 100% reduction in illness. At a retail guidance level of 100 *V. parahaemolyticus*/g, a 30% deviation from compliance reduces illness by approximately 90% as compared to the ~100% reduction predicted if 100% compliance were met. A rate of 50% deviation from compliance would result in approximately 74% reduction in illness versus the ~100% predicted if 100% compliance were met. If the guidance level was increased to 5, 000 *V. parahaemolyticus*/g, 50% compliance results in a larger decrease in the reduction of illness (approximately 63%) compared to ~100% predicted if there was 100% compliance.

At 10,000 and 100,000 *V. parahaemolyticus*/g the differences in illness reduction between 100% compliance and 70% compliance are larger than at 100 or 1,000. Therefore, as demonstrated in Figures A10-47 to A10-50, as the level of the microbiological criterion increases, the impact of compliance is more important on illness. Conversely, strict microbiological criteria must be matched with a high level of compliance if they are to be effective.

A deviation from compliance rate of 30% would substantially impact the reduction in risk of illness per serving (Table A10-11) for the higher guidance criteria. It is interesting to note that like at-harvest guidance, at 50% deviation from compliance of the lower guidance levels (100 and 1,000 *V. parahaemolyticus*/g), although the harvest is reduced by half of that at 100% compliance, reduction in illness is not equivalent. At the higher guidance levels, reduction in illness at 50% deviation from compliance is closer to half that at 100% compliance.

Effect of Deviation from Compliance on "At-Cooldown" Guidance Levels Scenarios

Table A10-11. Effect of Compliance Levels on the Effectiveness of Controlling Total *Vibrio parahaemolyticus* in Oysters at Cooldown for Gulf Coast Louisiana Summer

Total Vp/g At-Retail[a]	Compliance Level [b]	Reduction in Mean Risk per Serving (%)	Harvest Diverted (%)[c]	Illness Averted (%) [d]
100/g	50%	50.0%	49.0%	74.5%
	70%	70.1%	68.6%	90.6%
	90%	90.0%	88.2%	98.8%
	100%	~100%	98.0%	~100%
1,000/g	50%	50.0%	43.5%	71.7%
	70%	70.0%	60.9%	88.3%
	90%	90.0%	78.3%	97.8%
	100%	~100%	87.0%	~100%
5,000/g	50%	49.8%	34.5%	67.1%
	70%	69.9%	48.3%	84.4%
	90%	89.7%	62.1%	96.1%
	100%	99.6%	69.0%	99.9%
10,000/g	50%	49.5%	29.7%	64.6%
	70%	69.4%	41.5%	82.1%
	90%	89.2%	53.4%	95.0%
	100%	99.0%	59.3%	99.7%
100,000/g	50%	45.3%	13.9%	53.4%
	70%	63.4%	19.4%	71.2%
	90%	81.6%	25.0%	86.9%
	100%	90.6%	27.8%	94.1%

[a] Assumes that the level of *Vibrio parahaemolyticus* (Vp) is known in oysters at the time of harvest.
[b] The % of non-compliant oyster harvest which is removed from the raw consumption market; non-compliant oyster harvest consumed raw is assumed to have the same distribution of Vp/g (above the compliance level) as under the baseline (no mitigation) scenario.
[c] Refers to the harvest that would need to be diverted from the "raw market."
[d] Assuming that the volume of product available for raw consumption is impacted (i.e., reduced) according to the estimate of the % of harvest lost.

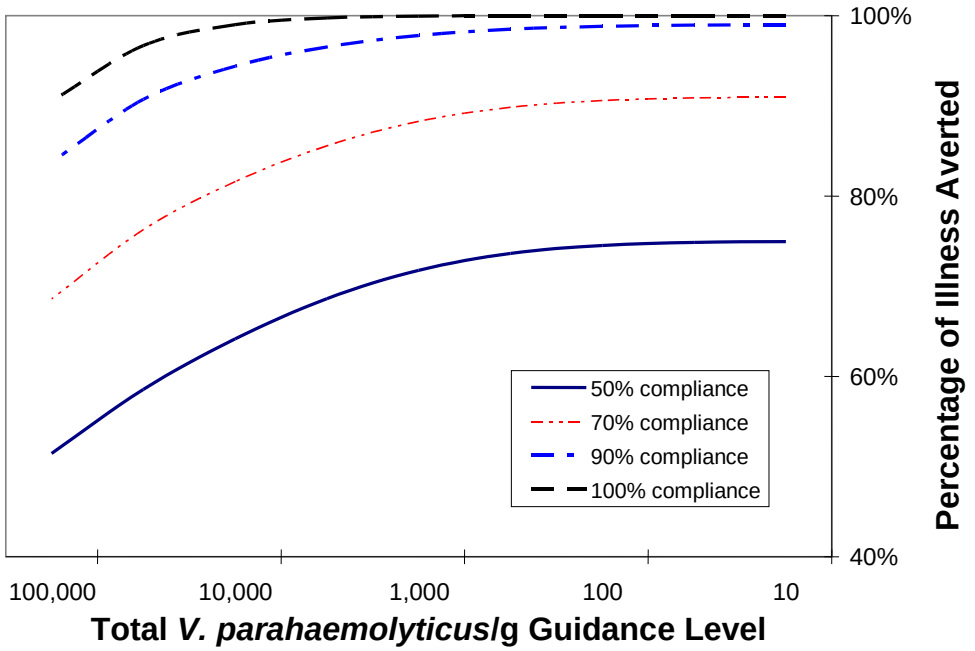


Figure A10-47. Percentage of Illnesses Averted

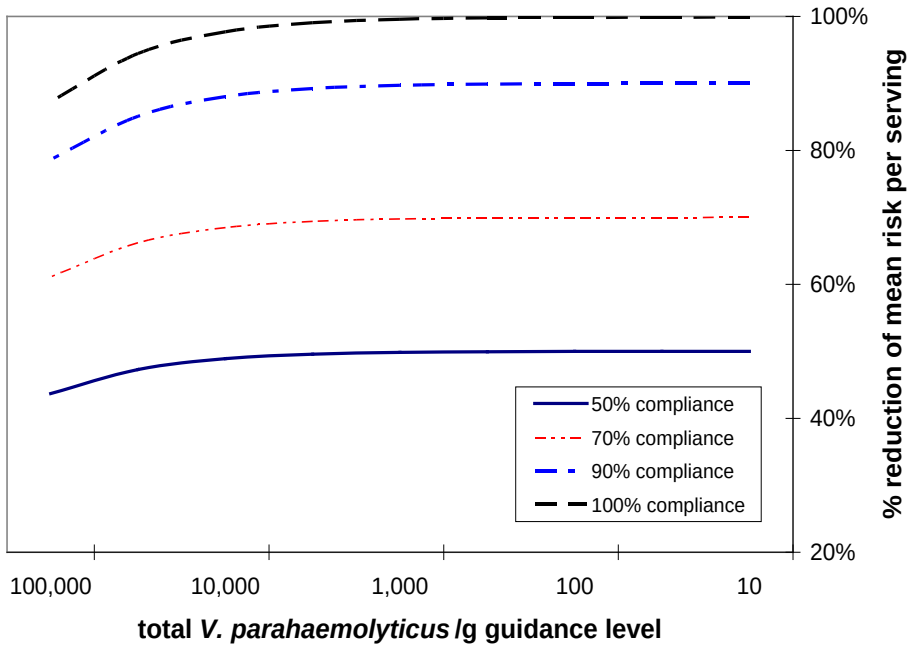


Figure A10-48. Percentage Reduction in Mean Risk per Serving.

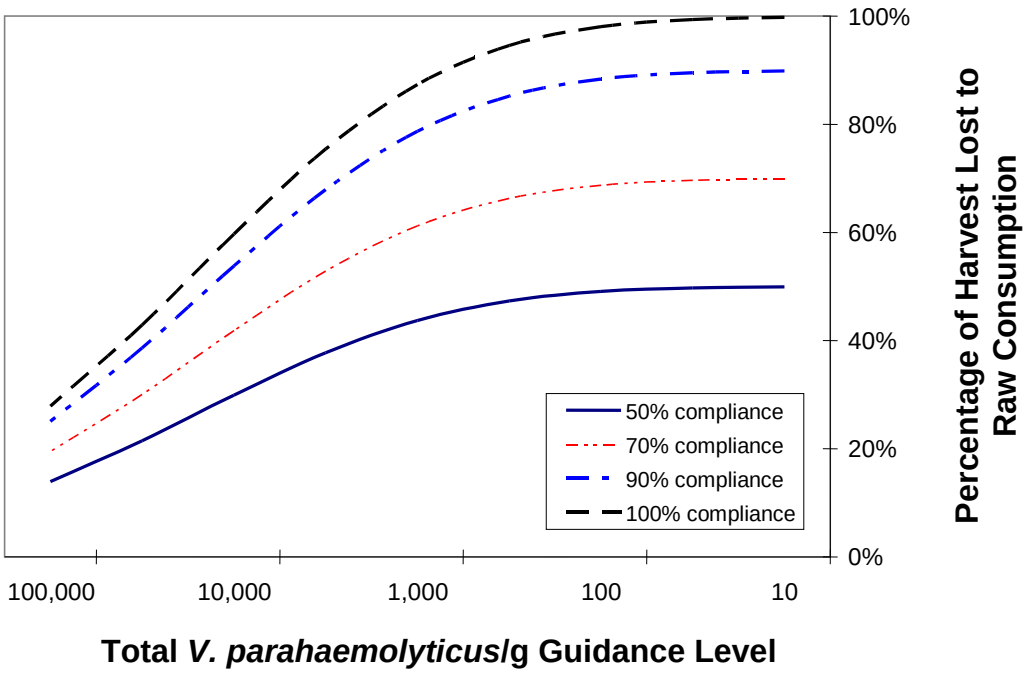


Figure A10-49. Percentage of Oyster Harvest Lost to Raw Consumption Market

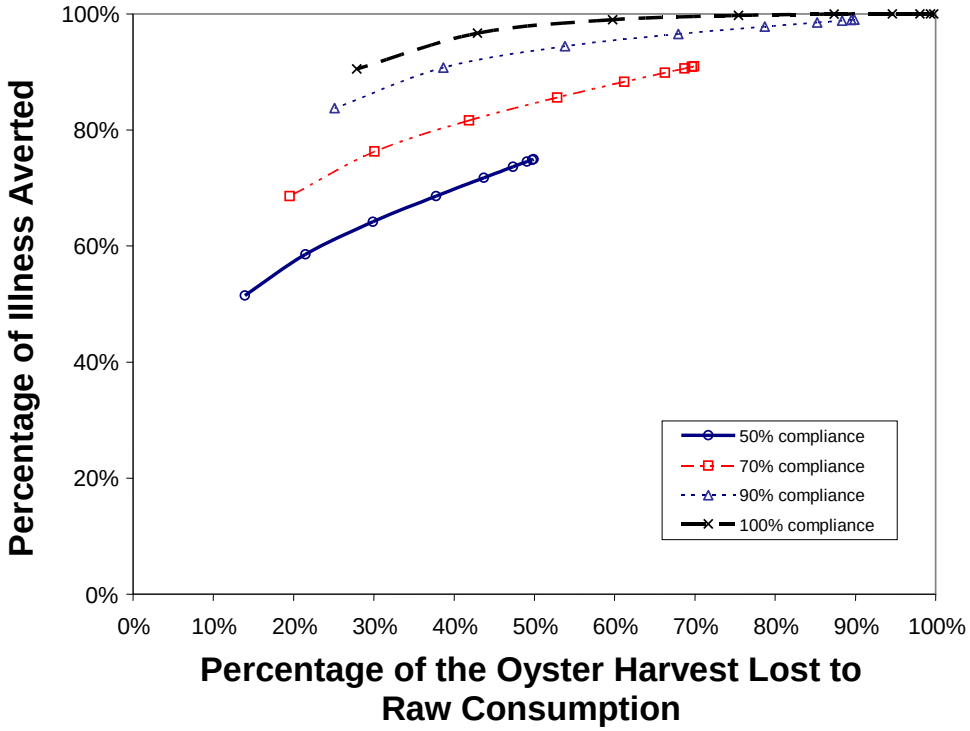


Figure A10-50. Percentage of Illnesses Averted versus Percentage of Harvest Lost to Raw Consumption Market

Effect of Deviation from Compliance on "At-Retail" Guidance Levels Scenarios

Table A10-12. Effect of Compliance Levels on the Effectiveness of Controlling Total *Vibrio parahaemolyticus* in Oysters at Retail for Gulf Coast Louisiana Summer

Total Vp/g At-Retail[a]	Compliance Level [b]	Reduction in Mean Risk per Serving (%)	Harvest Diverted (%)[c]	Illness Averted (%) [d]
100/g	50%	50.0%	47.0%	73.5%
	70%	70.0%	65.8%	89.7%
	90%	90.0%	84.6%	98.5%
	100%	~100%	94.0%	~100%
1000/g	50%	49.7%	37.4%	68.6%
	70%	69.8%	52.3%	85.6%
	90%	89.9%	67.2%	96.7%
	100%	99.8%	74.7%	~100%
5000/g	50%	49.3%	26.4%	62.8%
	70%	69.1%	36.9%	80.6%
	90%	88.8%	47.5%	94.2%
	100%	98.6%	52.8%	99.5%
10,000/g	50%	48.4%	21.5%	59.8%
	70%	68.1%	30.1%	77.9%
	90%	87.5%	38.7%	92.6%
	100%	97.2%	43.0%	98.6%
100,000/g	50%	39.7%	8.3%	45.6%
	70%	55.4%	11.7%	62.0%
	90%	71.4%	15.0%	77.2%
	100%	79.4%	16.7%	84.4%

[a] Assumes that the level of *Vibrio parahaemolyticus* (Vp) is known in oysters at the time of harvest.
[b] The compliance level is the percentage oyster harvest, which is removed form the raw oyster consumption market or subjected to preventive controls; this percentage is assumed to have the same distribution of Vp/g as under the baseline (no mitigation) scenario.
[c] Refers to the harvest that would need to be diverted from the "raw market" or subjected to preventive controls.
[d] Assuming that the volume of product available for raw consumption is impacted (i.e., reduced) according to the estimate of the % of harvest lost from the raw market or subjected to preventive controls.

In summary, as the levels increase, the percentage compliance for the at-harvest guidance is not as important in part because fewer numbers of illnesses are prevented at the higher guidance levels. When these same guidance levels are applied at-retail, however, a high percentage of illnesses is prevented, even when compliance is not 100%. For example, to obtain a 60% reduction in illness rates (assuming 50% compliance), the guidance level would need to be 100 at-harvest but at-retail could be as high as 10,000 *V. parahaemolyticus*/g.

Appendix 11: Data Gaps and Future Research Needs

The *Vibrio parahaemolyticus* risk assessment has provided a framework to significantly advance our ability to describe our current state of knowledge about this important foodborne pathogen, while simultaneously providing a framework for integrating and evaluating the impact of new scientific knowledge on enhancing public health. However, as demonstrated in the risk assessment, deficiencies of the current research with respect to risk assessment were identified. There are several uncertainties associated with the model due to insufficient or absent data. This has brought several future research needs or further data gathering to the forefront as discussed below, which would reduce the uncertainties and improve the risk assessment.

Incidence/frequency of pathogenic *V. parahaemolyticus* in water and shellfish

- More studies are needed to determine the relative abundance of pathogenic *V. parahaemolyticus* in the different regions, particularly the mid-Atlantic and Northeast Atlantic regions. A more accurate estimate of the incidence of pathogenic *V. parahaemolyticus* in these two latter regions would improve the risk assessment.
- Additional research is needed to determine the possibility of changes in the relative abundance of pathogenic *V. parahaemolyticus* during different seasons of the year in the different geographical regions, as well as the identification of associated environmental factors (e.g. temperature or salinity effects). Data on densities of total and pathogenic *V. parahaemolyticus* under a variety of conditions would considerably strengthen the VPRA. Further studies investigating (i.e., to either substantiate or refute) previous finding of higher ratios of pathogenic *V. parahaemolyticus* at lower water temperatures (DePaola *et al*., 2003a) would be particularly informative. Similar data on levels of pathogenic *V. parahaemolyticus* at the point of sale or consumption could provide more valid exposure estimates.
- There is a need for research on the dynamics and causes of temporal "spikes" in pathogenic levels and whether or not the interim monitoring plan, as devised, can identify these spikes as they occur (i.e., is it effective?)
- Information is also needed on the role of oyster physiology and immune status on levels of pathogenic *V. parahaemolyticus* in the oyster. There is a need to determine if there is any correlation between the number of pathogenic *V. parahaemolyticus* and the percentage of oysters diseased.
- It would be appropriate to further investigate *V. parahaemolyticus* O3:K6, and its incidence, because it has been shown to be more resistant to mitigation strategies and appears to require fewer microorganisms to cause illness than other pathogenic *V. parahaemolyticus*.

Impact of overnight submersion of intertidally harvested oysters

- Research is needed to determine whether the predicted level of 90% reduction in illness can be achieved when oysters are stacked in baskets and allowed to remain submerged in the water overnight.
-

Growth rate of *V. parahaemolyticus*

- Further knowledge of the growth rate of *V. parahaemolyticus* within oysters at temperatures other than 26 °C would help decrease the uncertainty with respect to the

difference between growth in the oyster vs. bacterial broth culture; including the issue of potential differences in the growth rate of pathogenic strains versus total *V. parahaemolyticus* populations.

Impact of hydrographic flushing

- Additional quantitative studies are needed on the rates of hydrographic flushing (water turnover) in shellfish harvest areas based on levels of freshwater flows, tidal changes, winds, depth of harvesting area to show how these factors may influence pathogenic *V. parahaemolyticus* levels.

Impact of post-harvest handling and processing

- Additional data on the genetic diversity that we are likely to encounter will enable better evaluation of the phenotypic characteristics that affect ability to tolerate mitigations, growth rates, acid tolerance, etc.
- Studies are needed to obtain more accurate estimates of the distribution of cooling rates of commercial oyster shellstock in an industry setting.
- Quantitative studies are needed to determine the effect of refrigerated wet storage with UV treatment (depuration under refrigerated conditions) as a means of further reducing *V. parahaemolyticus* post harvest.
- A multi-season, nationwide retail study would be required to determine the pathogenic *V. parahaemolyticus* density in market oysters.

Consumption

- A survey of the oyster retail market in the different regions would provide a better indication of the actual proportion of the oyster harvest that goes to the raw oyster market.
- Better consumption information would be helpful in determining the actual amount of oysters consumed per serving as well as per annum in the different regions.

Improved dose-response data

- More intensive investigations of shellfish foodborne disease outbreaks in such a way as to examine the relationships between the dose of contaminated food items ingested and the attack rate and severity of the resulting illness controlling for host factors.
- More research is needed to determine whether different pathogenic strains differ in virulence and in the levels of pathogen required to cause illness.
- More research on the potential virulence factors other than TDH (e.g., urease, TRH, enterotoxins, invasive ability) is needed to determine if the ability to cause disease is increased or decreased by the presence of additional virulence factors. *Vibrio parahaemolyticus* strains that do not produce TDH, TRH, or urease have been found to induce fluid accumulation in suckling mice and diarrhea in a ferret model after oral inoculation in a dose-dependent manner (Kothary *et al*., 2000). Correlation between clinical and environmental incidence of these strains is yet to be determined.
- Additional research is needed to determine the difference in virulence between the strains that have the above virulence properties, as well as between strains that are *tdh*+/*trh*- and *tdh*+/*trh*+. Research on the genetic diversity among pathogenic strains needs to be explored to determine if the degree of pathogenicity among pathogenic strains is

associated with additional genetic markers and the temporal and environmental dynamics related to the emergence of individual strains within the harvest areas. The current risk assessment assumes all *tdh*+ strains to be equally virulent but more recent reports indicate that strains with *tdh*+/*trh*+ have a different promoter sequence for the *tdh* gene and produce much less TDH than *tdh*+/*trh*- strains (Nishibuchi, 2004). This an important finding since ~95% of the *tdh*+ strains from Gulf and Atlantic oysters (and 100% from Pacific oysters) are *tdh*+/*trh*+. Nishibuchi's findings are further supported by CDC data that show that most US clinical isolates are *tdh*+/*trh*- even when O3:K6 (tdh+/trh-) are excluded.

Improved state surveillance systems

- More data from State surveillance systems would provide a better knowledge of the actual illnesses occurring due to consumption of raw oysters containing pathogenic V. *parahaemolyticus*. This would also help to better characterize the immune and general health status of individuals that become ill, as well as if there are other contributing factors such as taking stomach acid suppressors.
- There is a need to look at the seasonality of CDC illness data, especially for the Gulf. The illness peak in late spring is probably real as the reporting system should not vary seasonally. It may be that tdh levels peak then.

Impact of consumer handling of raw oysters

- More information is needed on post retail consumer handling of raw oysters, such as storage conditions (time and temperature), kitchen practices (possibility of cross-contamination), etc. This would provide some indication as to whether the consumer has a role in increasing or decreasing levels of *V. parahaemolyticus* in raw oysters at time of consumption.

Appendix 12: Response to Comments provided by a Review of the Modeling Techniques Used

In February of 2004 a review of the modeling of the risk assessment was conducted by two reviewers, one internal and one external with expertise in @RISK and Monte Carlo simulation. See copy of Carrington (2004) and Donahue (2004) for the full review. The VPRA team requested the reviewers to focus on the following issues:

1. The appropriateness of the general modeling approach adopted (e.g., the regional/seasonal "segmented" structure, no temporal structure within each region/season segment) and whether or not the level of model detail is consistent with the quality and quantity of data that was identified.

2. The appropriateness of assumptions made with respect to modeling and specification of variability and uncertainty distributions.

3. The appropriateness of selected parametric models used for summarizing available datasets, the methods of estimation used, and whether or not effects of model uncertainty are adequately addressed and discussed.

4. The appropriateness of the selected statistical methods of analysis for sensitivity assessment of influential variability and uncertainty factors.

5. The appropriateness and correctness of implementation of the model specification in @Risk (e.g., possible coding errors).

6. The appropriateness of selected sample sizes for Monte Carlo simulations (1,000 uncertainty samples versus 10,000 variability samples).

Several substantive comments were received from the reviewers with respect to these (and other) modeling issues. Below is a summary of the reviewer's major comments and FDA's response to these comments.

Comment 1
Geographical and seasonal variation currently described by segments (or scenarios) could be described (coded) in correlated distributions, which would facilitate evaluation of the effect of intervention strategies on an annual and national basis.

FDA Response to Comment 1
A separate simulation of correlated distributions for a national estimate of public health impact of baseline risk and mitigations is possible. We simulated the region-seasons separately because we wanted to see the impact of mitigations on a regional-seasonal basis. Since we had simulated the region-seasons, it was simple to get a national estimate from these data as opposed to a separate simulation for a national estimate. While the suggested approach of this comment is helpful in looking at national estimates apart from regional seasonal impacts, we concluded that

implementing the suggestion at the present time was not necessary in relation to achieving the stated goals of the risk assessment.

Comment 2
With respect to appropriate specification of the effect of uncertainties, the assessment does not include the range of all plausible interpretations of the data and this is particularly evident with respect to uncertainty of the dose-response and the growth rate model. In particular, the assessment evaluates three possible dose response models but the identified uncertainty is not carried forward in an integrated fashion.

FDA Response to Comment 2
We attempted to identify and appropriately include all relevant uncertainties in a consistent and balanced manner. With respect to uncertainty of the growth rate model we were limited in how this could be addressed because the raw data was not available (including effect on different strains). Predictions were therefore based on the summary model fit information provided in the cited reference (a $\log_{10}$-linear primary growth model with a secondary model of the square-root type). The extent to which use of alternative models would produce substantially different predictions depends on the degree of extrapolation away from the range of the experimental conditions and there is relatively little extrapolation away from the time-temperature range of these data. The primary extrapolation is from broth cultures to growth conditions within the oyster, with a relatively large uncertainty being specified for this extrapolation. As to the identification of dose-response uncertainty we did not carry forward model uncertainty for two principle reasons. First, of the three models considered, the Beta-Poisson is the only one which is low-dose linear; a characteristic which is reasonable *a priori* based on mechanistic considerations (FAO/WHO, 2003). Second, after anchoring each model (in turn) to the epidemiological data it was found that the residual uncertainty of risk predictions for Gulf Coast summer (the region/season with the largest number of attributed illnesses) was comparable across these three different models (Appendix 4). Anchoring each model separately was considered appropriate since, in this instance, the epidemiological estimate of average annual illness burden is effectively being utilized as a "datum" for the purpose of estimation.

Comment 3
With respect to sensitivity analysis, a method that examines the correlation between input percentiles (rather than values) and the output variable may be preferable. Any appropriate method applied to the uncertainty dimension is useful for planning research but in the variability dimension such analyses may not be useful unless targeted at distributions (or portions of distributions) that can be controlled.

FDA Response to Comment 3
The observation that sensitivity analyses may not be useful when applied to variability factors that are not controllable is a valid point. Here we have used sensitivity analysis as applied to variability factors as a means of summarizing the behavior of the model rather than limiting the analysis to just the controllable factors per se. Thus, while we have identified total *V. parahaemolyticus*/g in individual servings as an important variability factor we do recognize that this not controllable on a serving by serving basis. More refined sensitivity analyses limited to controllable variability factors could be developed at a later time. As to preference of a method

comparing the output to percentiles of the input it is our understanding that this is most relevant when there are pronounced thresholds and discontinuities (e.g., growth/no-growth boundaries). With the exception of some low temperature region/seasons, such threshold behavior is atypical of the present model.

Comment 4
With respect to appropriateness of selected sample sizes for Monte Carlo simulations, use of the median rather than the mean as a central estimate of the distribution of uncertainty in output variables would mitigate any concerns that the central estimate is driven by potentially erroneous expression of the tail of the uncertainty distribution.

FDA Response to Comment 4
We have not looked at the effect of anchoring the dose-response with respect to the median as opposed to the mean of the uncertainty distribution. Future work with the risk assessment will examine this issue.

Comment 5
The general segmented structure of the model (region/season) is justified based on the data that modelers had to work with but the justification of this region/season approach could be better documented in the technical document.

FDA Response to Comment 5
We have amended the document to better justify the region/season approach.

Comment 6
With respect to appropriateness of selected parametric distributions used for modeling, the distributions (i.e., Normal) used to model the water temperature are not as accurate and precise as they could be and this may impact on the predicted densities of total *V. parahaemolyticus* and the number of pathogenic *V. parahaemolyticus*. As shown in Appendix 4 these data are (typically) skewed and this fact, compounded with the uncertainty arising from selection of only one point (or buoy) to represent the temperature of an area may have a significant impact on the modeling results. Other models of water temperature (such as a bounded Beta variate) may be appropriate given that the sensitivity analysis in Appendix 5 shows that the water temperature parameters are significant.

FDA Response to Comment 6
Although a parametric distribution could be utilized that better represents the skewness of the temperature data, there is a trade-off between fidelity of representation of the data and utility of the model. The choice of the Normal distribution to summarize the water temperature data for the model simulations was based on the judgment that the discrepancy of predictions resulting from use of a fitted Normal rather than the empirical distribution of the data was a relatively minor "cost" to pay for more utility or ease of use (e.g., interpretability). On a practical level, the model would be much more cumbersome if the empirical distributions of water temperature data (or bounded Beta variates) were used rather that the Normal approximation. With respect to utility and interpretability, the potential effect of year-to-year variations of temperature distributions (i.e., extreme temperature events such as El Niño or La Niña) was initially

identified as a potentially important factor to be considered in the assessment. Appropriate assessment of the effect of year-to-year variability of temperature distributions requires an effective summary of year-to-year differences in the temperature data. It is unclear how this could be effectively accomplished based on either empirical distributions of a limited number of years of temperature data or the parameters of bounded Beta variates fitted to these data. As to the magnitude of the impact of using a Normal approximation rather than the empirical distribution of the temperature data, simulations where conducted using the NBDC Gulf Coast temperature data and the maximum likelihood estimate of the *V. parahaemolyticus*/g versus water temperature regression relationship. The simulations indicated that the alternative specifications of water temperature distributions result in predictions of mean $\log_{10}$ *V. parahaemolyticus*/g at time of harvest which have a relative difference of <1% across all years and seasons of the temperature data. Relative differences in mean *V. parahaemolyticus*/g at time of harvest are larger with a range of up to a 10% relative difference for some of the year and season specific data; however, the average relative difference was only 2%. Thus any infidelity of representation of the skewness of the water temperature data (within a given year and season) does not appear to have a substantial impact, and this is further validated by the comparison of model simulation output to data on *V. parahaemolyticus*/g at time of consumption.

Comment 7

The estimation of the dose-response deserves further attention. As illustrated by the sensitivity analyses, the impact of the dose-response uncertainty is substantial. As such, other sources of dose-response data should be considered. In the absence of better data or modeling methods, the impact of this uncertainty (as a weakness of the model) should be better identified in the technical document and interpretive summary.

FDA Response to Comment 7

We have amended the document to better explain our use of dose-response data and the impact of the dose-response uncertainty on the estimates of risk. Since the goal of the risk assessment was to (1) examine the factors that contribute to the risk, (2) examine the differences between different regions/seasons, and (3) evaluate the impact of potential mitigations, the dose response curve is not something that is varied in any manner during the risk assessment. Accordingly, it can almost be viewed as a constant for the risk assessment that was not changed, so the uncertainty was a constant for all factors and "what-if scenarios".