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 the risk assessment and request for comment (66FR 13544)	Public

Date	Activity	Reviewers
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 10,000th 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 1st 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 c[JCB1]hanged for each uncertainty simulation. Predicted growth (presented as $\log_{10}$) (cell F40) is added [JCB2]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	16.36246		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.	
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 to	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						
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	0.000756876
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
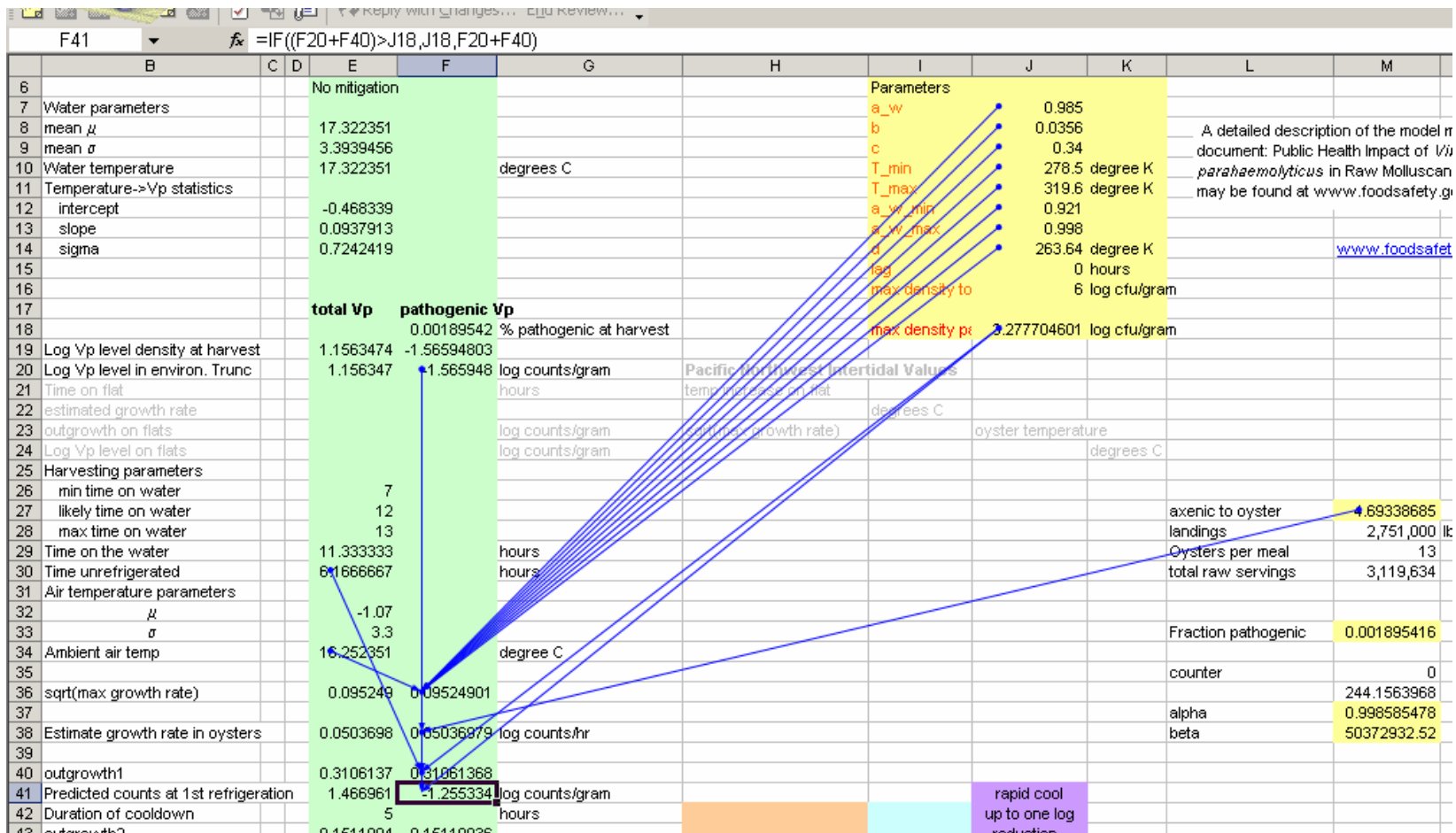
Figure A3-3. Screen Shot of @RISK Spreadsheet Showing How *Vibrio parahaemolyticus* Levels at First Refrigeration were Derived

F54   fx =RiskOutput("prob ill baseline") + 1-(1+F52/M38)^(-M37)

	B	C	D	E	F	G	H	I	J	K	L	M
26	min time on water			7							axenic to oyster	4.69338685
27	likely time on water			12							landings	2,751,000 lb:
28	max time on water			13							Oysters per meal	13
29	Time on the water			11.333333		hours					total raw servings	3,119,634
30	Time unrefrigerated			6.1666667		hours						
31	Air temperature parameters											
32	μ			-1.07								
33	σ			3.3							Fraction pathogenic	0.001895416
34	Ambient air temp			16.252351		degree C						
35											counter	0
36	sqrt(max growth rate)			0.095249	0.09524901							244.1563968
37											alpha	0.998585478
38	Estimate growth rate in oysters			0.0503698	0.05036979	log counts/hr					beta	58372932.52
39												
40	outgrowth1			0.3106137	0.31061368							
41	Predicted counts at 1st refrigeration			1.466961	-1.255334	log counts/gram			rapid cool			
42	Duration of cooldown			5		hours			up to one log			
43	outgrowth2			0.1511094	0.15110936				reduction			
44	Predicted counts after cooldown			1.618070	-1.104225		four.five log	two log	-1.414839			
45	Length of refrigeration time			7.7		days	reduction	reduction				
46												
47	Predicted level after die off			1.121270	-1.601025	log counts/gram	-6.101025	-3.601025	-1.911639			
48												see Spreadsheet
49	Grams oysters consumed			168.50063		grams						
50												
51	Vp exposure per meal			2492.2018	472375987	mean count per serving	0.000149378	0.047237599	2.310330604			
52	Pathogenic Vp consumed				5	counts	0	0	2			
53					0.69997	log mean counts						
54	probability of illness				9.9119E-08	per this serving	0	0	3.96477E-08			
55							0	0	3.96477E-08			
56												
57												
58							percent pathogenic				rate fraction	Water
59							alpha	beta			for axenic to oyster	temperature mear
60	last edited: 2004-Jul-16						0.463656827	244.1563968			4.693387	17.32235148 3
61							0.336556923	187.4095706			3.786338	13.89422526
62							1.181277883	923.8980358			3.27427	14.57524617 3

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			88.5006		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
64	dsheet requires the user to						0.567710601

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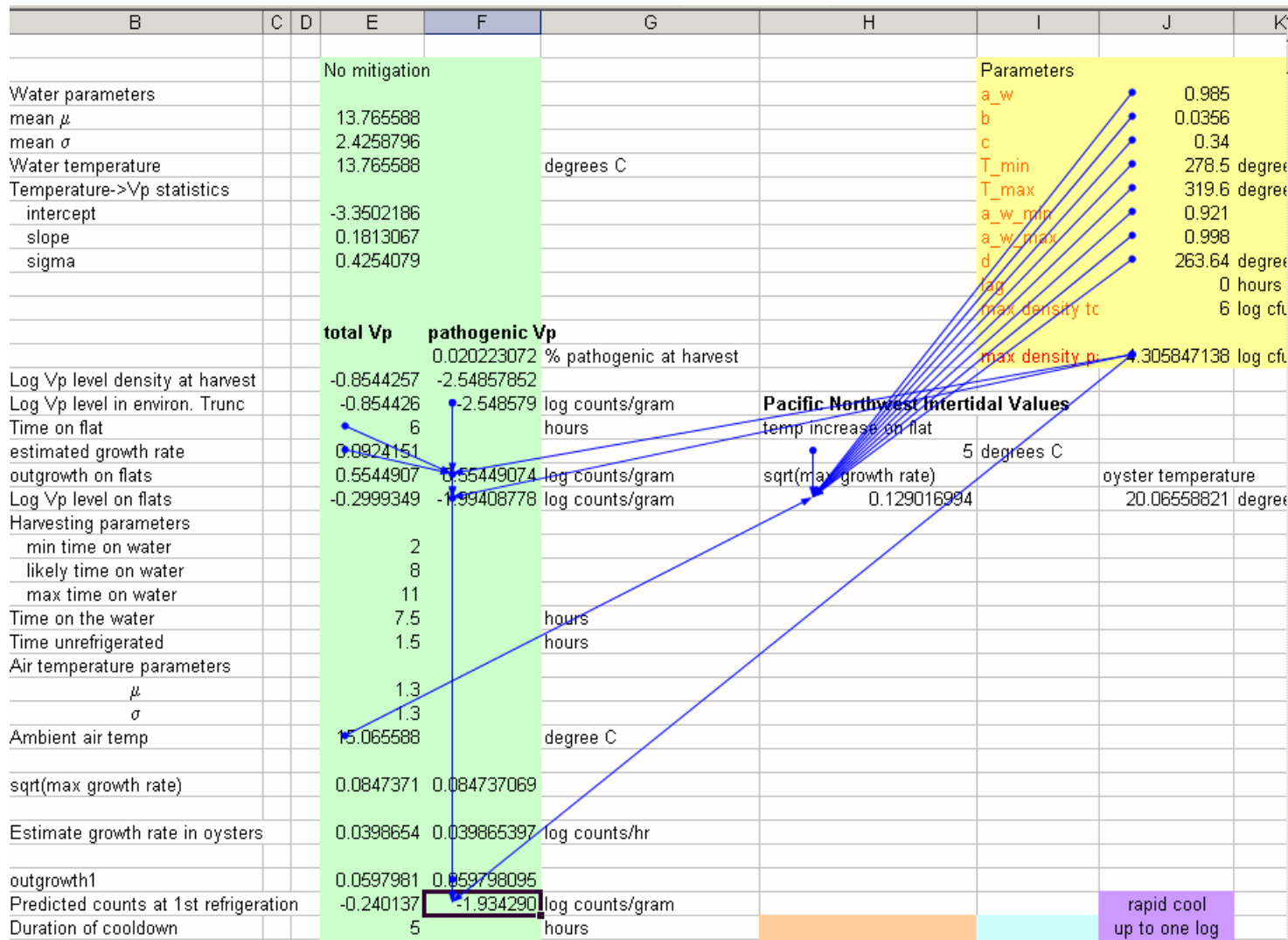
Figure A3-6. Screen Shot of @RISK Spreadsheet Showing the Effect of Intertidal Harvesting on Levels of *Vibrio parahaemolyticus* in Oysters in the Pacific Northwest

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	$\Pr(ill \mid d) \quad = \quad 1 - (1 + \dfrac{d}{\beta})^{-\alpha}$
Probit[a]	$\Pr(ill \mid d) \quad = \quad \Phi\big(\alpha + \beta * f(d)\big)$
Gompertz[1]	$\Pr(ill \mid d) \quad = \quad 1 - \exp\big[-\exp[\alpha + \beta * f(d)]\big]$

[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 $\beta \gg \alpha$ and $\beta \gg 1$. 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**	**1.31×10^6**	**-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.
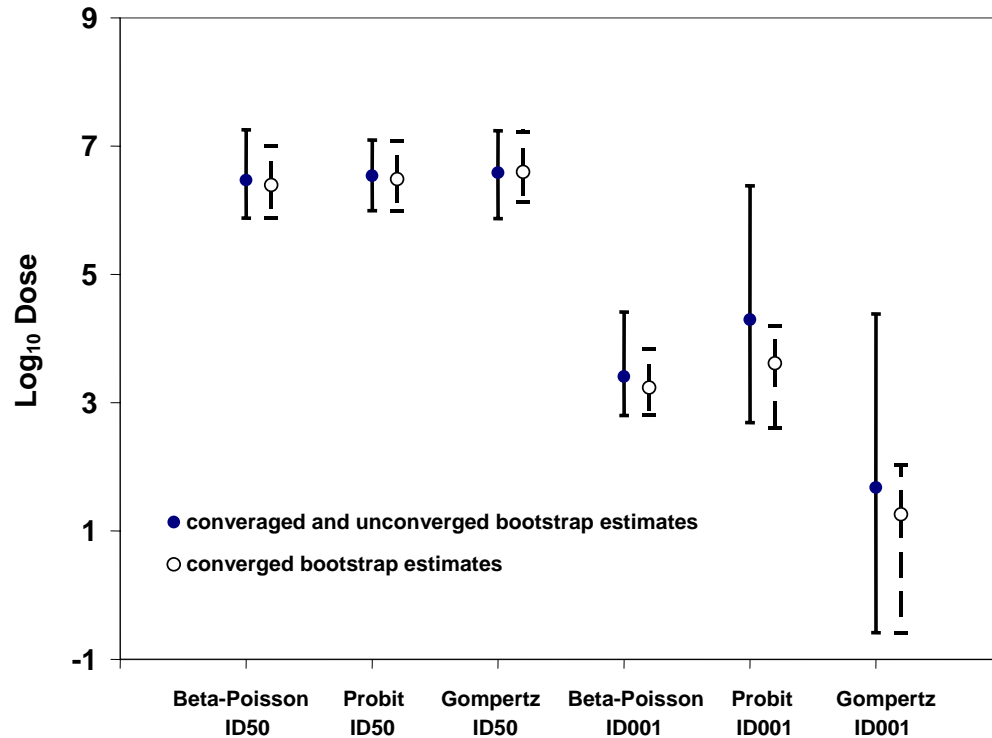
Figure A4-1. Bootstrap Estimates of the 2.5%-tile, the Mean, and the 97.5%-tile of the Uncertainty Distribution of ID_{50} and ID_{001} Based on Fit of the Beta-Poisson, Probit and Gompertz Models to the Human Feeding Trials Data (With and Without Unconverged Parameter Estimates Being Retained)

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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:

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

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

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

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(septicemia | immunocompromised) = 0.12

Pr(septicemia | healthy) = 0.0165

Pr(septicemia) = 0.047

Pr(death | 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:

$$Pr(CC) = Pr(CC|S) * Pr(S) + Pr(CC | \bar{S}) * Pr(\bar{S})$$

and

$$Pr(S | CC) = \frac{Pr(CC|S) * Pr(S)}{Pr(CC|S) * Pr(S) + Pr(CC | \bar{S}) * Pr(\bar{S})}$$

where

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

$$Pr(S) = Pr(immunocompromised)$$

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

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

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

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

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:

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

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

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

$$\Pr(septicemia \mid S) = \Pr(septicemia \mid S \ \& \ CC) * \Pr(CC \mid S) = 0.12 * 0.21 = 0.025$$
$$\Pr(septicemia \mid \overline{S}) = \Pr(septicemia \mid \overline{S} \ \& \ CC) * \Pr(CC \mid \overline{S}) = 0.0165 * 0.038 = 0.00063$$

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(septicemia | 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 3.4×10^{4}, 4.6×10^{4}, and 6.5×10^{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 Sate 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.
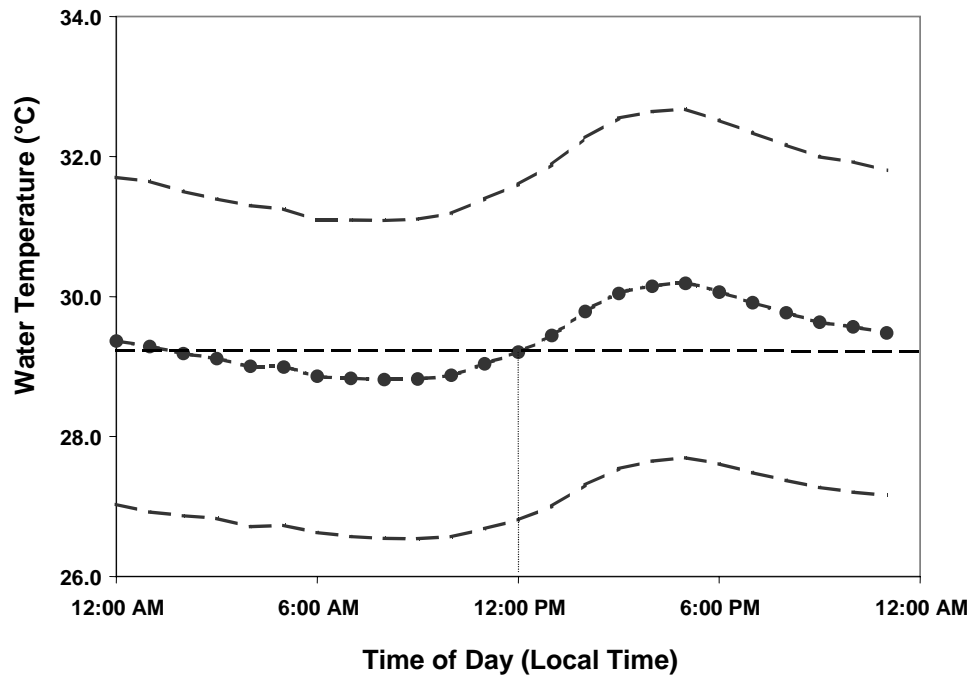
Figure A5-1. Mean and Percentiles (2.5% and 97.5%) of Hourly Water Temperature Profile for Dauphin Island, AL (NBDC, July – Sept 1997)
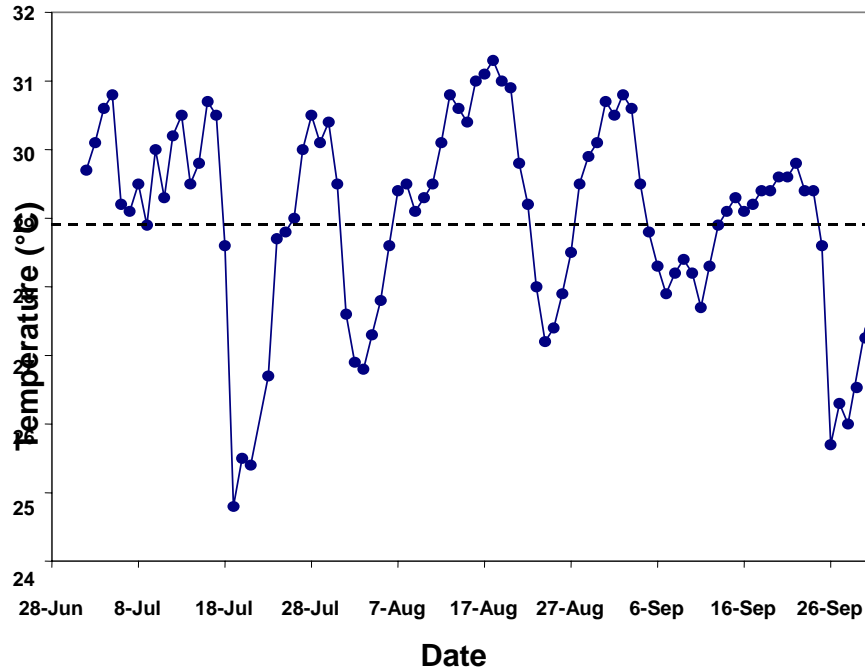
Figure A5-2. Temporal Pattern of Day-to-Day Variation of MiddayWater Temperature Profile for Dauphin Island, AL (NBDC, July – Sept 1997)
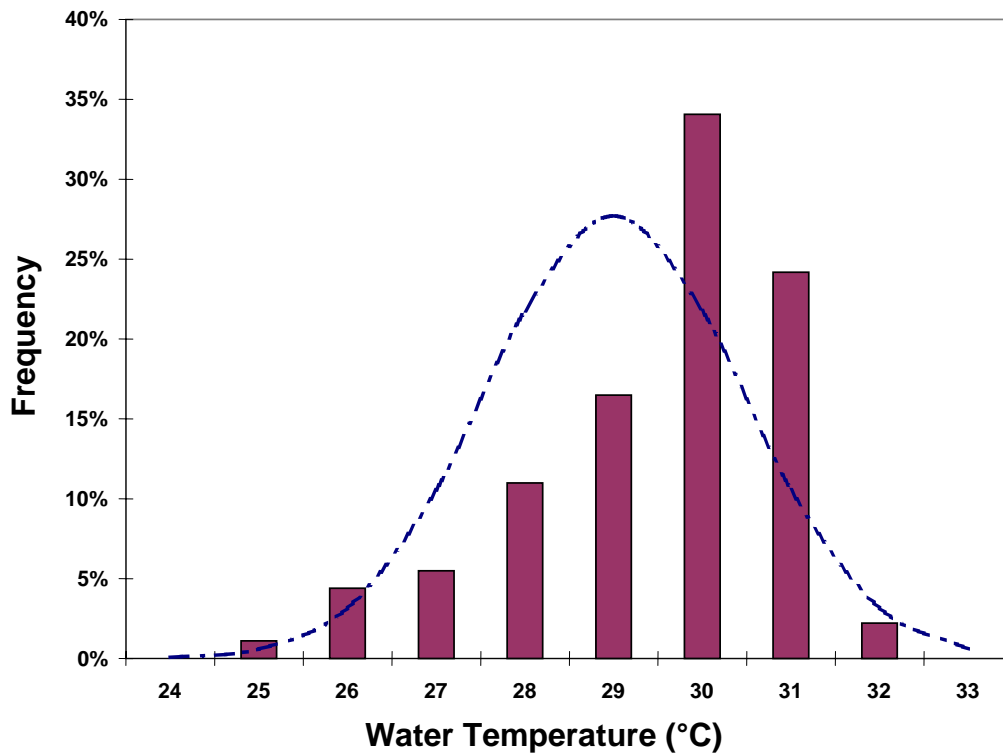
Figure A5-3. Histogram of Day-to-Day Variation of Midday Water Temperature Profile and Approximating Normal Distribution Summary for Dauphin Island, AL (NBDC, July – Sept 1997)

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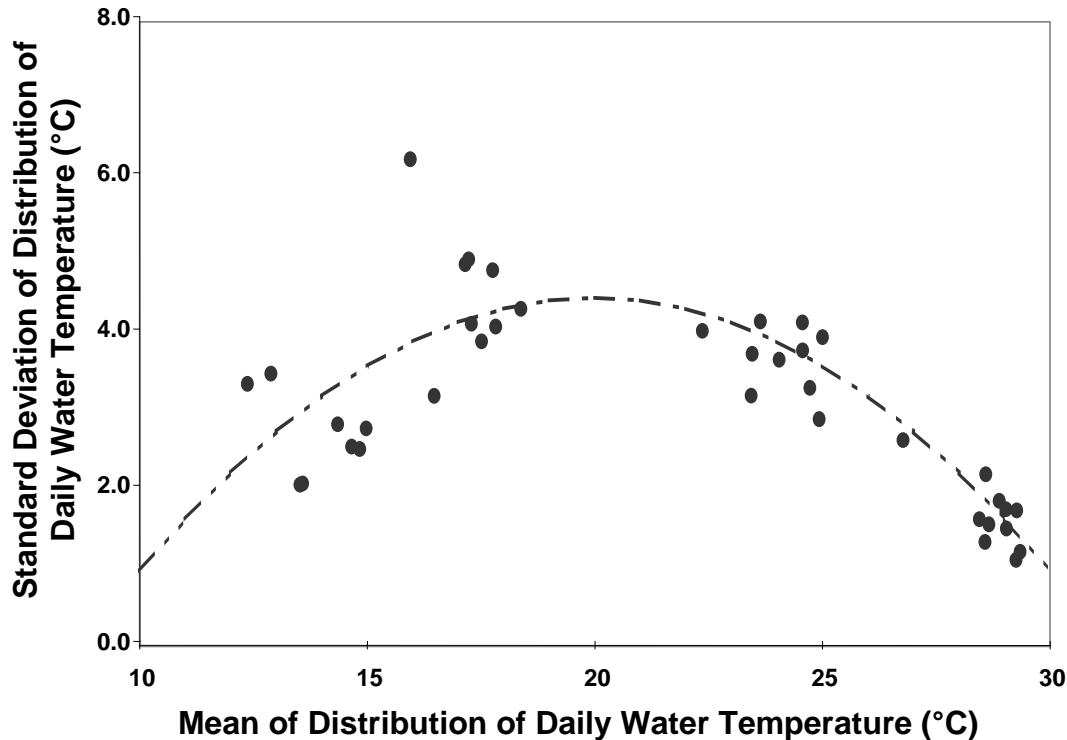
Figure A5-4. Interannual Variation of the Mean and Standard Deviation of Within Season Water Temperature Distributions for Dauphin Island, AL (NBDC, 1997).

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

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

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

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

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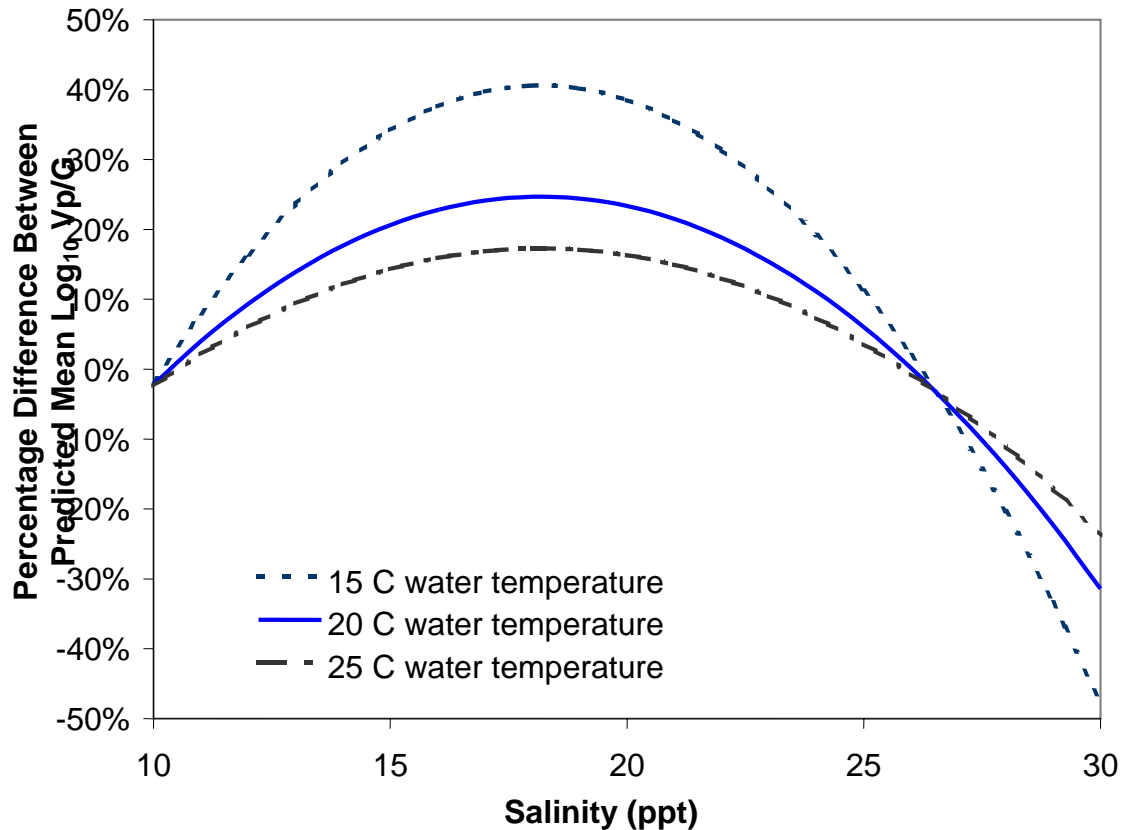
Figure A5-5. Effect of Salinity on Predicted Mean log$_{10}$ *Vibrio parahaemolyticus* Density in Oysters Relative to Predicted Density at Optimal Salinity (22 ppt).

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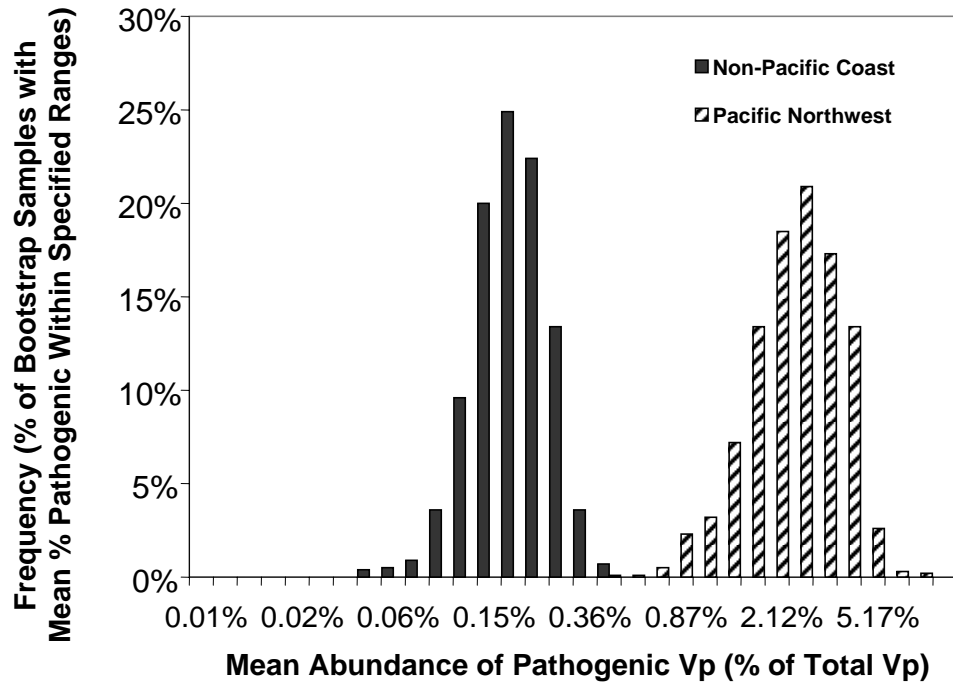
Figure A5-6. Histograms of Bootstrap Uncertainty Distributions of Mean Percentage Pathogenic Based on the Beta Distribution Model of Sample-to-Sample Variation of Percentage Pathogenic *Vibrio parahaemolyticus* (Pacific Northwest and Non-Pacific Coastal regions).
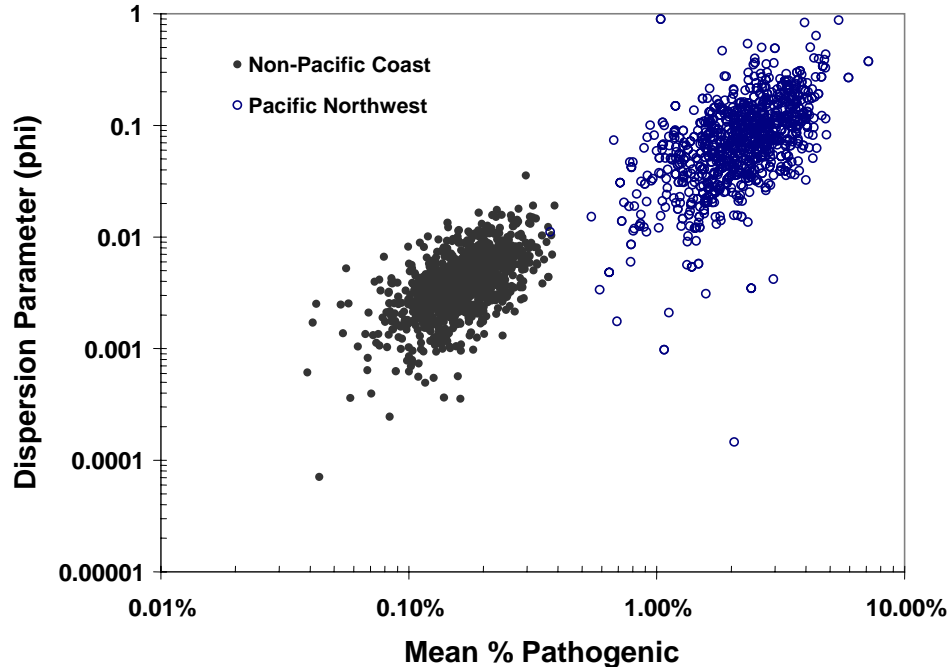

Figure A5-7. Bootstrap Samples of Uncertainty of Mean Percentage Pathogenic and Dispersion Parameter of the Beta Distribution Model of Sample-to-Sample Variation of Percentage Pathogenic *Vibrio parahaemolyticus* (Pacific Northwest and non-Pacific Coastal regions).

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.

<u>Distribution of Air Temperature at Oyster Harvest</u>
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.
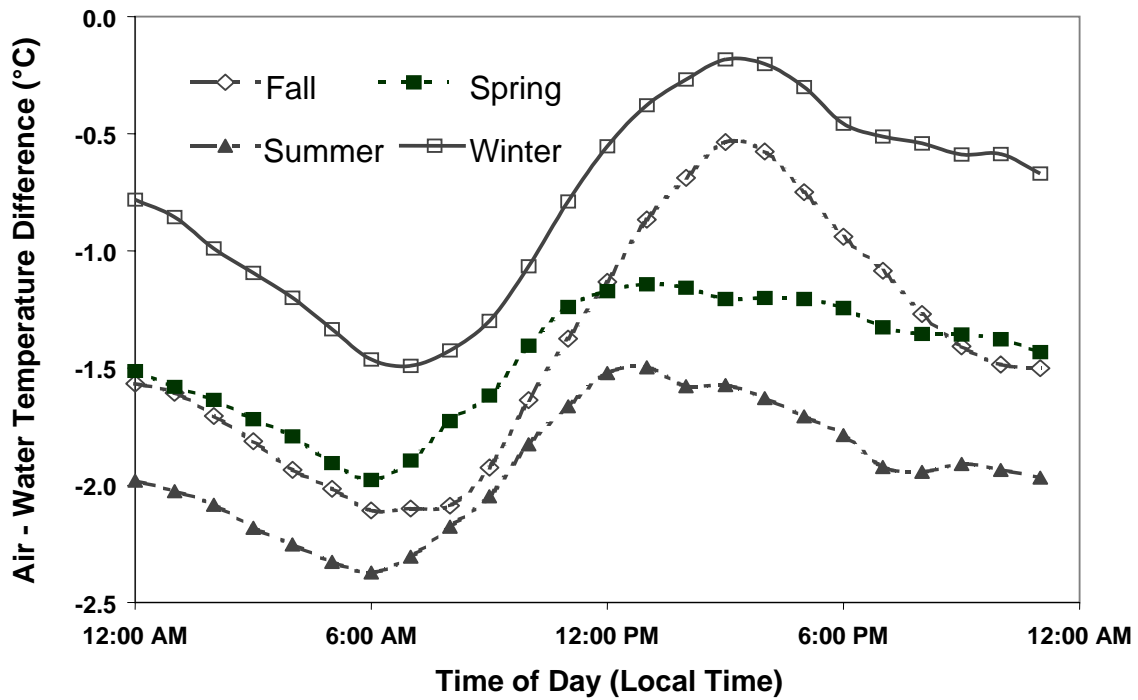
Figure A5-8. Variation of mean hourly air-water temperature differences for Dauphin Island, Alabama Buoy (NBDC, 1997).
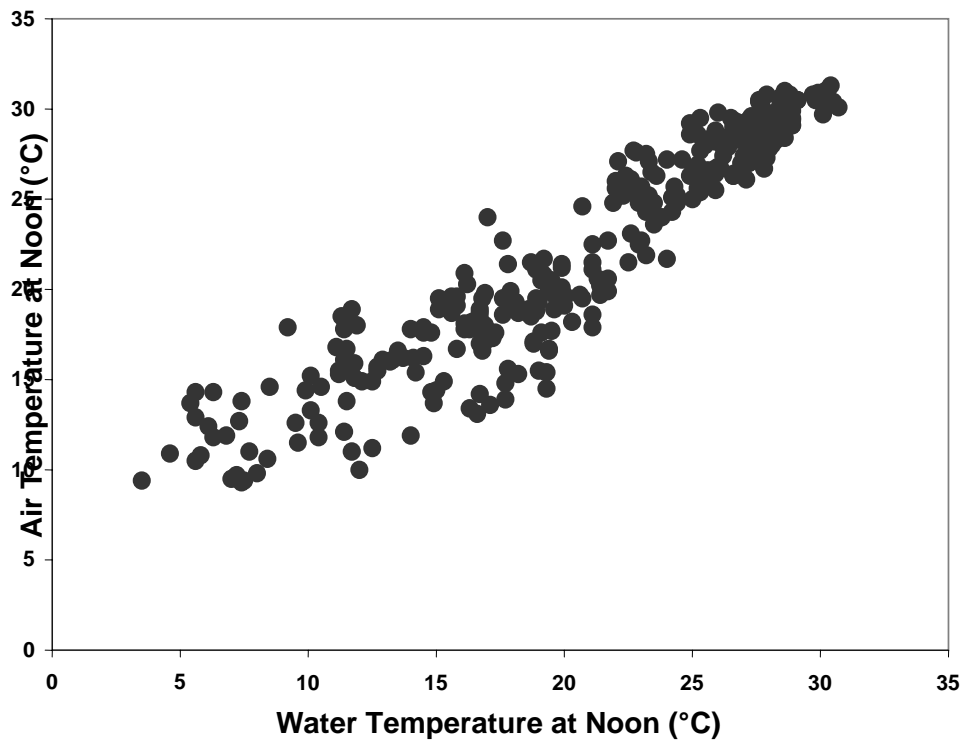

Figure A5-9. Correlation of Daily Midday Air and Water Temperature Measurements for Dauphin Island, AL (NBDC, 1997).

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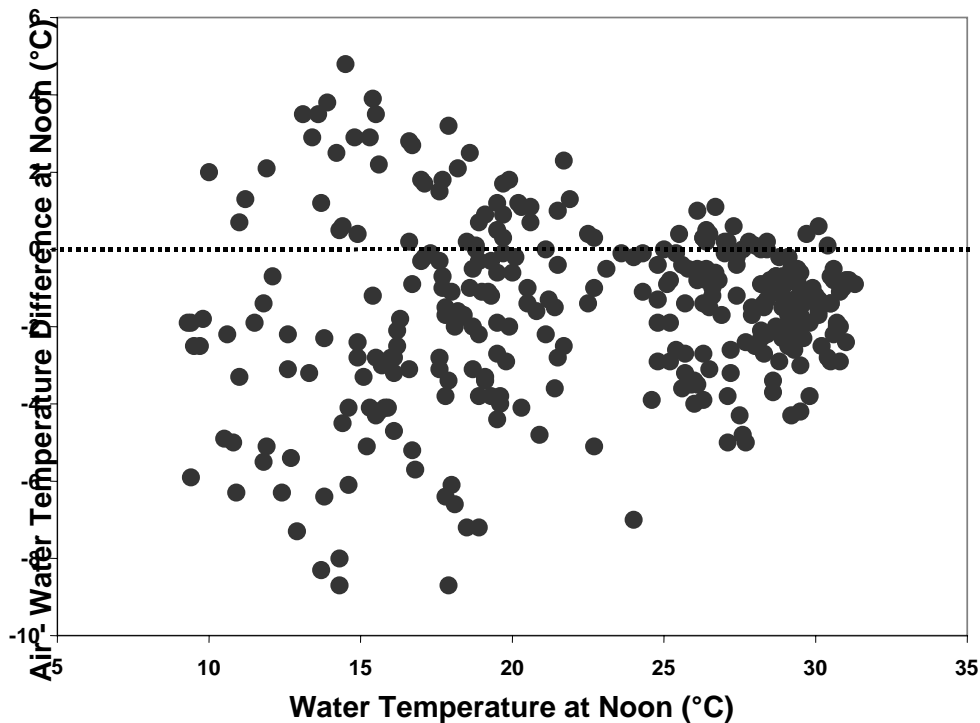
Figure A5-10. Differences Between Midday Air and Water Temperatures as a Function of Water Temperature for Dauphin Island, AL (NBDC, 1997).

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 $^{\circ}$C (78.8 $^{\circ}$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 $^{\circ}$C (78.8 $^{\circ}$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 ith 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.
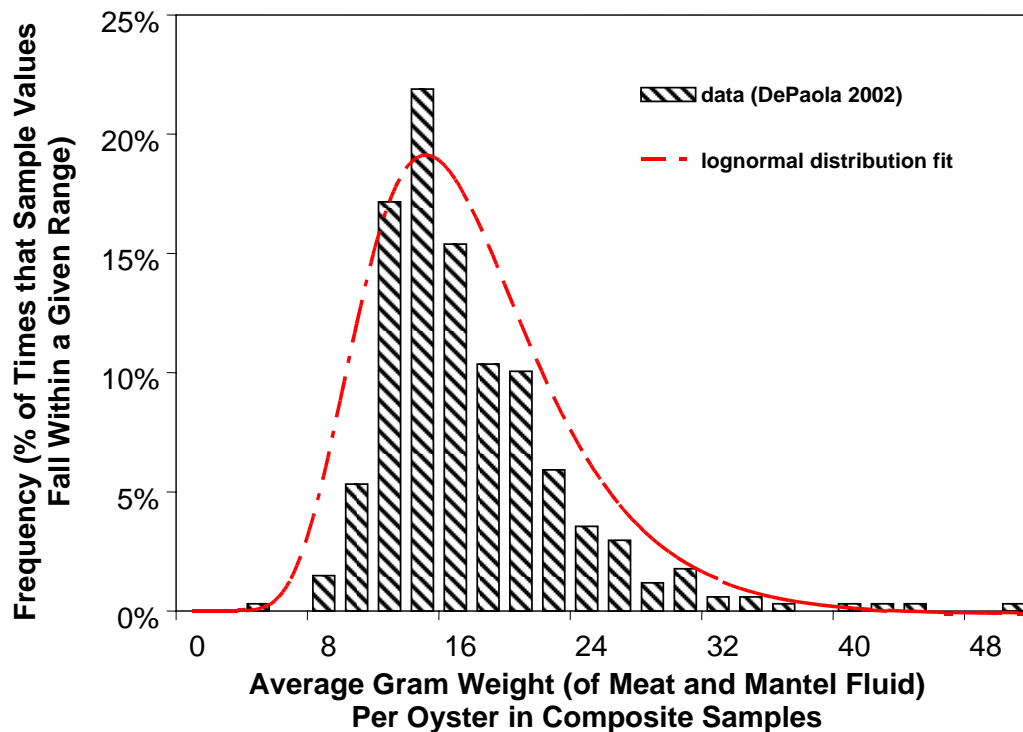

Figure A5-11. Distribution of Average Oyster (Meat and Mantle Fluid) Weight Over Samples of Composites of 4-15 Oysters Collected From Retail Establishments (FDA/ISSC, 2000; DePaola, 2002).

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.
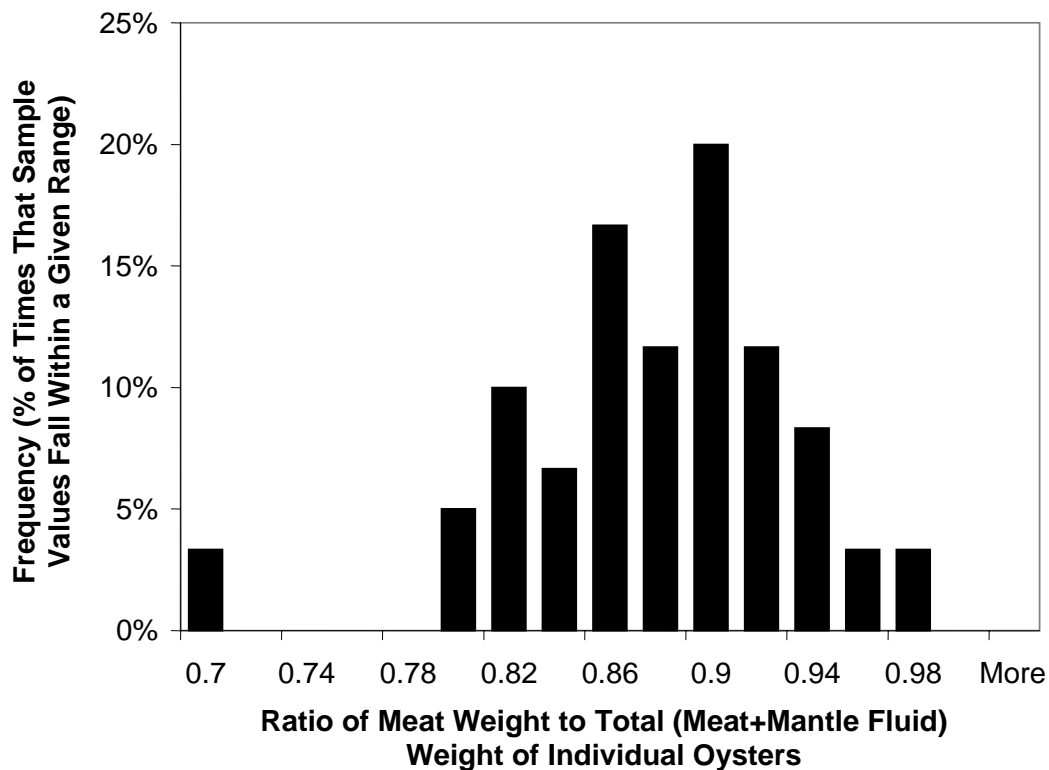

Figure A5-12. Distribution of Oyster Meat Weight as the Percentage of Total Oyster (Meat and Mantle Fluid) Weight Over Samples of Individual Oysters (Kaufman et al., 2003).

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 2nd and 3rd 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 1st 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 = 5th %-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

Table A7-3. Mean total Vp/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

Table A7-4. Mean pathogenic Vp/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

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

Region	Season	mean	median	5th %-tile	95th %-tile
	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
Gulf LA	fall	5670.00	4506.72	1284.31	13710.14
	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
Gulf non-LA	fall	2496.79	1845.00	443.81	6622.02
	winter	1.49	1.11	0.31	3.42
	spring	510.58	319.67	50.57	1667.50
Northeast	summer	2458.30	1782.36	501.60	6808.45
Atlantic	fall	51.94	30.37	9.45	160.20
	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
Mid-Atlantic	fall	305.85	171.56	22.89	994.08
	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
PNW dredged	fall	0.2302	0.1316	0.0369	0.6721
	winter	0.0169	0.0102	0.0019	0.0556
	spring	150.14	18.99	0.66	778.34
PNW	summer	1651.17	870.28	117.41	6080.52
intertidal	fall	3.9387	0.9567	0.1526	17.1876

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

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

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

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

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

Table A7-11. Number of septicemia cases

Region	Season	Mean	Median	5th %-tile	95th %-tile
	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
Gulf LA	fall	0.3089	0.1710	0.0150	1.0943
	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
Gulf non-LA	fall	0.1185	0.0608	0.0047	0.4194
	winter	0.000062	0.000033	0.000003	0.000194
	spring	0.0069	0.0028	0.0002	0.0282
Northeast	summer	0.0321	0.0163	0.0015	0.1234
Atlantic	fall	0.0039	0.0016	0.0001	0.0159
	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
Mid-Atlantic	fall	0.0088	0.0031	0.0002	0.0406
	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
PNW dredged	fall	0.000056	0.000018	0.000001	0.000199
	winter	0.0000078	0.0000030	0.0000003	0.0000302
	spring	0.0426	0.0040	0.0001	0.1905
PNW	summer	0.4034	0.1417	0.0089	1.7430
intertidal	fall	0.002443	0.000360	0.000019	0.010002

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

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

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

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

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

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

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

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

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			

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%
	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%
Gulf non-LA	fall	0.2279%	0.0900%	0.0100%	0.9200%
	winter	0.0004%	0.0000%	0.0000%	0.0000%
	spring	0.0261%	0.0000%	0.0000%	0.1300%
Northeast	summer	0.2057%	0.0600%	0.0100%	0.8815%
Atlantic	fall	0.0154%	0.0000%	0.0000%	0.0800%
	winter	0.0004%	0.0000%	0.0000%	0.0000%
	spring	0.2021%	0.0800%	0.0100%	0.7905%
Mid-Atlantic	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.
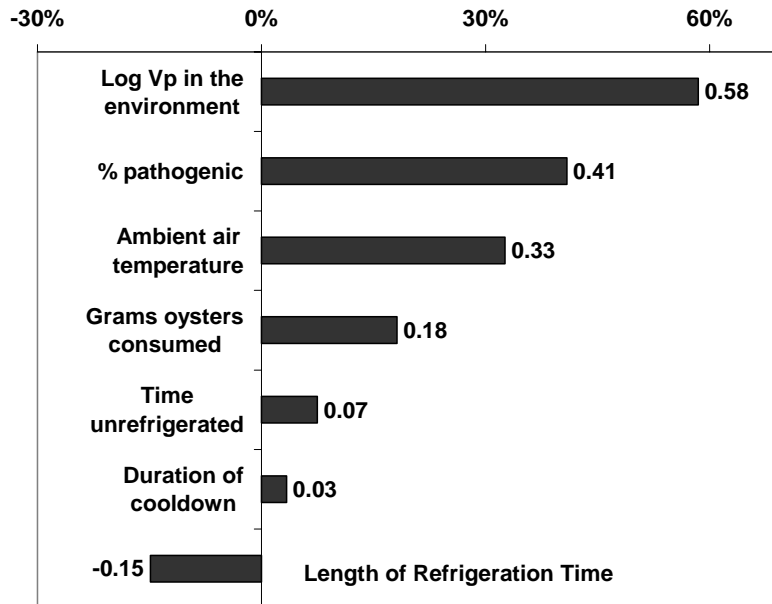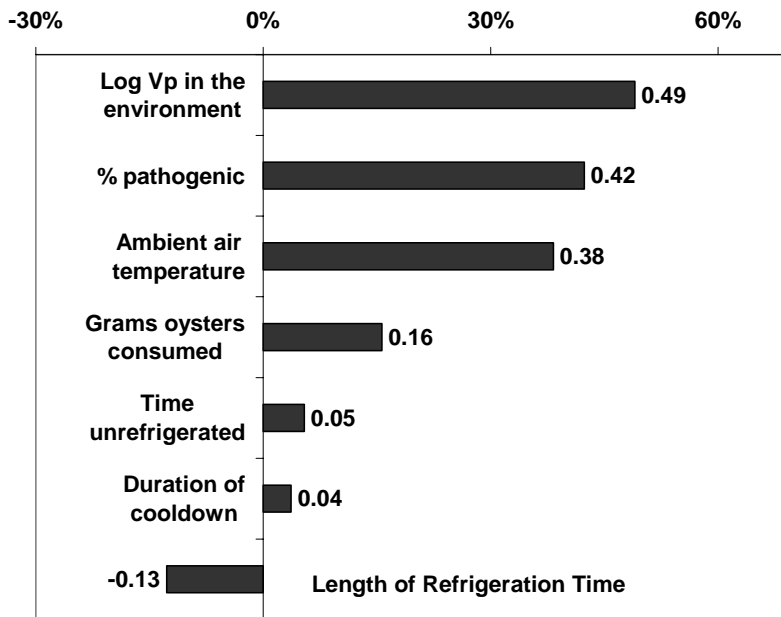

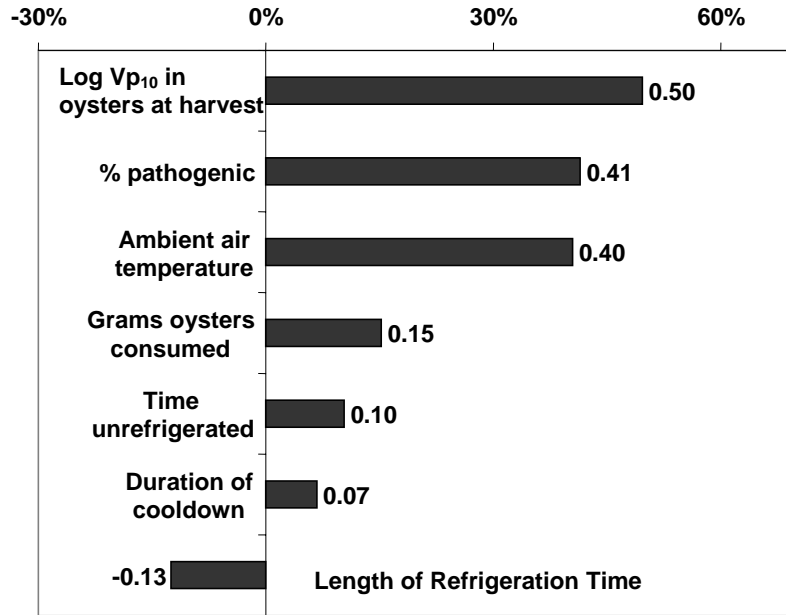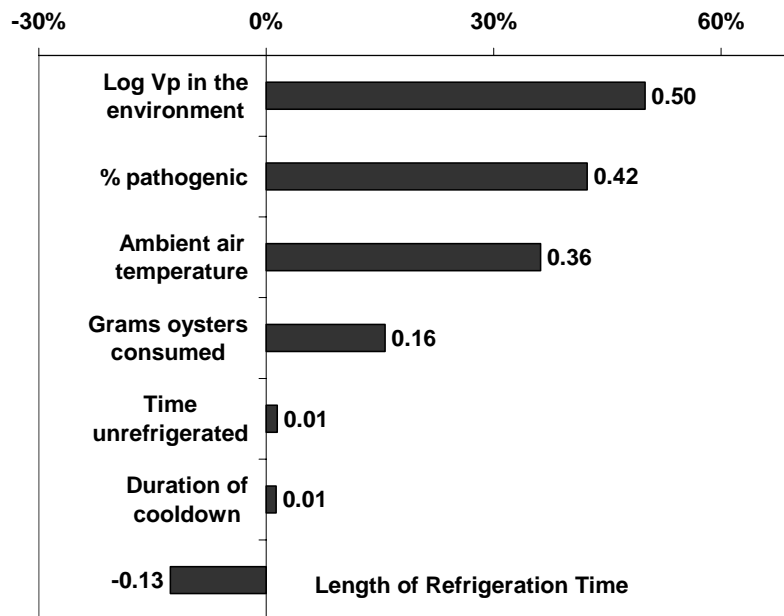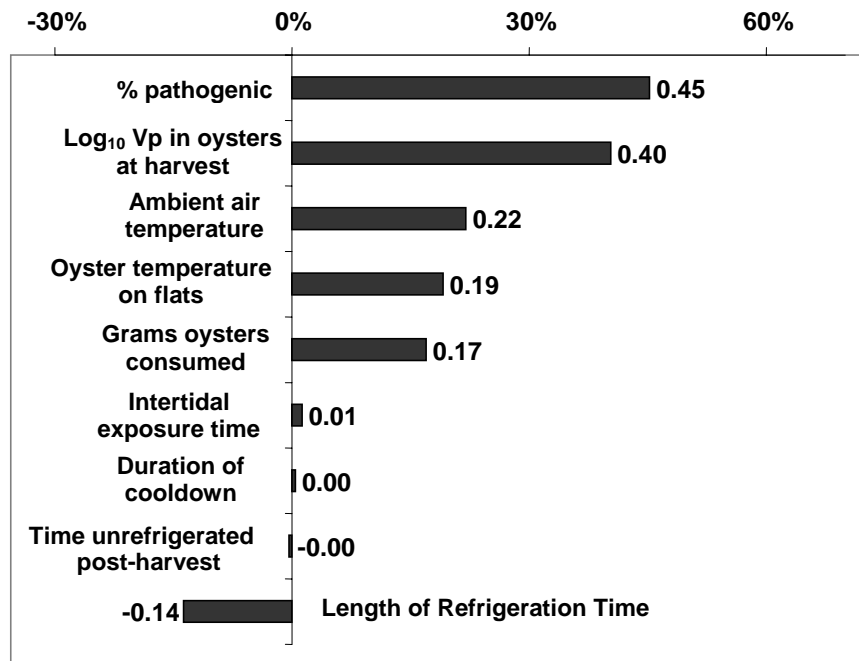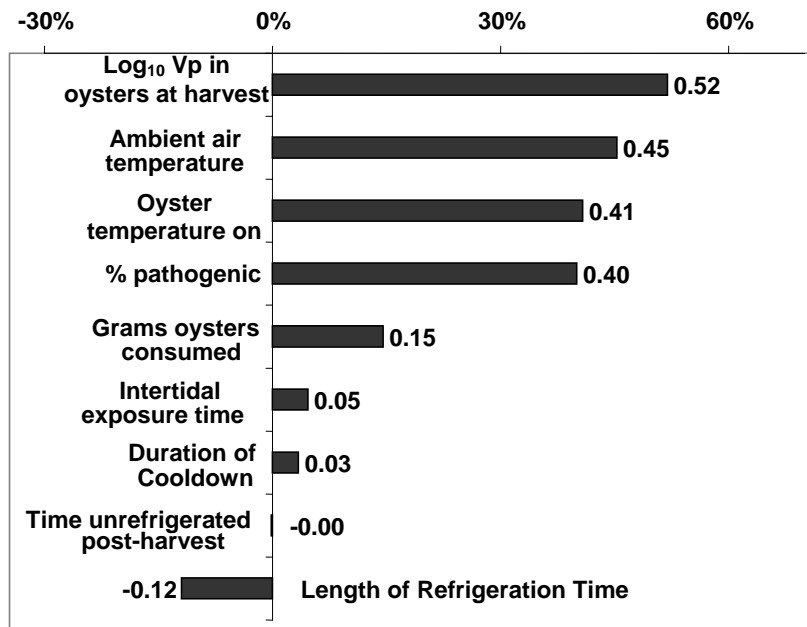**Figure A8-1. Tornado Plot of Influential Variability Parameters on log$_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (Louisiana) Winter Harvest**
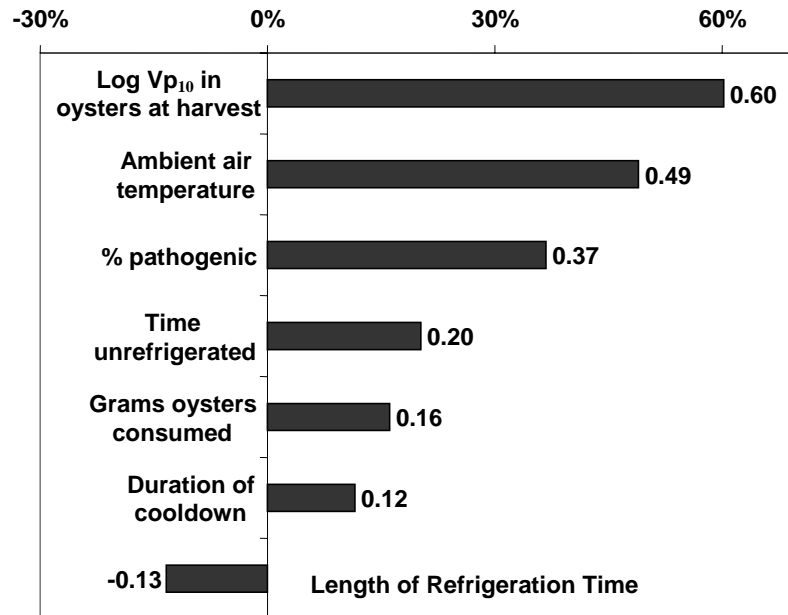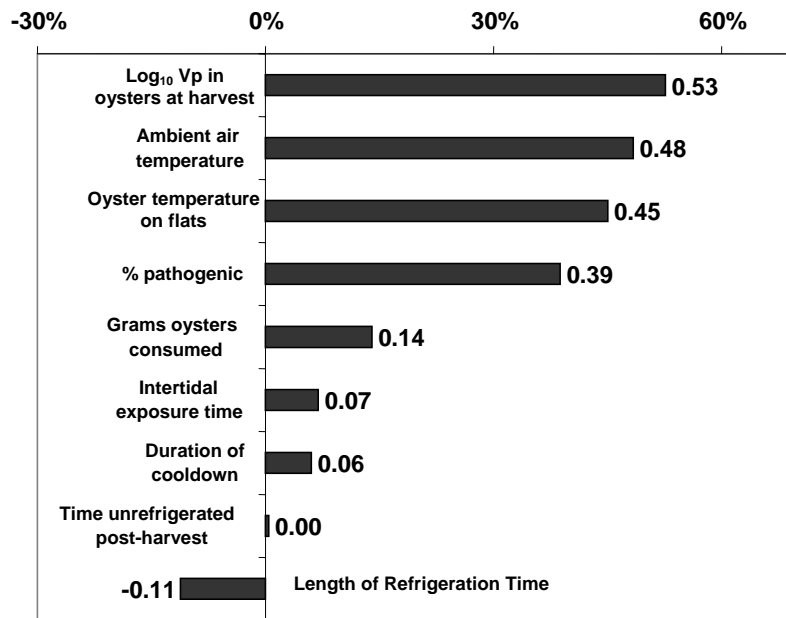
Figure A8-2. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (Louisiana) Spring Harvest
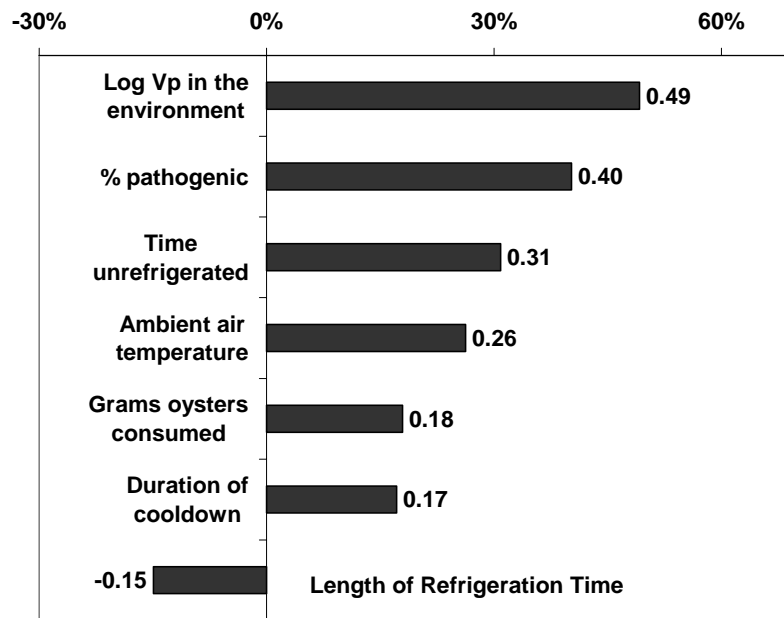
Figure A8-3. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (Louisiana) Summer Harvest
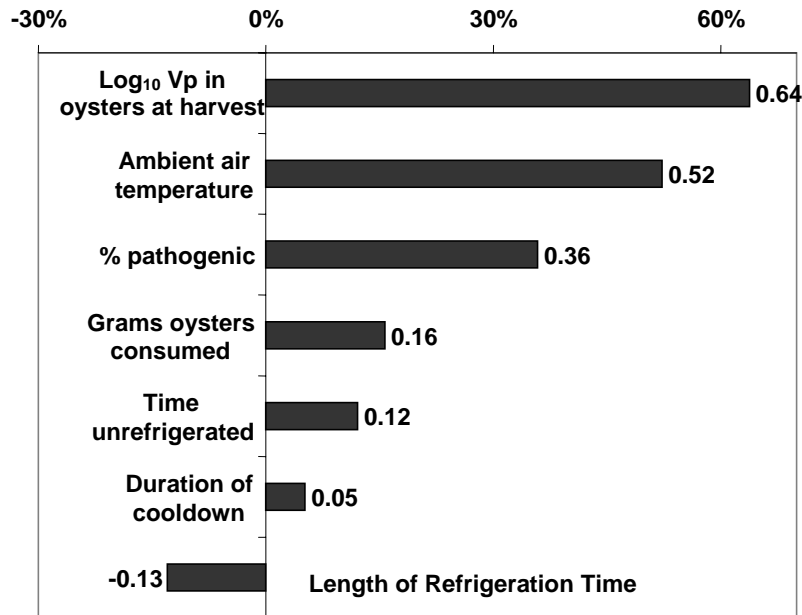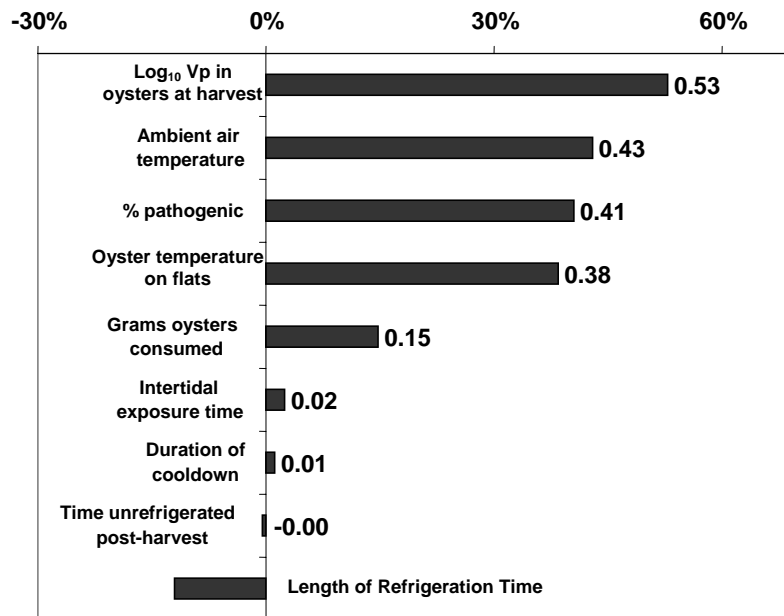
Figure A8-4. Tornado Plot of Influential Variability Parameters on log$_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (Louisiana) Fall Harvest
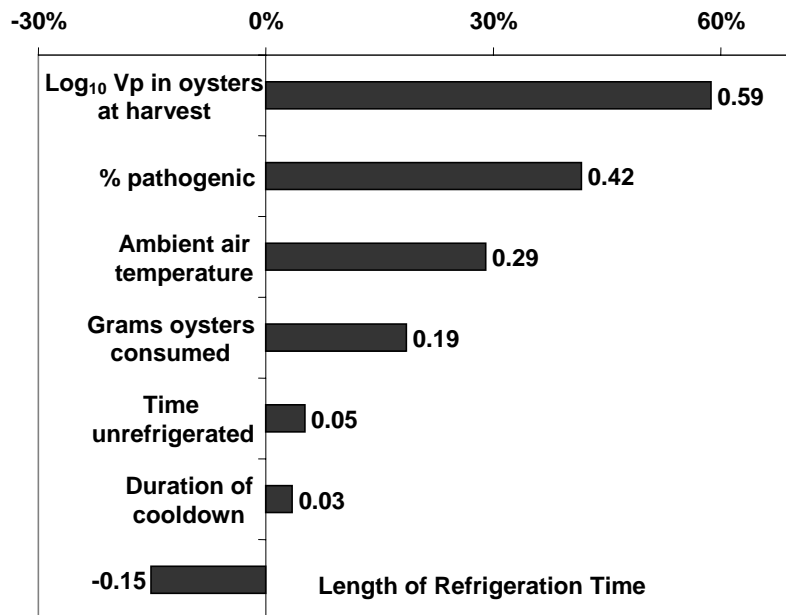
Figure A8-5. Tornado Plot of Influential Variability Parameters on log$_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (non-Louisiana) Winter Harvest
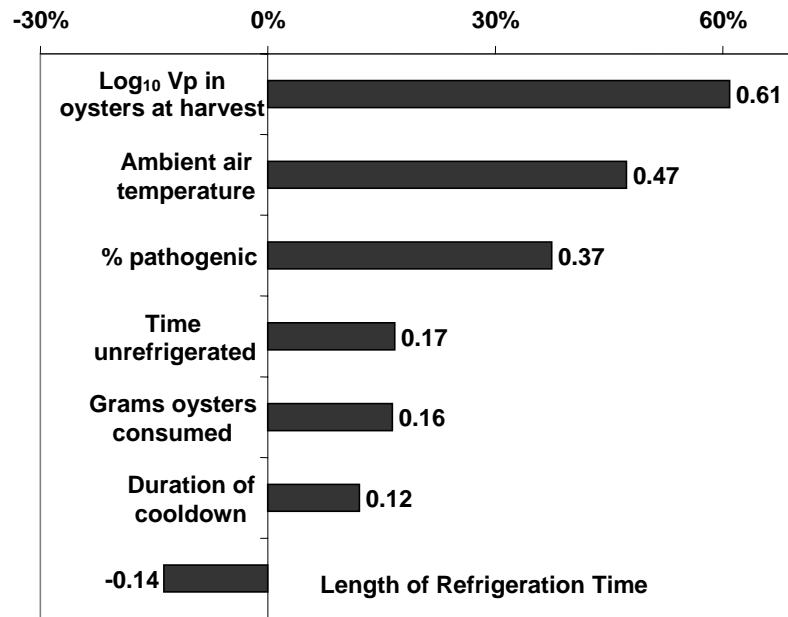
Figure A8-6. Tornado Plot of Influential Variability Parameters on log$_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (non-Louisiana) Spring Harvest
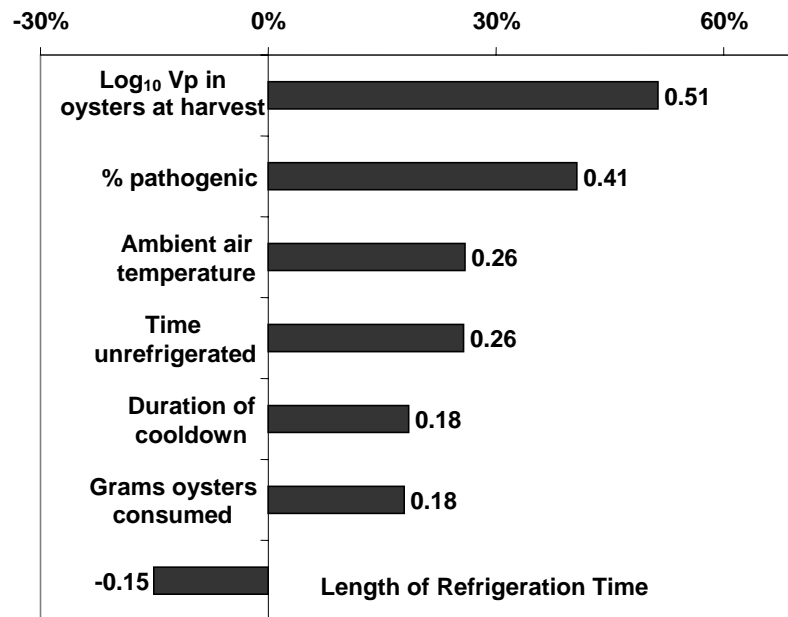
Figure A8-7. Tornado Plot of Influential Variability Parameters on log$_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (non-Louisiana) Summer Harvest
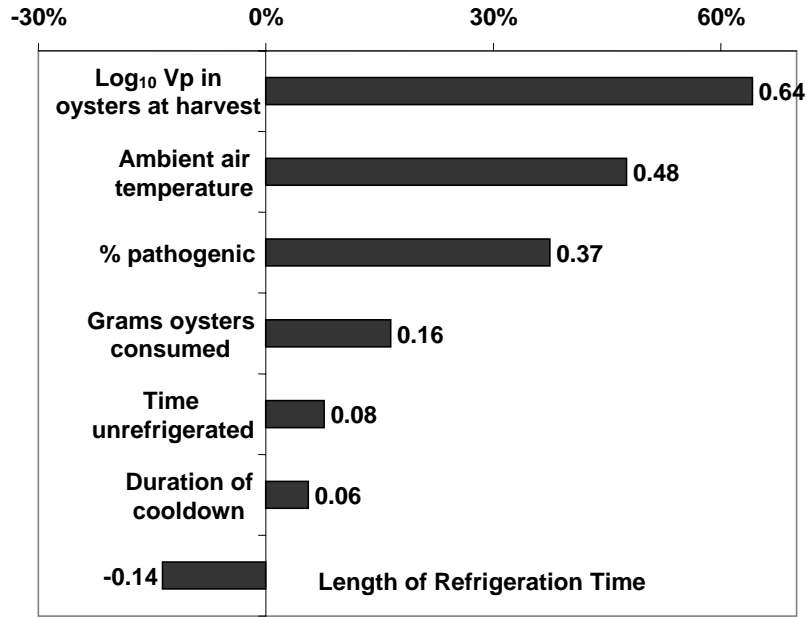
Figure A8-8. Tornado Plot of Influential Variability Parameters on log₁₀ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Gulf Coast (non-Louisiana) Fall Harvest
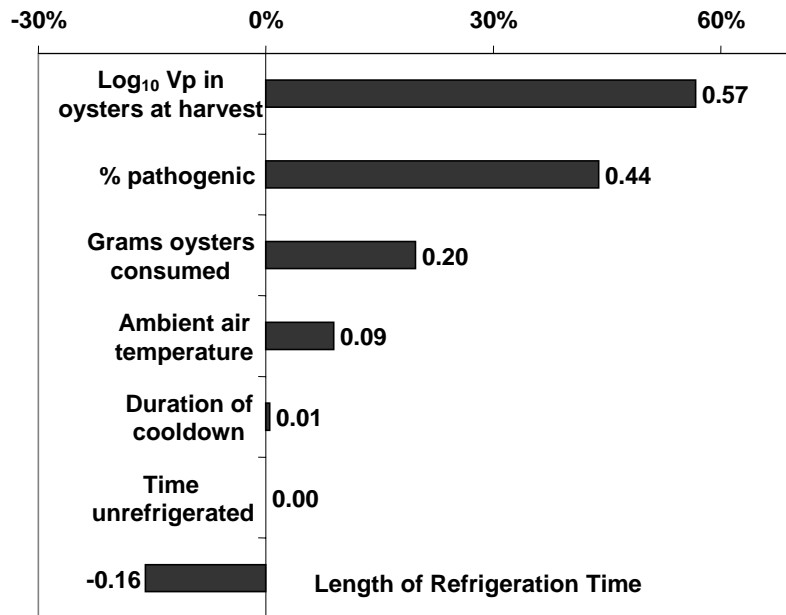
Figure A8-9. Tornado Plot of Influential Variability Parameters on log₁₀ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Mid-Atlantic Winter Harvest
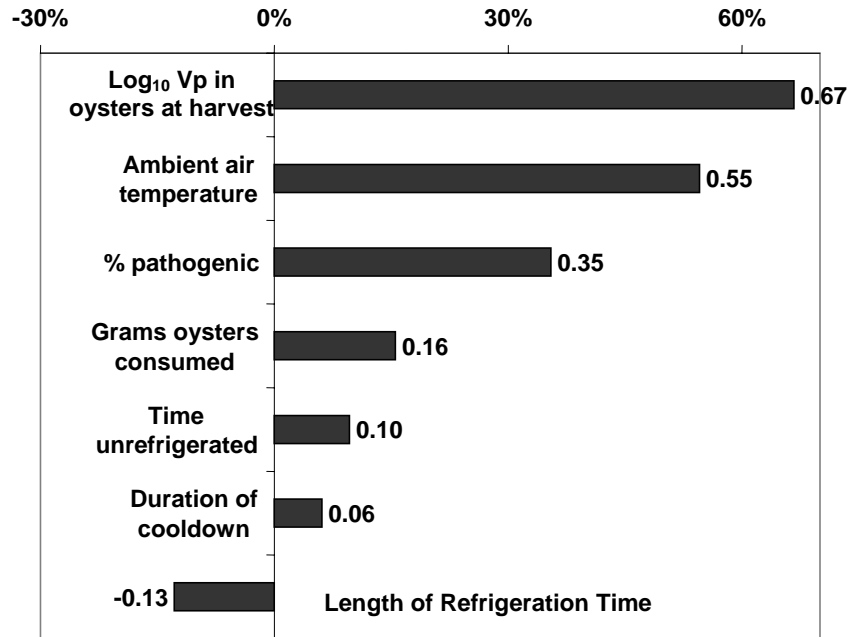
Figure A8-10. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Mid-Atlantic Spring Harvest
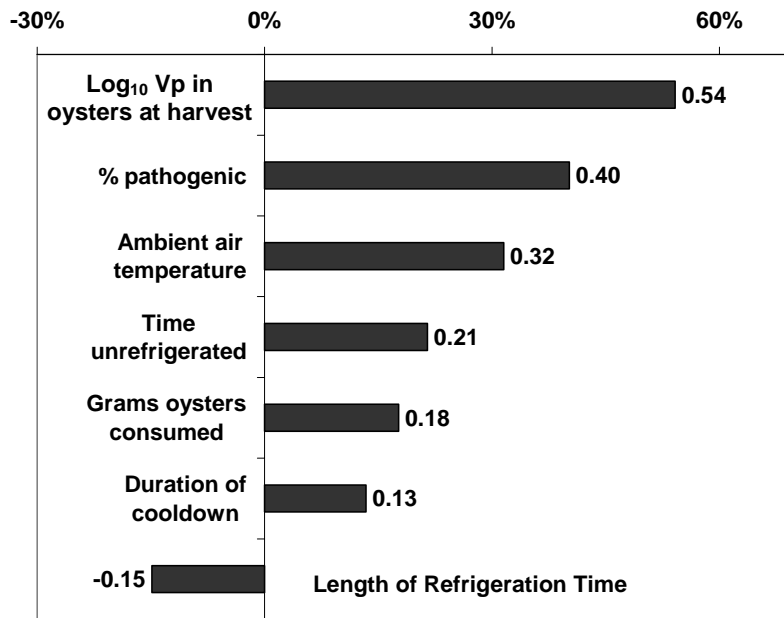
Figure A8-11. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Mid-Atlantic Summer Harvest
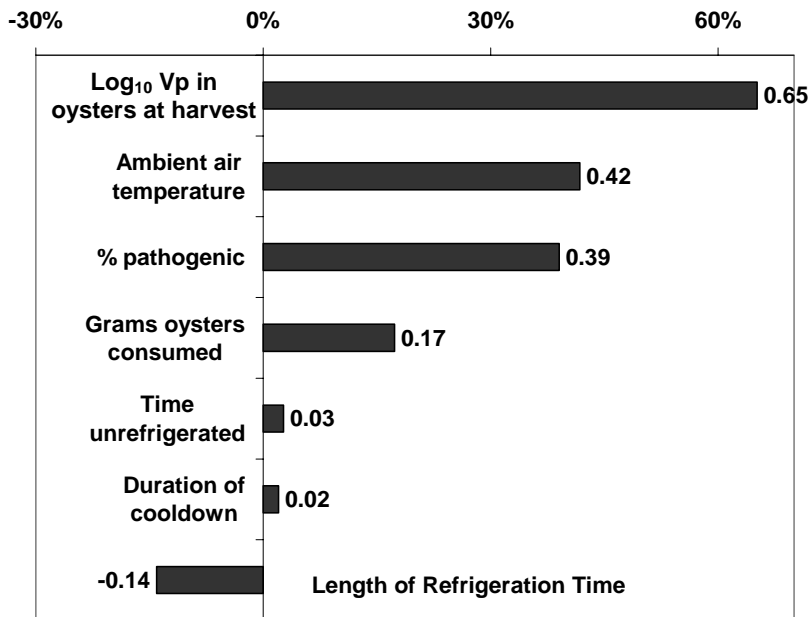
Figure A8-12. Tornado Plot of Influential Variability Parameters on $\log_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Mid-Atlantic Fall Harvest
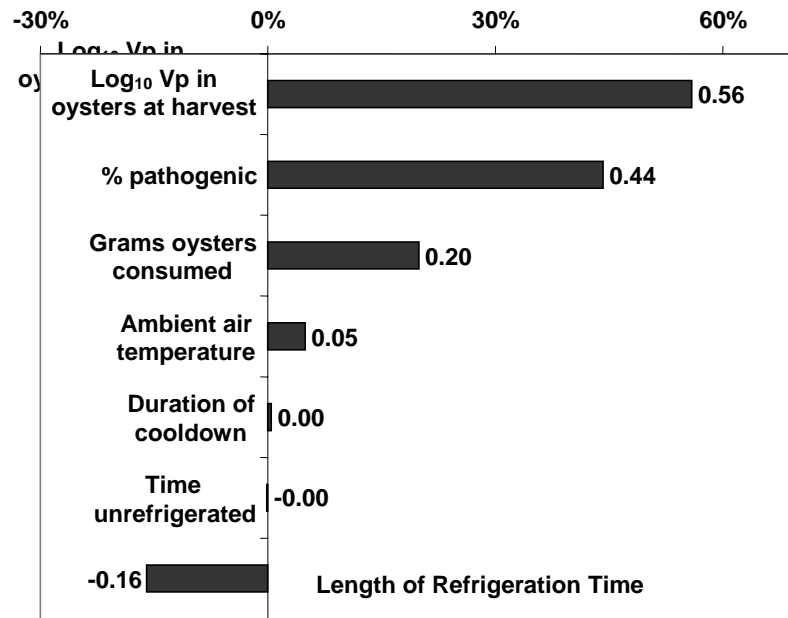
Figure A8-13. Tornado Plot of Influential Variability Parameters on log$_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Northeast Atlantic Winter Harvest
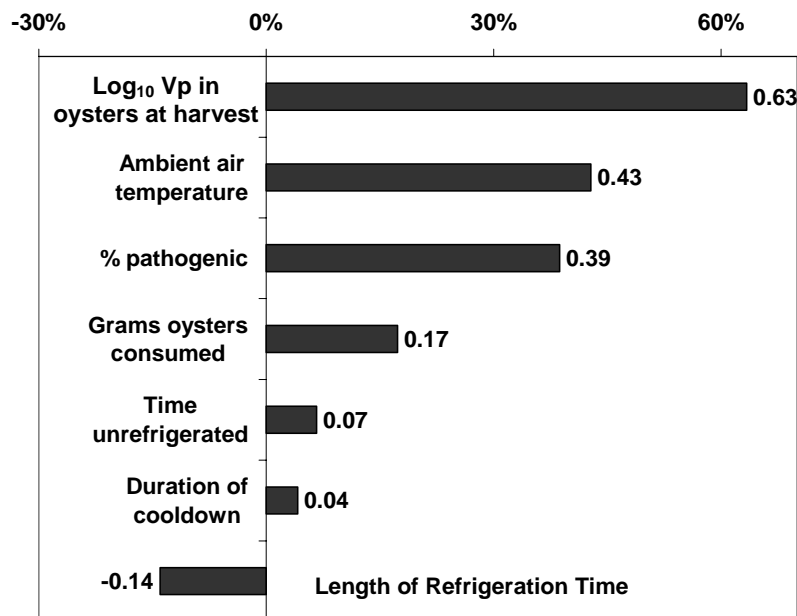
Figure A8-14. Tornado Plot of Influential Variability Parameters on log$_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Northeast Atlantic Spring Harvest
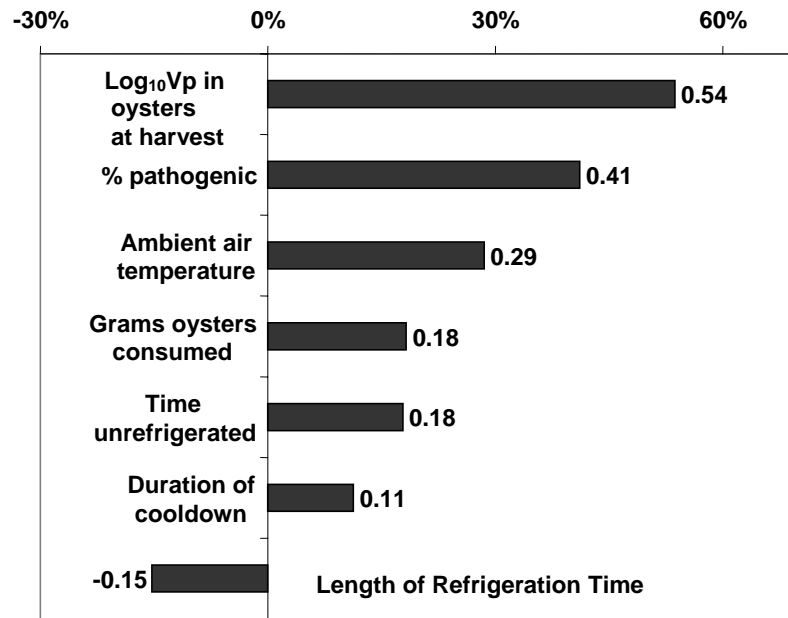
Figure A8-15. Tornado Plot of Influential Variability Parameters on log$_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Northeast Atlantic Summer Harvest
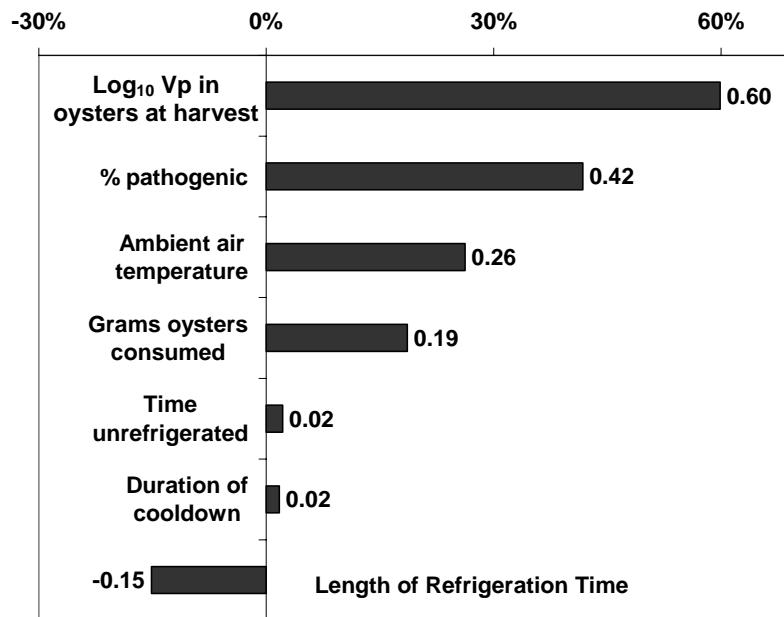
Figure A8-16. Tornado Plot of Influential Variability Parameters on log$_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Northeast Atlantic Fall Harvest

-30%   0%   30%   60%

% pathogenic � 0.46

Log$_{10}$ Vp in
oysters at harvest � 0.39

Grams oysters
consumed � 0.17

Ambient air
temperature � 0.17

Duration of
cooldown � 0.00

Time
unrefrigerated � -0.00

-0.14 � Length of Refrigeration Time
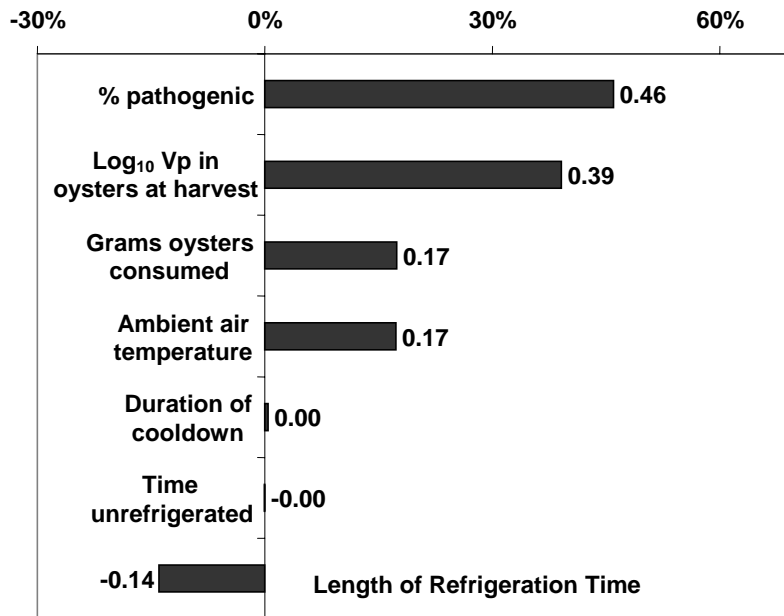
Figure A8-17. Tornado Plot of Influential Variability Parameters on log$_{10}$ Risk of *Vibrio parahaemolyticus* Illness per Serving of Raw Oysters from the Pacific Northwest (Dredged) Winter Harvest

-30%   0%   30%   60%

Log Vp in the
environment � 0.49

% pathogenic � 0.42

Ambient air
temperature � 0.38

Grams oysters
consumed � 0.16

Time
unrefrigerated � 0.05

Duration of
cooldown � 0.04

-0.13 � Length of Refrigeration Time

Figure A8-18. Tornado Plot of Influential Variability Parameters on log$_{10}$ 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 log$_{10}$ 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.
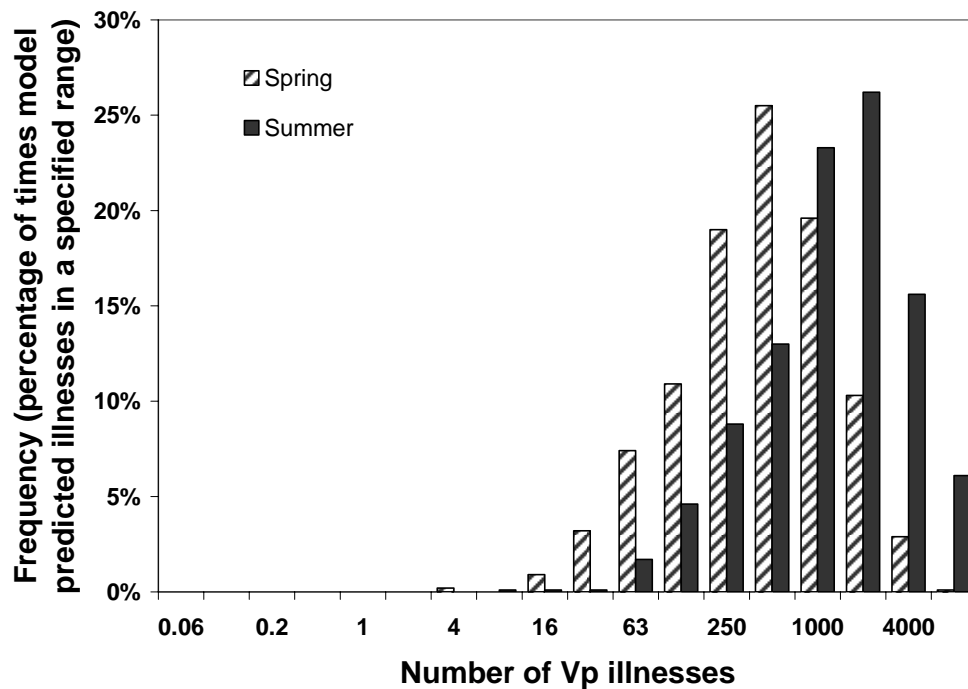
Figure A8-25. Uncertainty distributions of the annual number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Gulf Coast (Lousianna) Harvests.
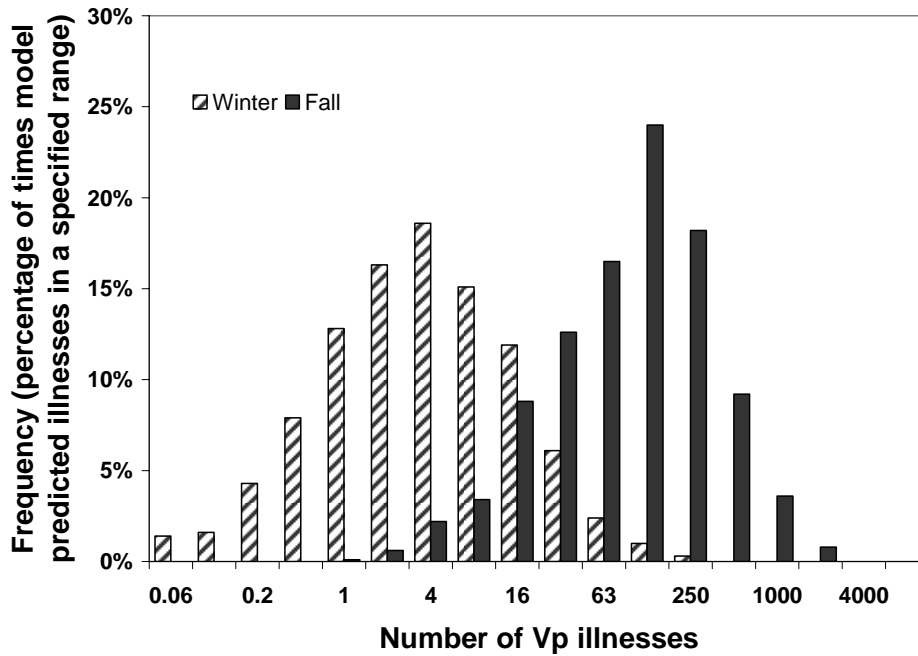
Figure A8-26. Uncertainty distributions of the annual number of *Vibrio parahaemolyticus* Illnesses Associated with Fall and Winter Gulf Coast (Lousianna) Harvests.
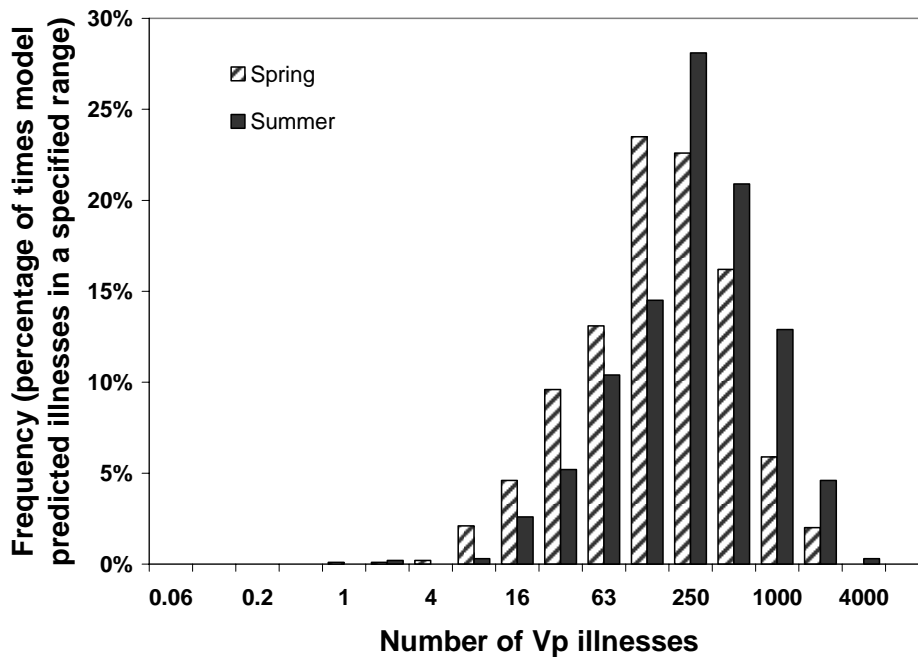
Figure A8-27. Uncertainty distributions of the annual number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Gulf Coast (Non-Lousianna) Harvests.
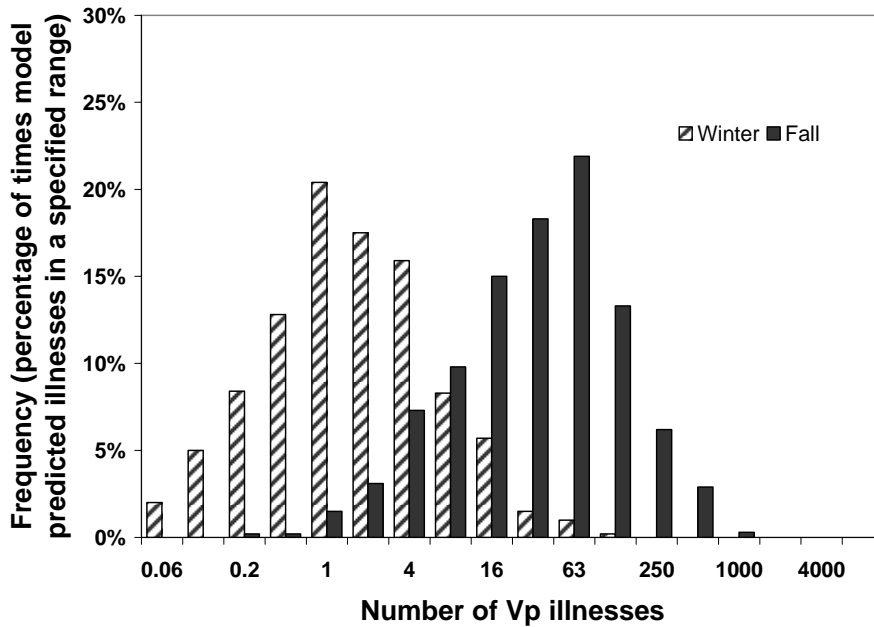
Figure A8-28. Uncertainty distributions of the annual number of *Vibrio parahaemolyticus* Illnesses Associated with Fall and Winter Gulf Coast (Non-Lousianna) Harvests.
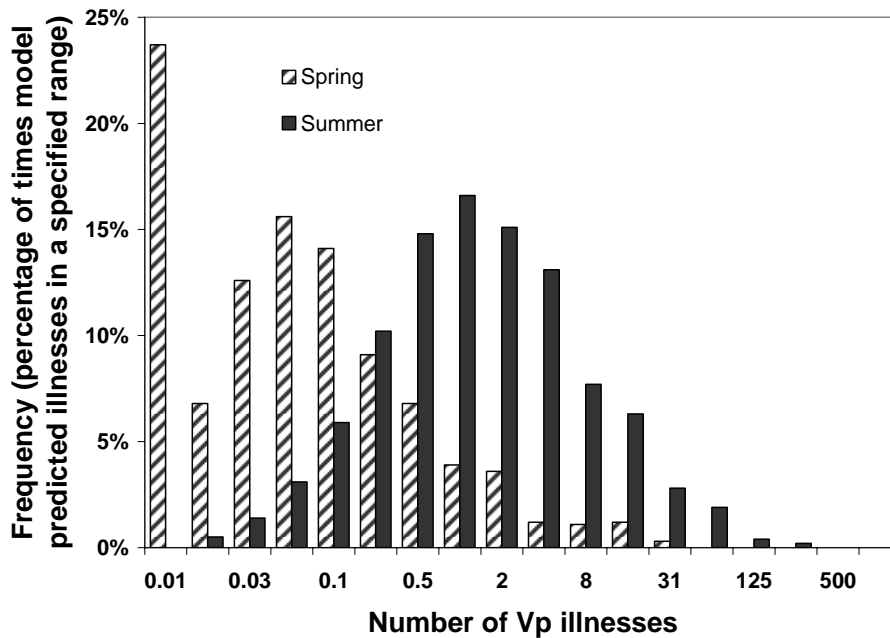
Figure A8-29. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Pacific Coast (dredged) Harvests.
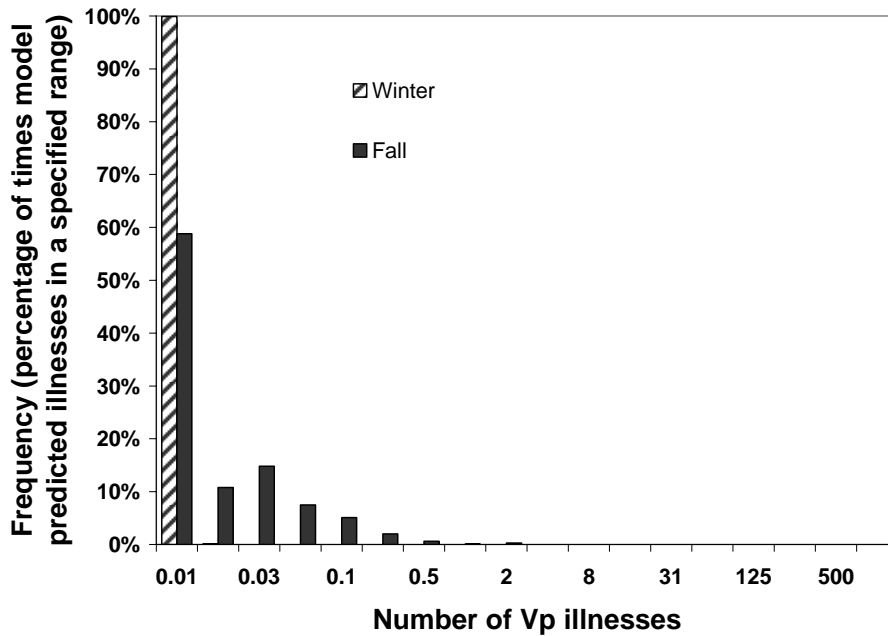
Figure A8-30. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Fall and Winter Pacific Coast (dredged) Harvests.
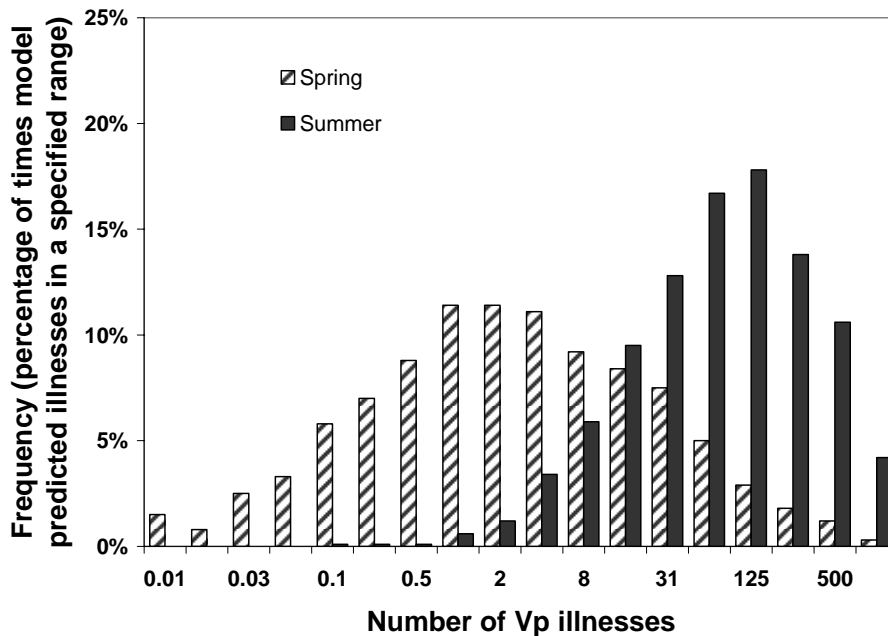
Figure A8-31. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Pacific Coast (intertidal) Harvests.
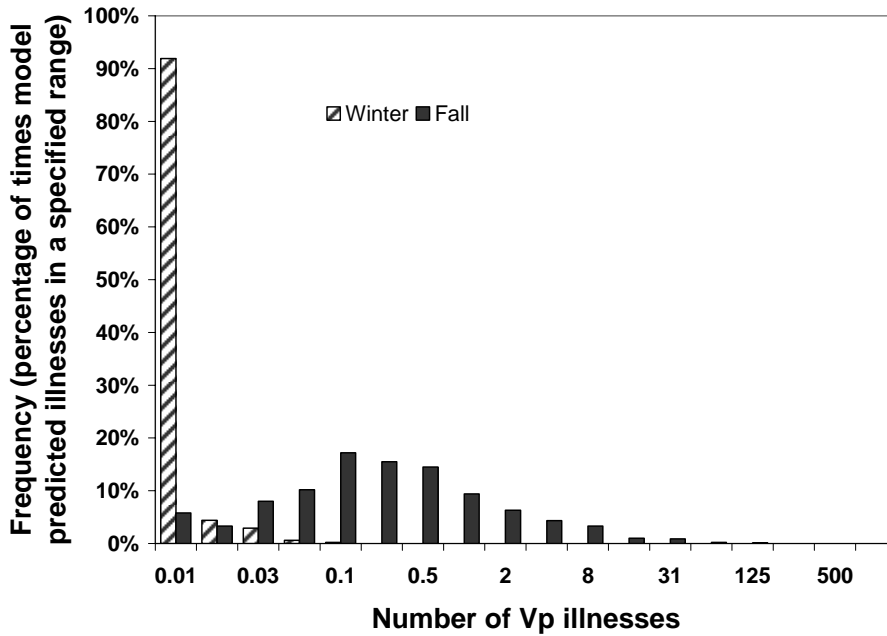
Figure A8-32. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Fall and Winter Pacific Coast (intertidal) Harvests.
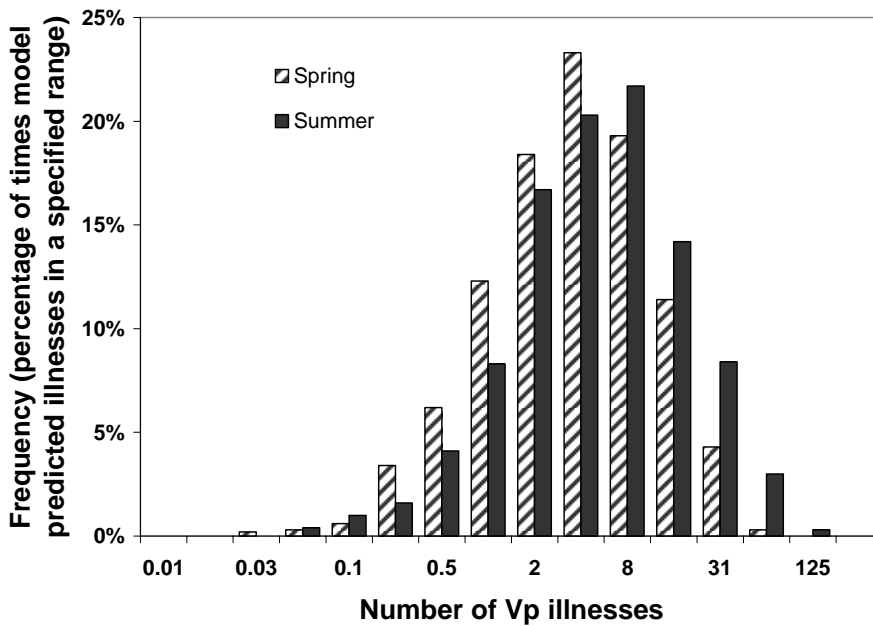
Figure A8-33. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Mid-Atlantic Harvests.
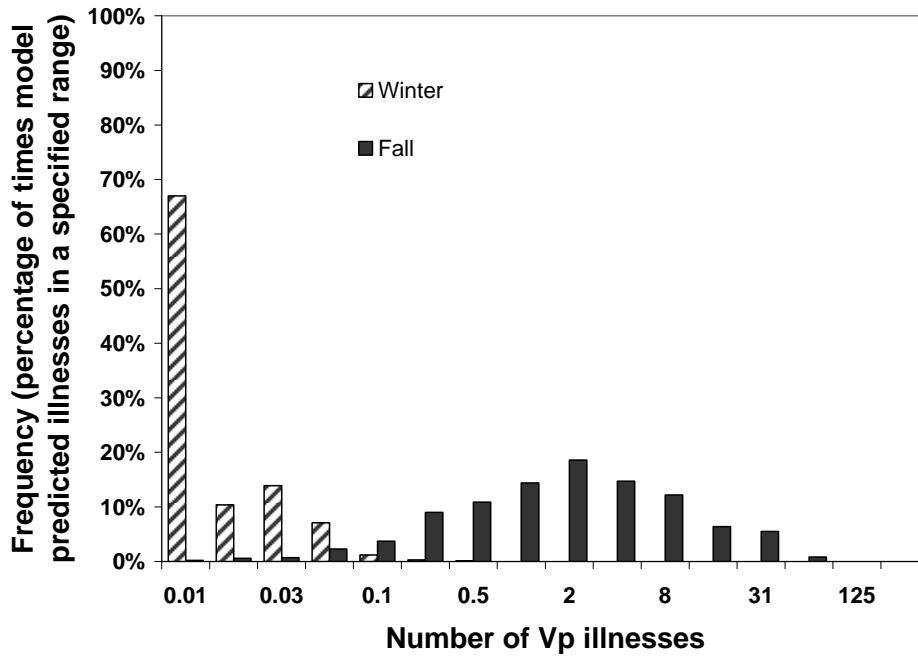
Figure A8-34. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Fall and Winter Mid-Atlantic Harvests.
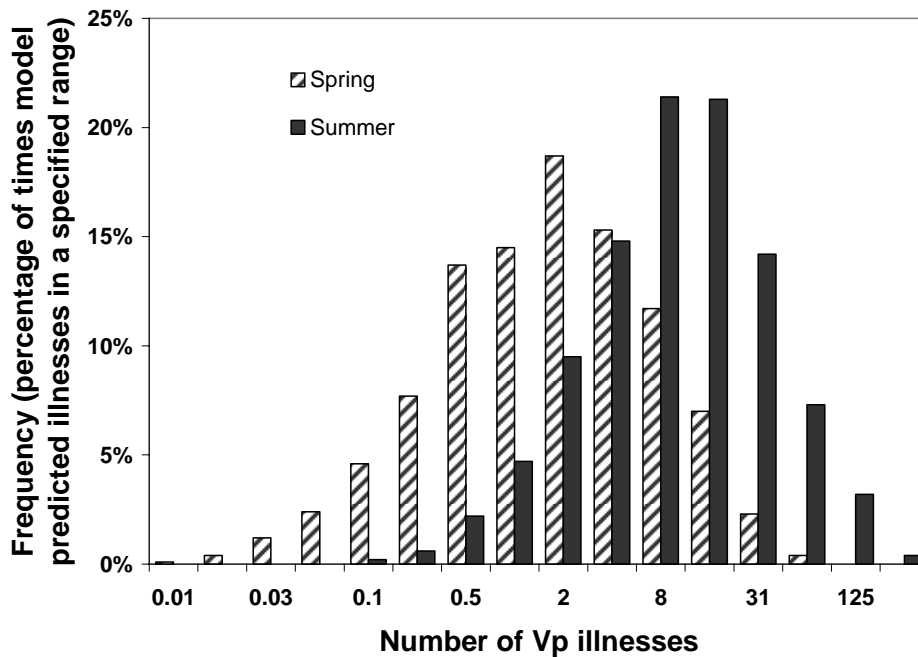
Figure A8-35. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Spring and Summer Northeast Atlantic Harvests.
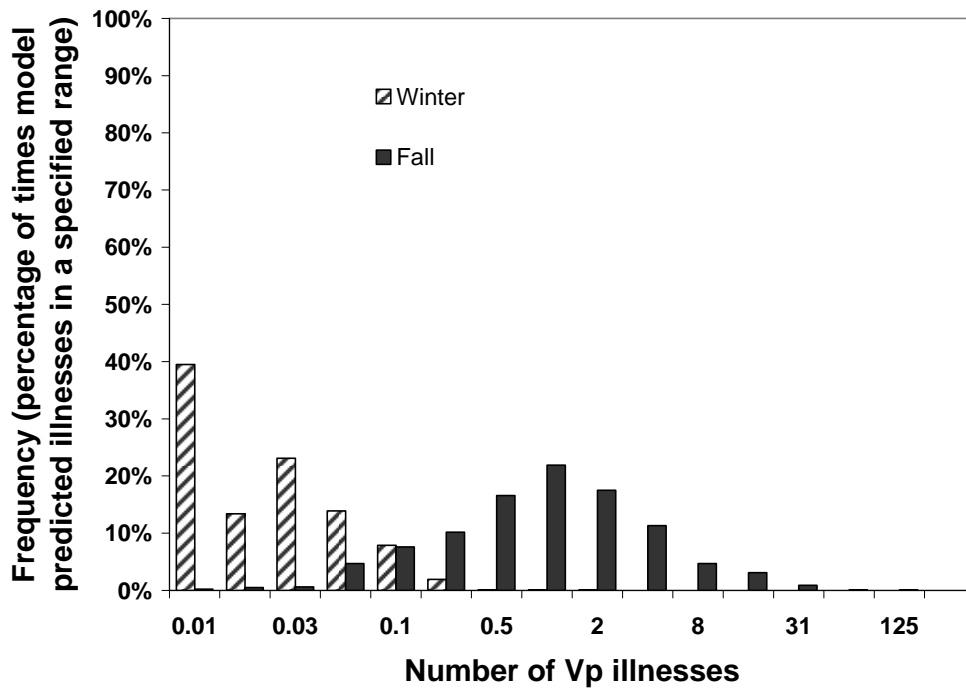
Figure A8-36. Uncertainty Distributions of the Annual Number of *Vibrio parahaemolyticus* Illnesses Associated with Fall and Winter Northeast Atlantic Harvests.

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 Coast[c]	26.4%
Pacific Coast[d]	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 Coast[d]	7%	93%	0%	0%
Pacific Coast[e]	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

Patient Residence	Oyster Harvest Region[a]			
	Atlantic[b]	Gulf Coast	Pacific Northwest[c]	Other Pacific
Atlantic[b]	6.6%	11.5%	3.2%	0%
Gulf Coast[d]	1.8%	24.6%	0%	0%
Pacific Coast[e]	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)	$4.4x10^{-4}$ ($2.1x10^{-5}$, $1.6x10^{-3}$)

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
Pacific Northwest (Dredged)	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})
	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

Region	Season	Predicted Mean Levels of Pathogenic *Vibrio parahaemolyticus* per gram[a]			
		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	7.9×10^3 (2.3×10^3, 1.8×10^4)	840 (170, 2.4×10^3)	78 (22, 180)	0.25 (0.072, 0.57)
	Summer	720	2.1×10^4 (7.5×10^3, 4.4×10^4)	2.0×10^3 (470, 5.8×10^3)	210 (73, 440)	0.66 (0.23, 1.4)
	Fall	80	2.0×10^3 (320, 5.1×10^3)	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)	3.2×10^{-3} (2.0×10^{-4}, 0.012)
Gulf Coast (Non-Louisiana)	Spring	320	5.6×10^3 (1.5×10^3, 1.3×10^4)	850 (170, 2.4×10^3)	56 (15, 130)	0.18 (0.048, 0.41)
	Summer	720	1.5×10^4 (4.9×10^3, 3.2×10^4)	2.0×10^3 (480, 5.7×10^3)	150 (49, 320)	0.47 (0.15, 1.0)
	Fall	80	880 (110, 2.5×10^3)	130 (19, 430)	8.6 (1.1, 25)	0.027 (3.7×10^{-3}, 0.08)
	Winter	18	47 (5.1, 160)	12 (2.7, 35)	0.46 (0.054, 1.5)	1.5×10^{-3} (1.0×10^{-4}, 4.9×10^{-3})
Mid-Atlantic	Spring	66	1.5×10^3 (330, 3.5×10^3)	180 (27, 550)	15 (3.1, 35)	0.047 (0.01, 0.11)
	Summer	260	4.3×10^3 (750, 1.1×10^4)	520 (92, 1.5×10^3)	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)	3.2×10^{-3} (2.0×10^{-4}, 0.012)
	Winter	1.2	0.48 (0.09, 1.2)	0.46 (0.08, 1.1)	4.9×10^{-3} (7.0×10^{-4}, 0.012)	1.5×10^{-5} (0.0, 1.0×10^{-4}
Northeast Atlantic	Spring	14	180 (12, 620)	20 (2.9, 59)	1.8 (0.13, 6.5)	5.7×10^{-3} (3.0×10^{-4}, 0.02)
	Summer	78	860 (130, 2.5×10^3)	100 (22, 300)	8.5 (1.4, 23)	0.027 (4.2×10^{-3}, 0.074)
	Fall	12	17 (2.4, 57)	6.1 (1.4, 16)	0.20 (0.024, 0.68)	6.4×10^{-4} (0.0, 2.4×10^{-3})
	Winter	1.2	0.5 (0.09, 1.2)	0.47 (0.085, 1.2)	4.9×10^{-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, 2.1×10^3)	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)	9.9×10^{-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 (3.9×10^{-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, 3.7×10^4)	380 (1.9, 2.0×10^3)	7.1 (0.025, 40)	0.022 (8.0×10^{-5}, 0.13)
	Summer	3.0×10^3	7.5×10^3 (370, 3.0×10^4)	4.1×10^3 (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 (5.6×10^{-3}, 0.47)	4.4×10^{-4} (2.0×10^{-5}, 1.5×10^{-3})
	Winter	0.18	0.08 (0.01, 0.28)	0.075 (6.6×10^{-3}, 0.26)	8.0×10^{-4} (7.0×10^{-5}, 2.8×10^{-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	1.7×10^{-9}
	Spring	1.3×10^{-5}
	Summer	1.4×10^{-4}
	Fall	3.9×10^{-7}
Overnight Submersion of Intertidal Harvest[a]	Winter	8.1×10^{-10}
	Spring	8.7×10^{-7}
	Summer	1.0×10^{-5}
	Fall	2.7×10^{-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	1.6×10^3	2.5×10^3	3.8×10^3	2.3×10^4
	summer	2.3×10^3	3.8×10^3	6.1×10^3	9.1×10^3	6.0×10^4
	fall	170	270	400	610	5.7×10^3
Gulf non-Louisiana	winter	26	31	36	42	140
	spring	970	1.6×10^3	2.4×10^3	3.4×10^3	1.6×10^4
	summer	2.3×10^3	3.8×10^3	5.8×10^3	8.3×10^3	4.2×10^4
	fall	180	270	380	530	2.5×10^3
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	2.5×10^3
	fall	14	16	18	20	52
Mid-Atlantic	winter	1.3	1.3	1.3	1.3	1.4
	spring	190	320	500	750	4.2×10^3
	summer	680	1.0×10^3	1.5×10^3	2.1×10^3	1.2×10^4
	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	1.7×10^3
	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	1.1×10^3	1.9×10^3	2.9×10^3	4.4×10^3	4.6×10^4
	summer	3.8×10^3	6.8×10^3	1.1×10^4	1.8×10^4	2×10^5
	fall	160	210	280	370	2.8×10^3
Gulf non-Louisiana	winter	35	39	44	48	84
	spring	1.2×10^3	1.9×10^3	2.8×10^3	3.9×10^3	2.6×10^4
	summer	3.8×10^3	6.7×10^3	1.1×10^4	1.6×10^4	1.2×10^5
	fall	160	210	270	330	1.0×10^3
Northeast Atlantic	winter	2.3	2.3	2.3	2.3	2.3
	spring	27	33	39	45	100
	summer	240	330	440	560	2.5×10^3
	fall	18	19	21	22	28
Mid-Atlantic	winter	2.1	2.1	2.1	2.1	2.2
	spring	140	190	260	330	1.3×10^3
	summer	990	1.5×10^3	2.2×10^3	3.1×10^3	2.2×10^4
	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] ^aLevels 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	4.0×10^3	6.2×10^3	8.9×10^3	1.2×10^4	2.2×10^4
	summer	9.8×103	1.5×10^4	2.3×10^4	3.1×10^4	6.0×10^4
	fall	620	950	1.4×10^3	1.9×10^3	5.7×10^3
Gulf non-Louisiana	winter	43	55	68	82	140
	spring	4.0×10^3	6.1×10^3	8.6×10^3	1.1×10^4	1.6×10^4
	summer	9.8×10^3	1.5×10^4	2.2×10^4	2.8×10^4	4.2×10^4
	fall	620	930	1.3×10^3	1.7×10^3	2.5×10^3
Northeast Atlantic	winter	1.4	1.4	1.4	1.4	1.5
	spring	90	140	200	270	510
	summer	460	670	930	1.2×10^3	2.5×10^3
	fall	21	25	30	35	52
Mid-Atlantic	winter	1.3	1.3	1.4	1.4	1.4
	spring	860	1.3×10^3	1.9×10^3	2.5×10^3	4.2×10^3
	summer	2.4×10^3	3.7×10^3	5.2×10^3	6.9×10^3	1.2×10^4
	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	1.2×10^3	1.4×10^3	1.5×10^3	1.6×10^3	1.7×10^3
	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	4.3×10^3	7.3×10^3	1.2×10^4	1.8×10^4	4.7×10^4
	summer	1.9×10^4	3.4×10^4	5.5×10^4	8.3×10^4	2.0×10^5
	fall	340	470	650	880	2.8×10^3
Gulf non-Louisiana	winter	48	56	63	70	84
	spring	4.3×10^3	7.2×10^3	1.1×10^4	1.6×10^4	2.6×10^4
	summer	1.9×10^4	3.3×10^4	5.3×10^4	7.5×10^4	1.3×10^5
	fall	340	470	610	760	1.0×10^3
Northeast Atlantic	winter	2.3	2.3	2.3	2.3	2.3
	spring	45	57	68	80	100
	summer	590	840	1.1×10^3	1.5×10^3	2.5×10^3
	fall	22	23	25	26	28
Mid-Atlantic	winter	2.1	2.2	2.2	2.2	2.2
	spring	330	480	650	830	1.3×10^3
	summer	3.3×10^3	5.3×10^3	7.9×10^3	1.1×10^4	2.2×10^4
	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)
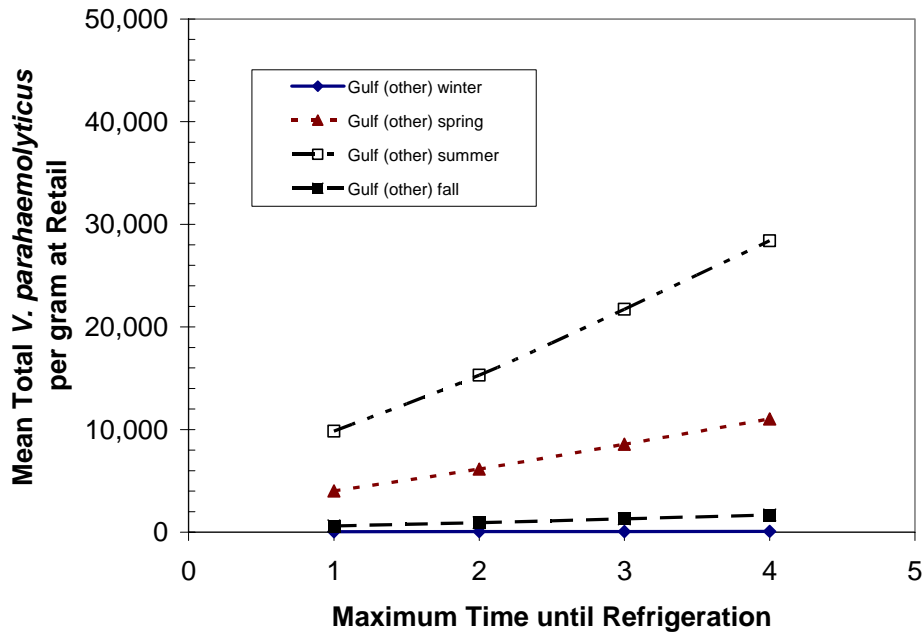
Figure A10-1. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Non-Louisiana Harvest).
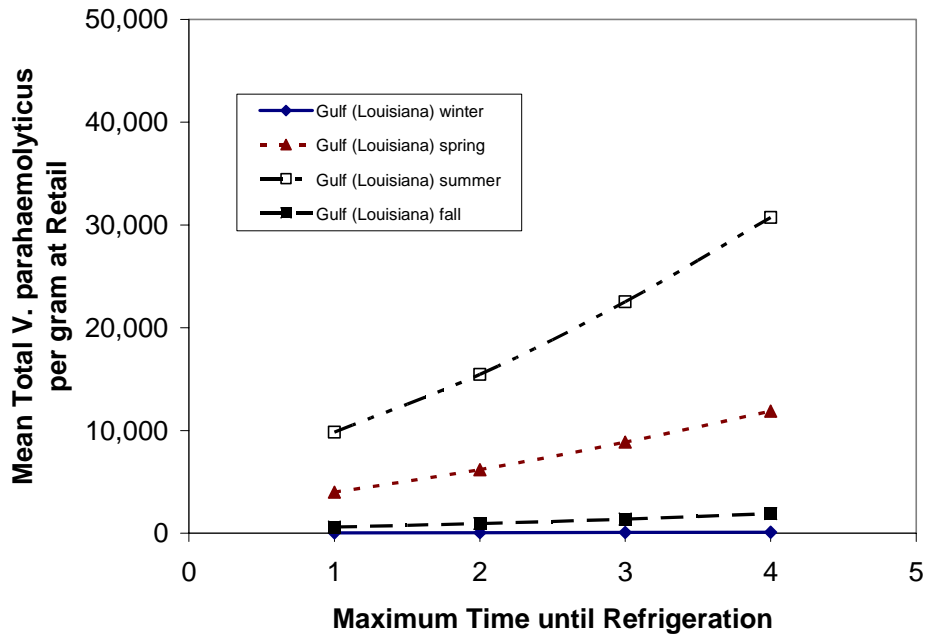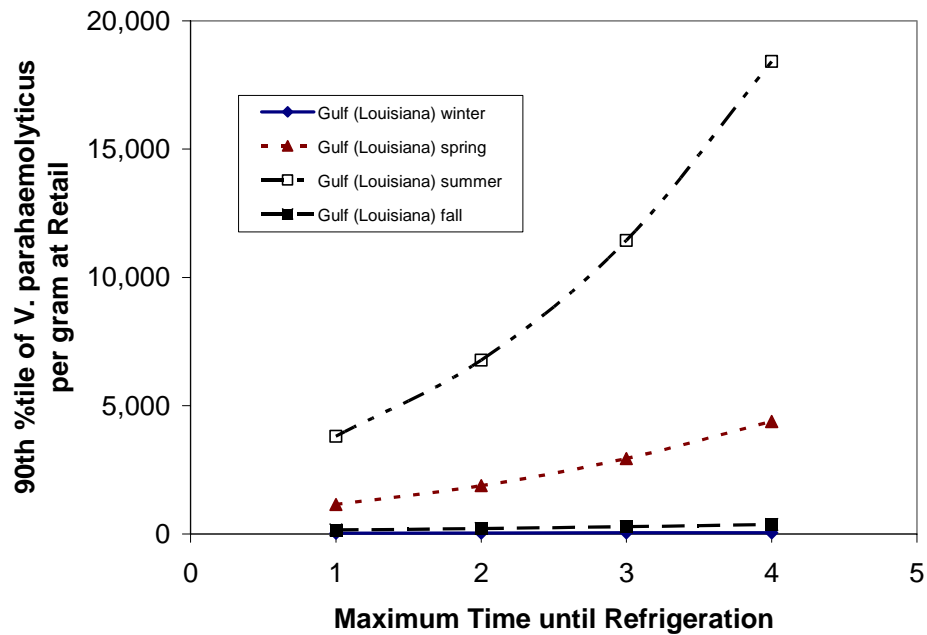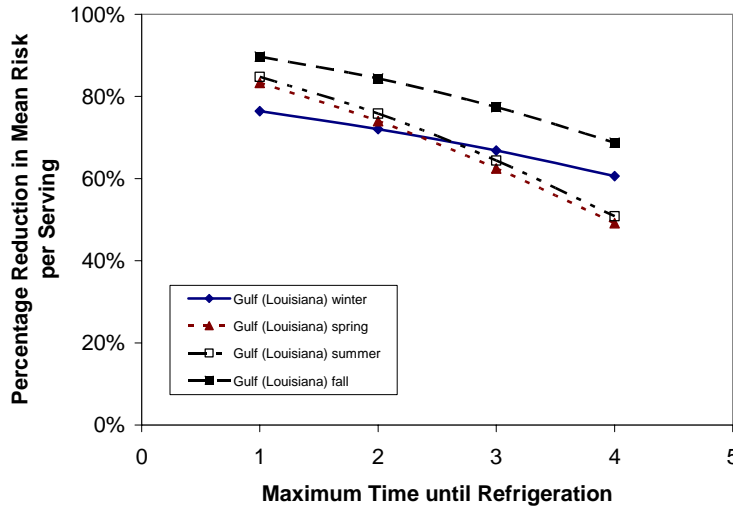
Figure A10-2. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest).
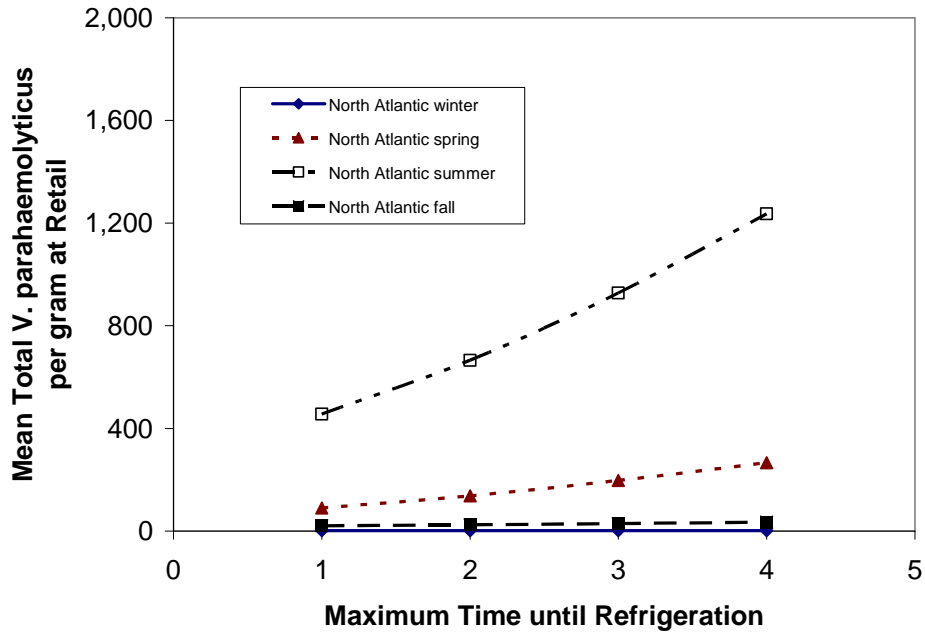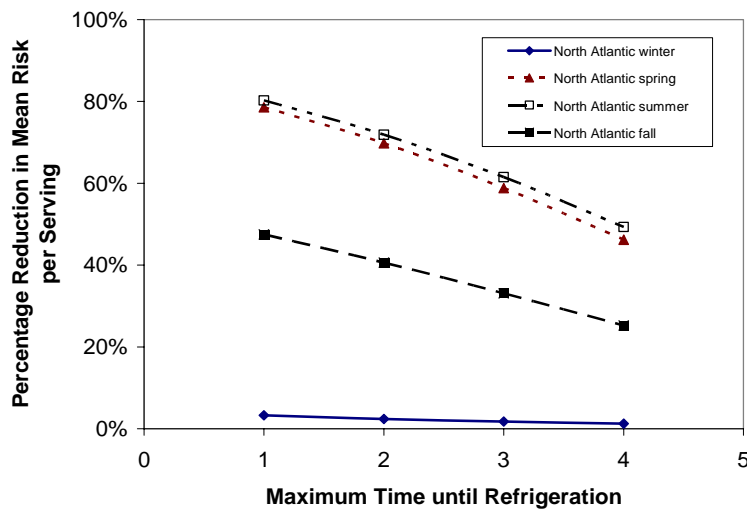

Figure A10-3. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).
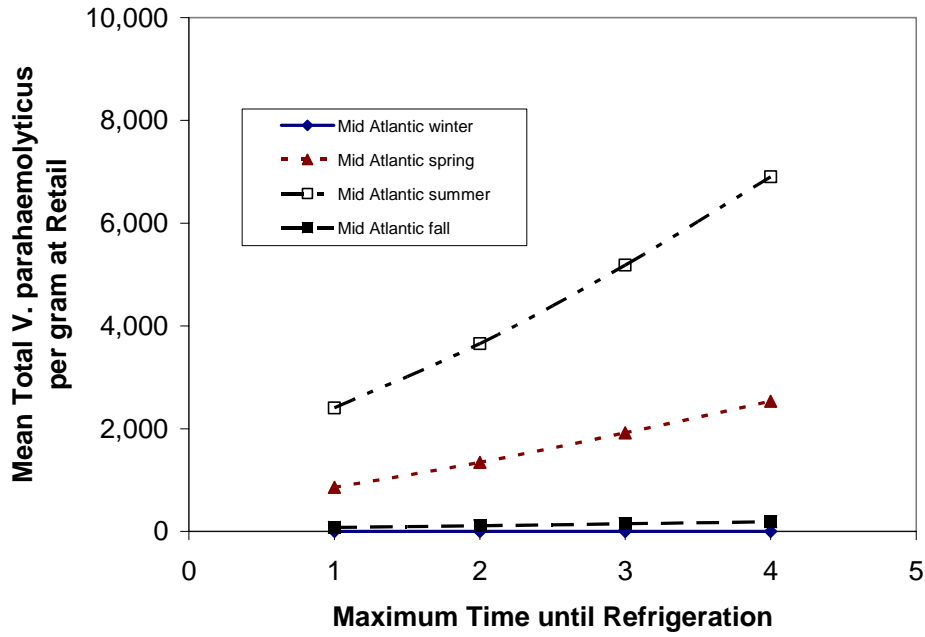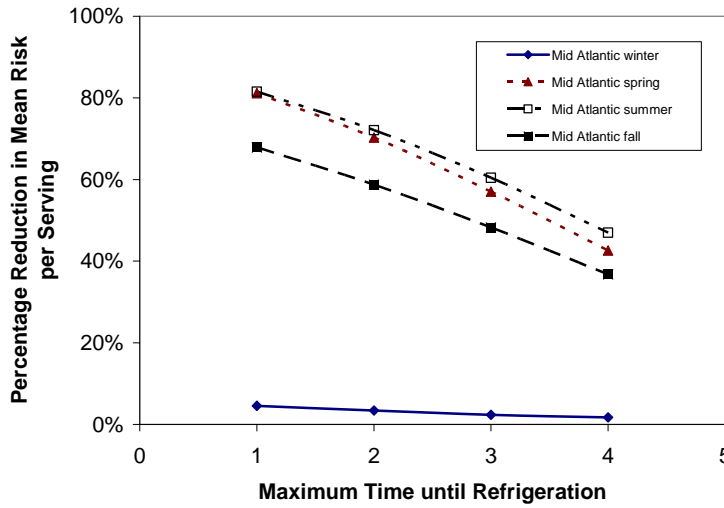
Figure A10-4. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Mid-Atlantic Harvest)
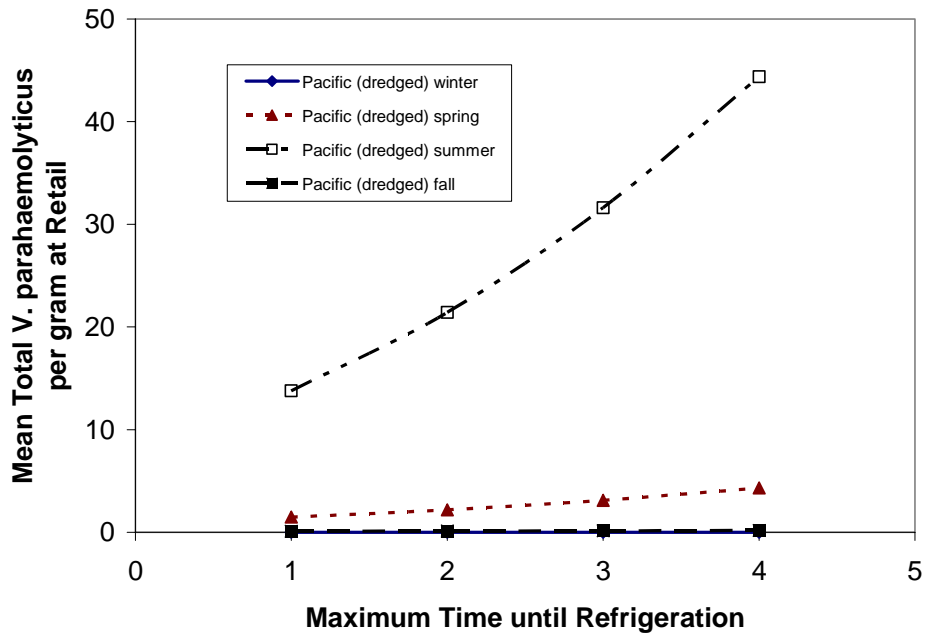
Figure A10-5. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Dredged Harvest).
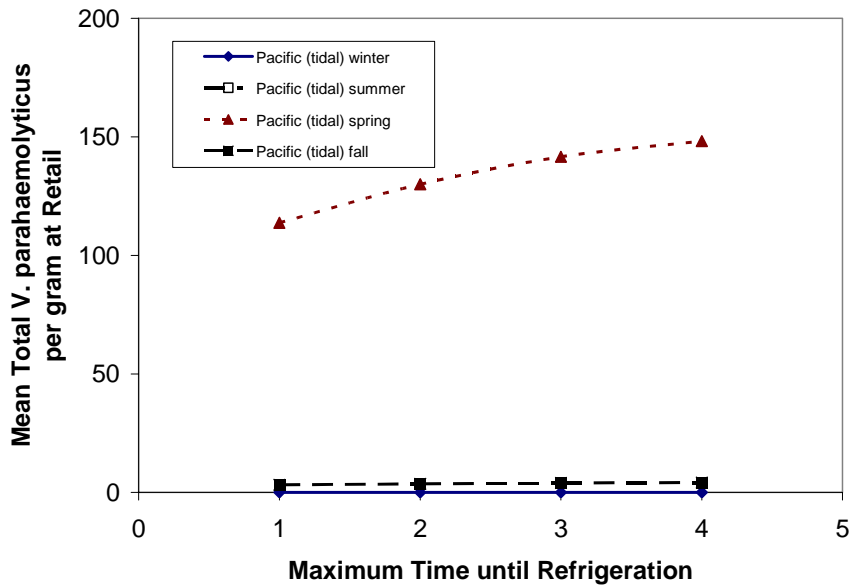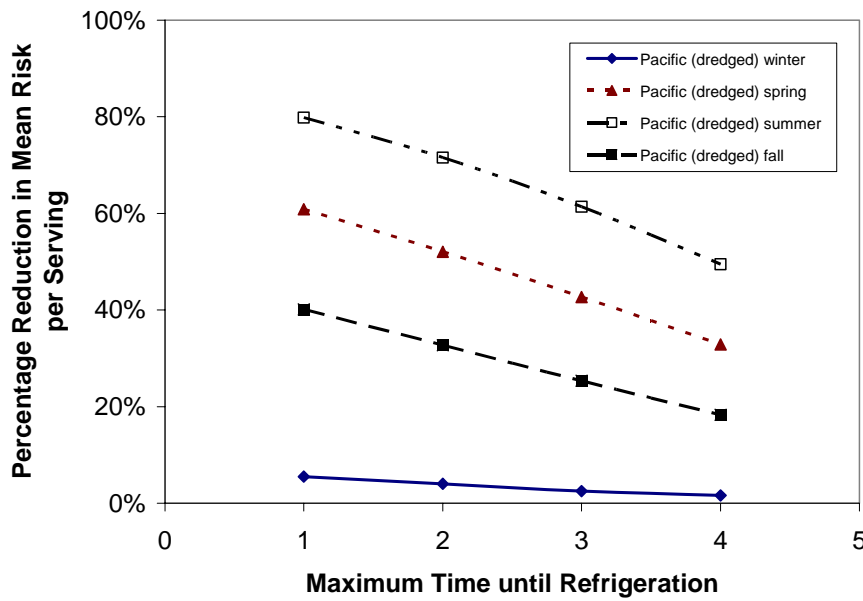
Figure A10-6. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Intertidal Harvest).
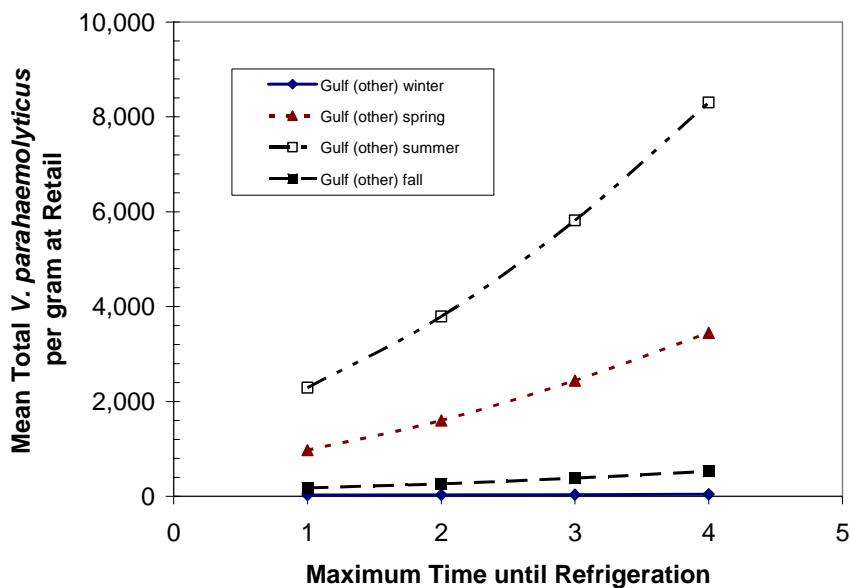
Figure A10-7. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Non-Louisiana Harvest).
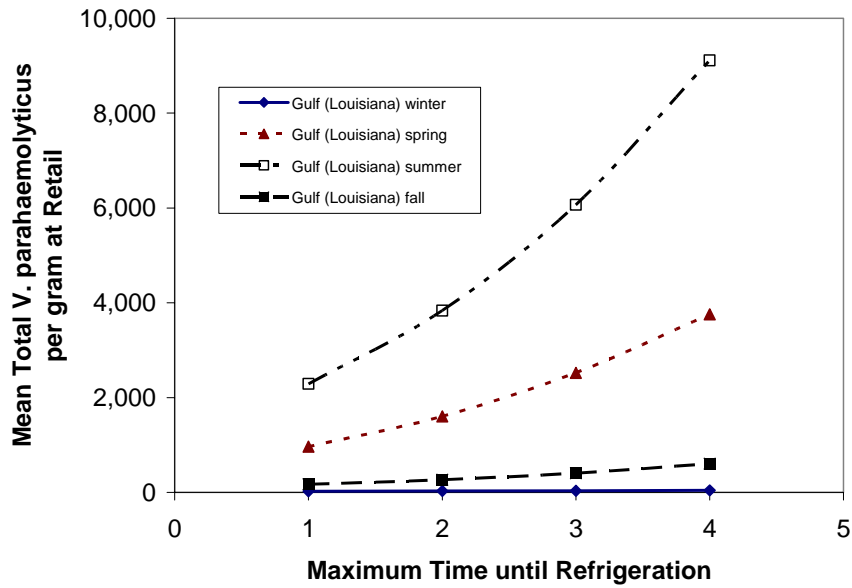
Figure A10-8. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest).
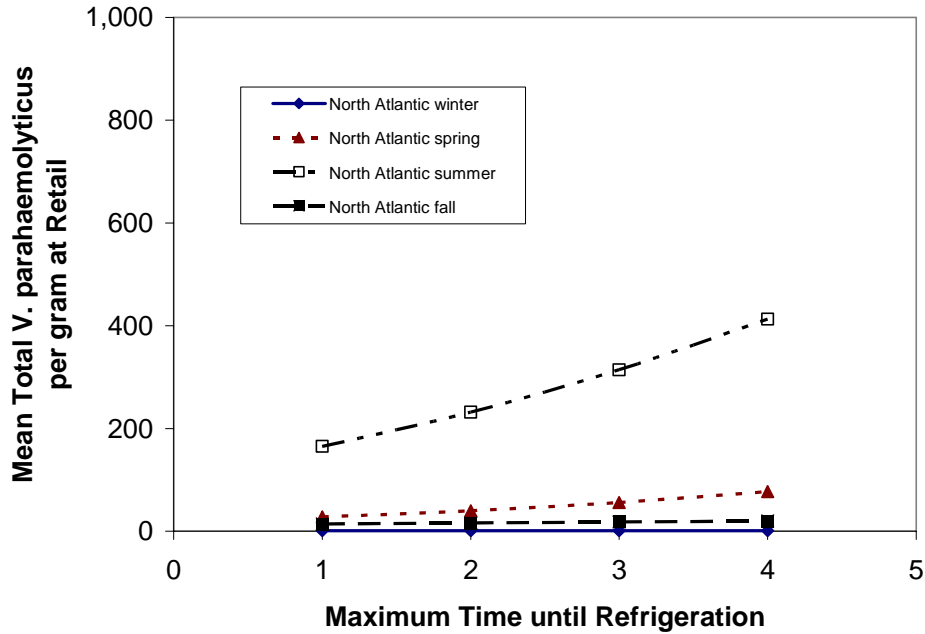
Figure A10-9. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).
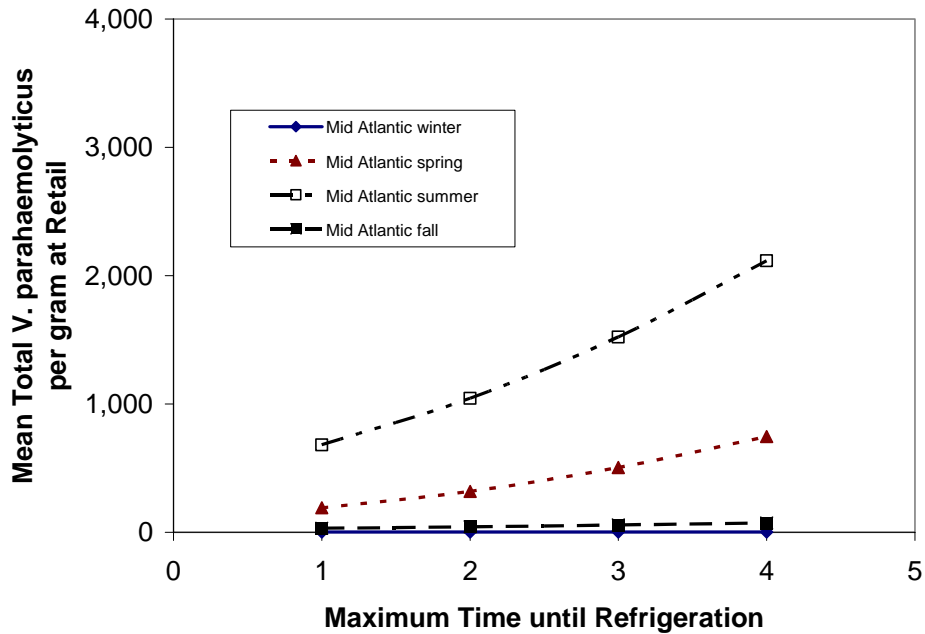
Figure A10-10. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Mid-Atlantic Harvest).
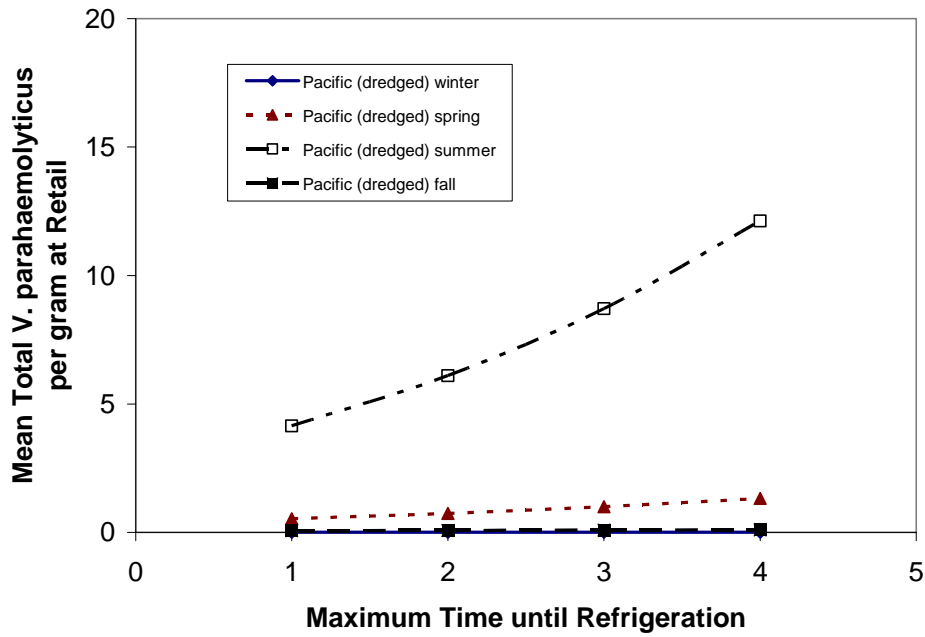
Figure A10-11. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Pacific Northwest Dredged Harvest).
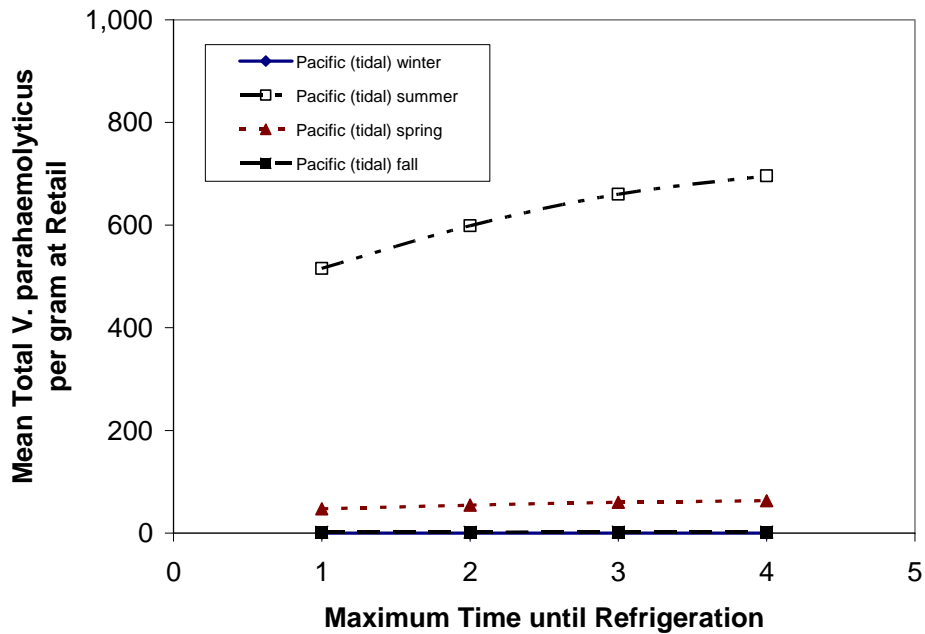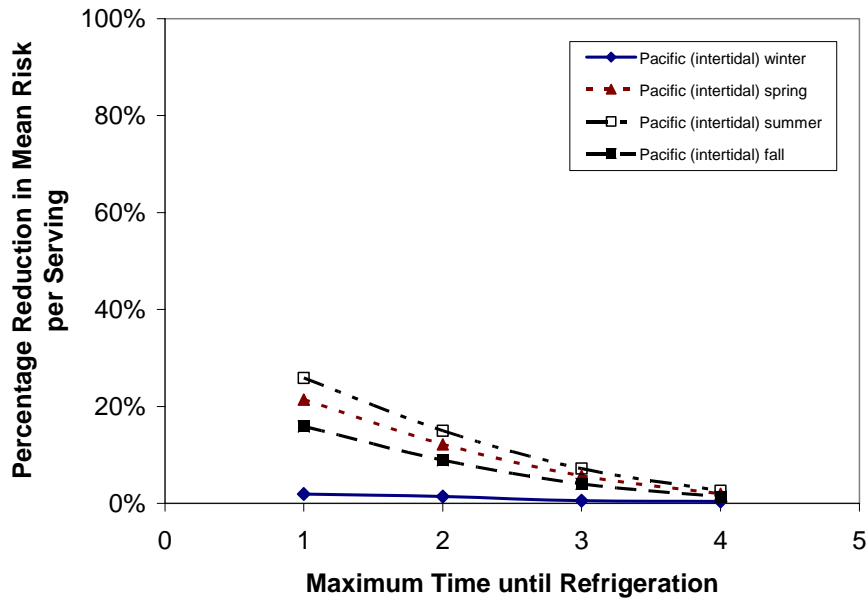

Figure A10-12. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Pacific Northwest Intertidal Harvest).

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)
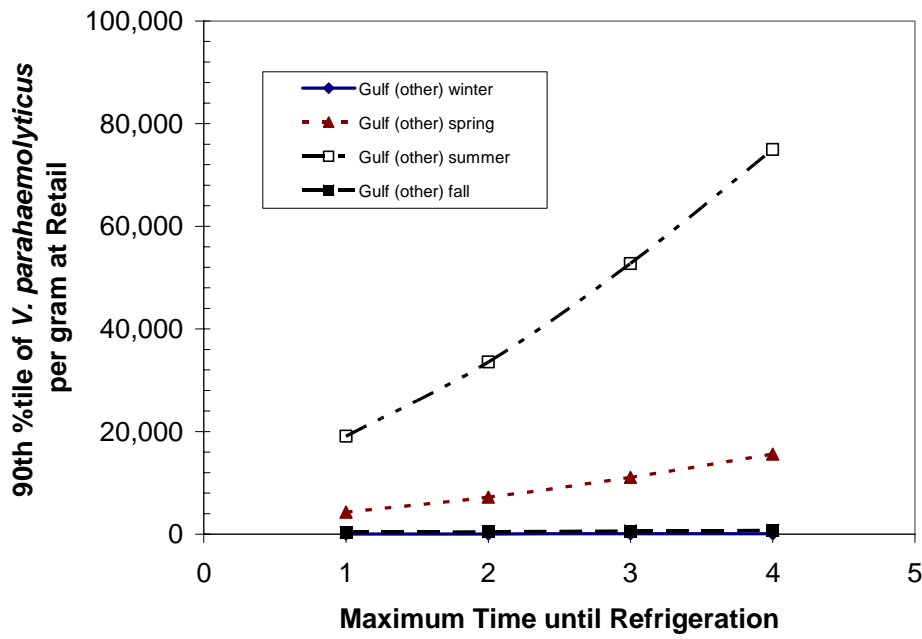
Figure A10-13. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Non-Louisiana Harvest).
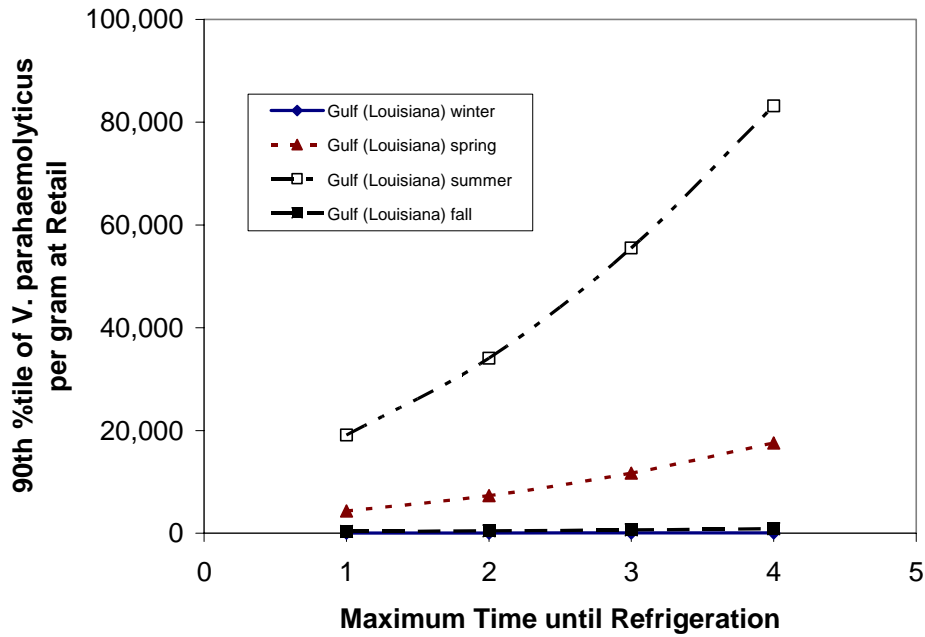
Figure A10-14. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest).
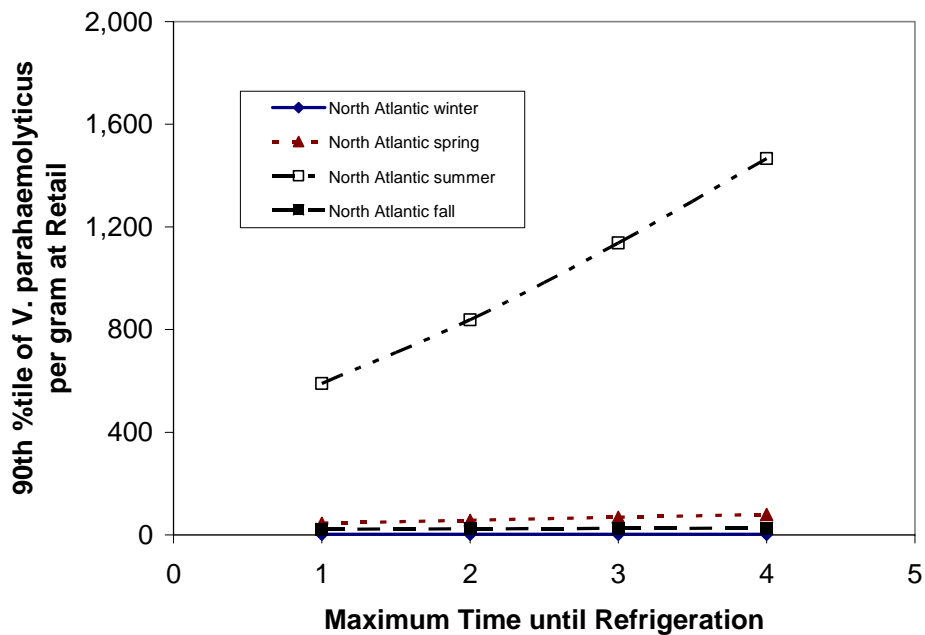
Figure A10-15. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).
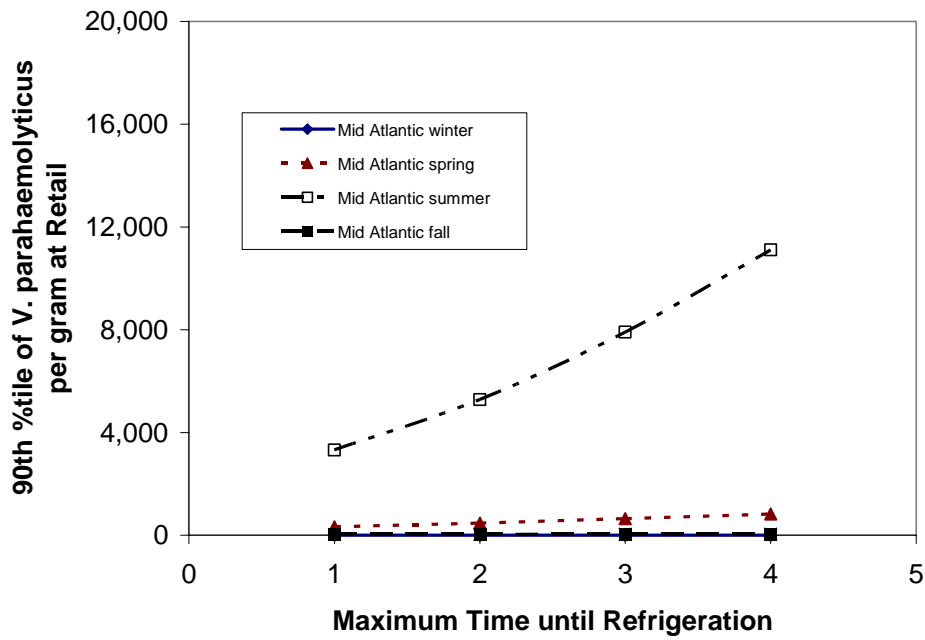
Figure A10-16. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Mid-Atlantic Harvest).
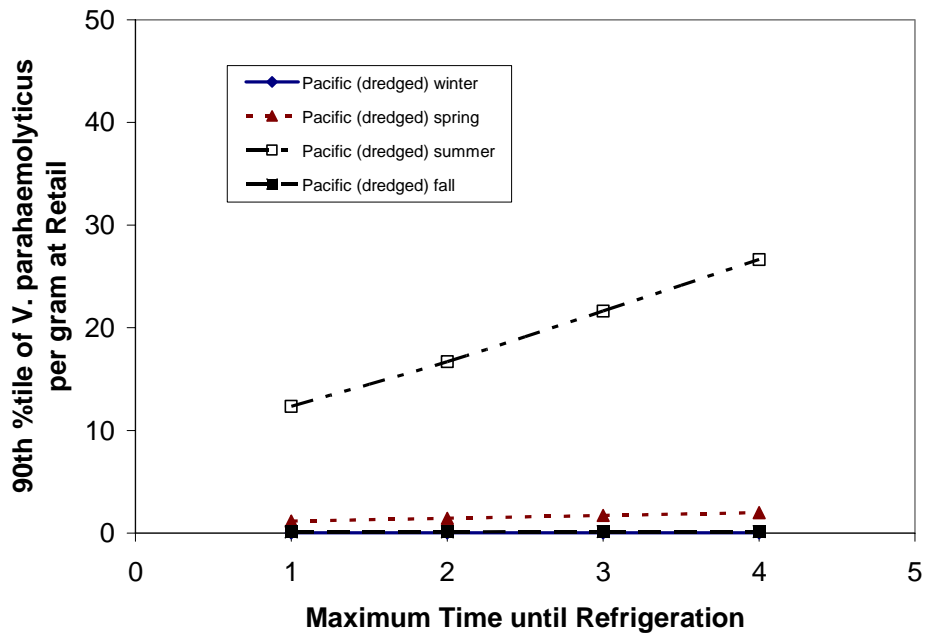
Figure A10-17. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Dredged Harvest).
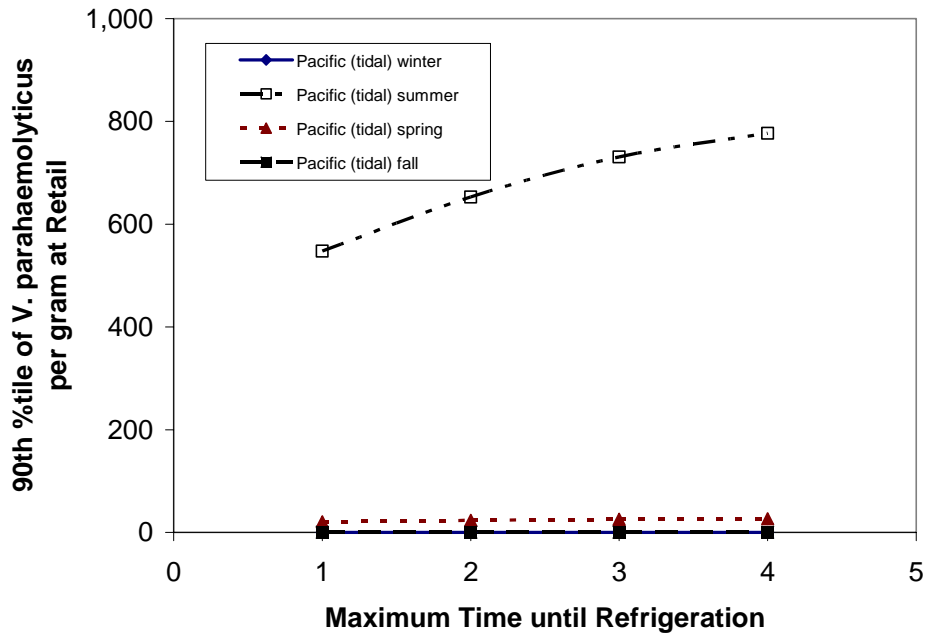
Figure A10-18. Predicted Effect of Maximum Time to Refrigeration with Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Intertidal Harvest).
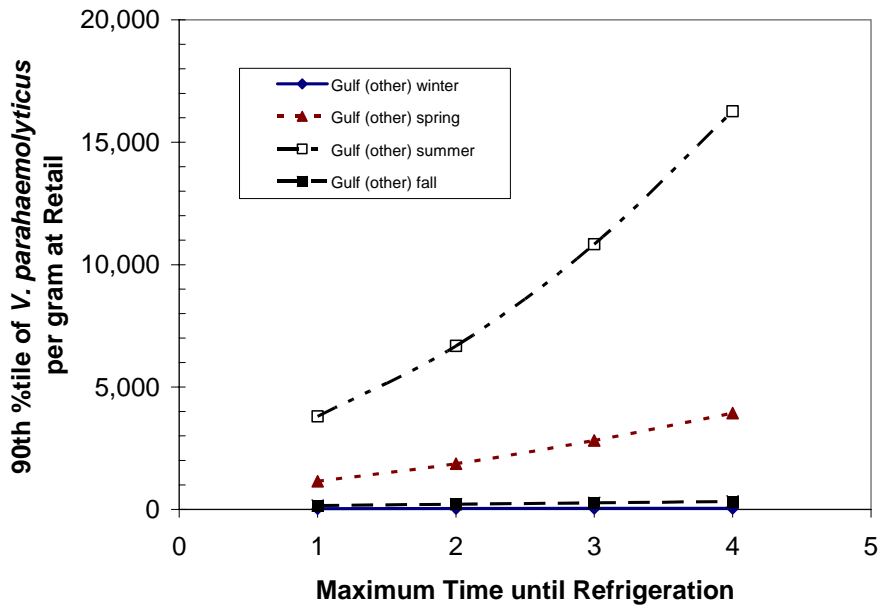
Figure A10-19. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Non-Louisiana Harvest).
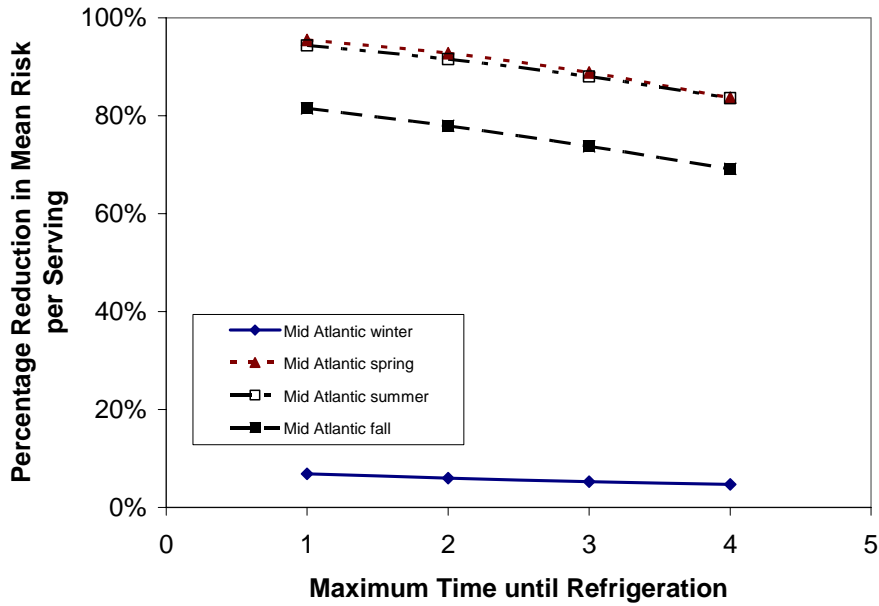

Figure A10-20. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest).
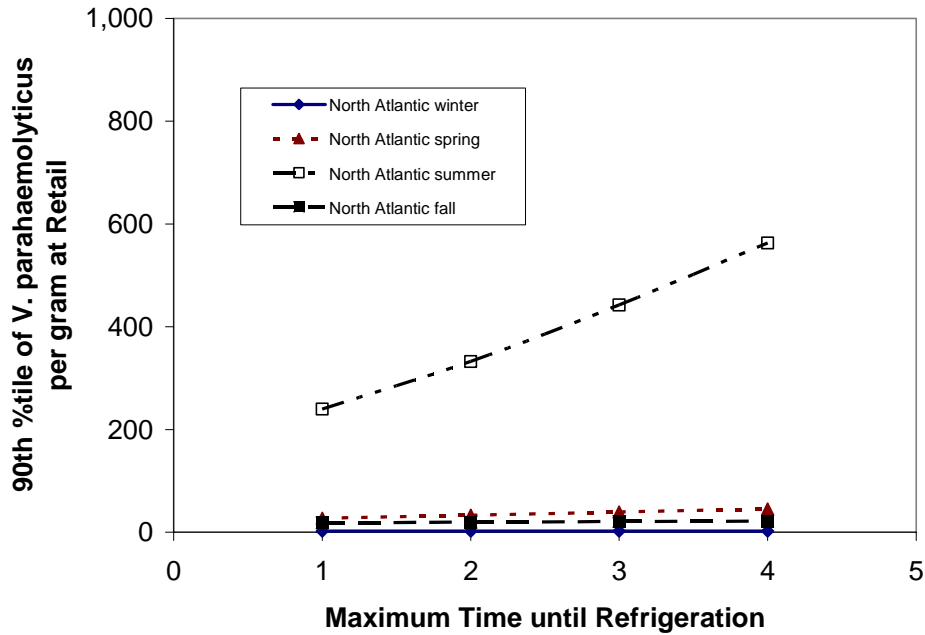
Figure A10-21. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).
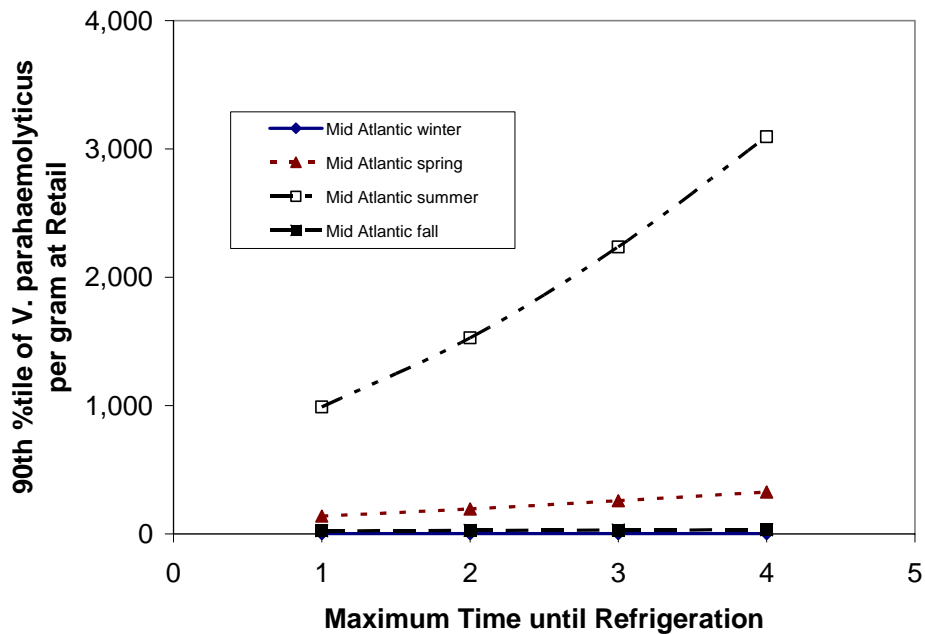
Figure A10-22. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Mid-Atlantic Harvest).
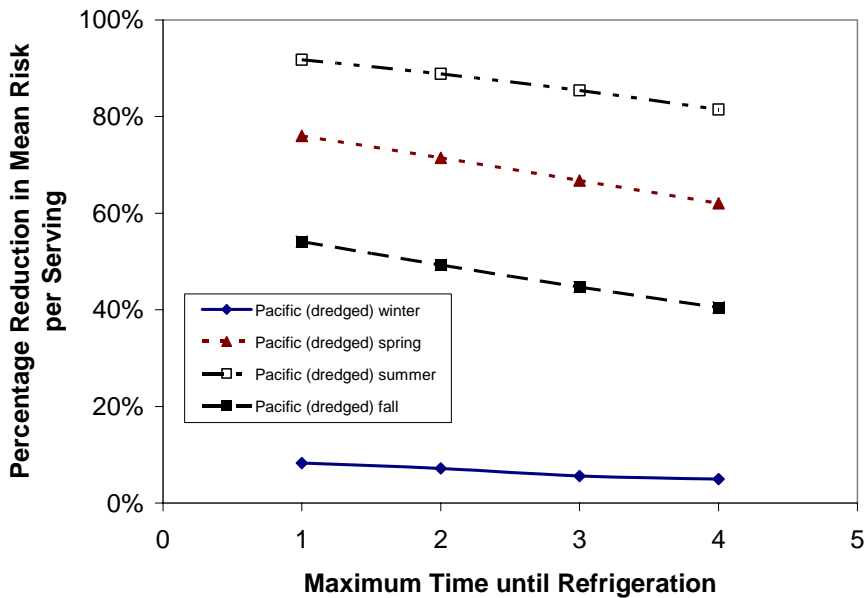
Vibrio parahaemolyticus Risk Assessment
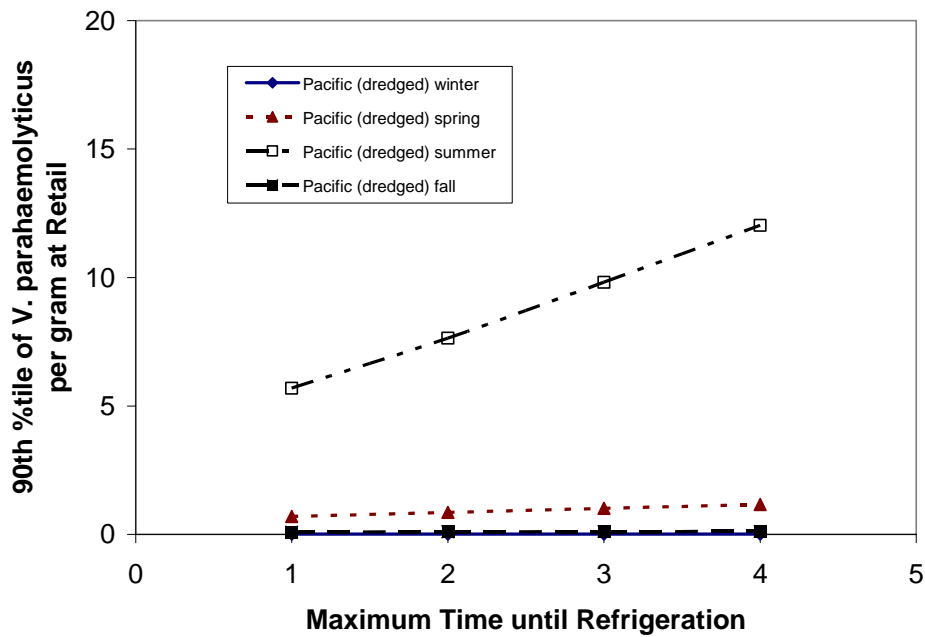
Figure A10-23. Predicted Effect of Maximum Time to Refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Pacific Northwest Dredged Harvest).
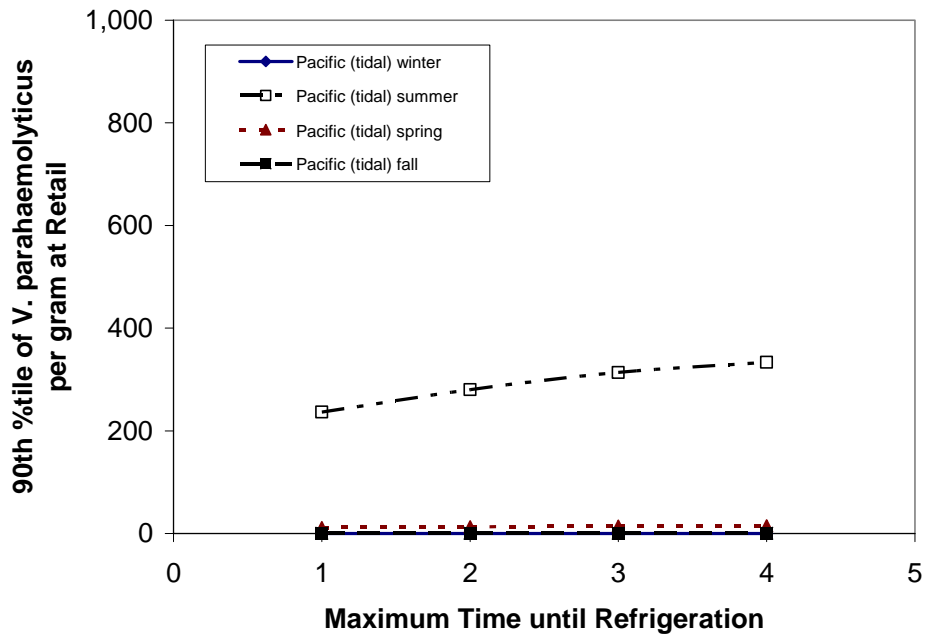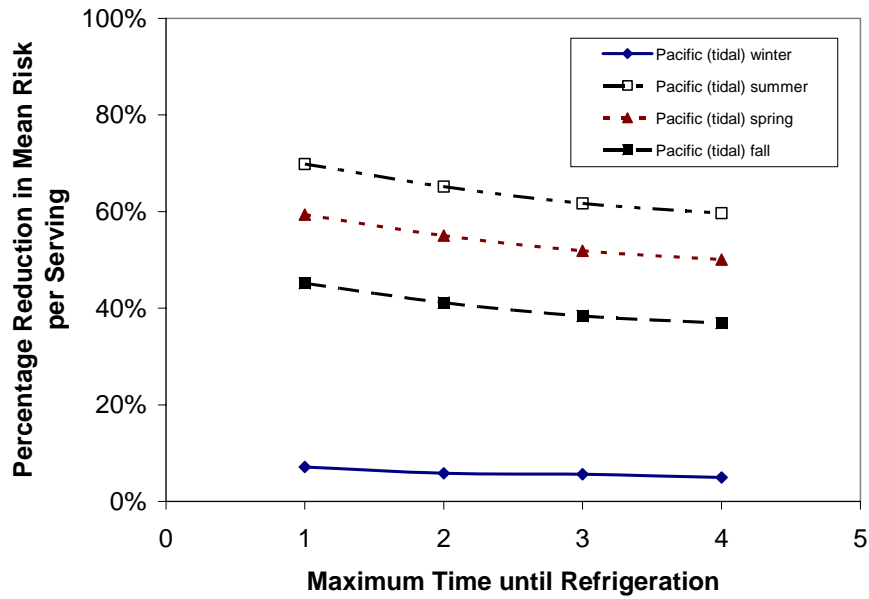

Figure A10-24. Predicted Effect of Maximum Time to Refrigeration with Rapid (on (on ice) Cooling of Oyster Shellstock (Pacific Northwest Intertidal Harvest).

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.

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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).
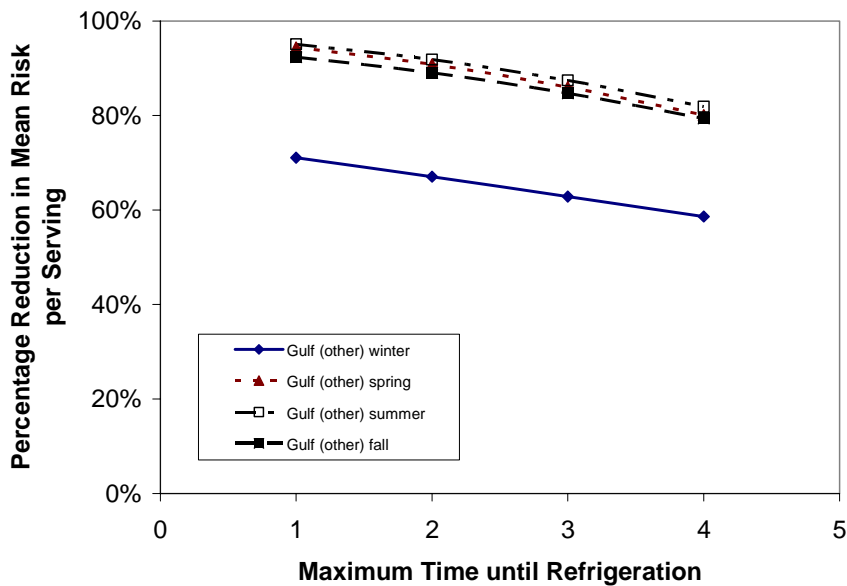
Figure A10-30. Predicted Effect of Maximum Time-to-refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Non-Louisiana Harvest).
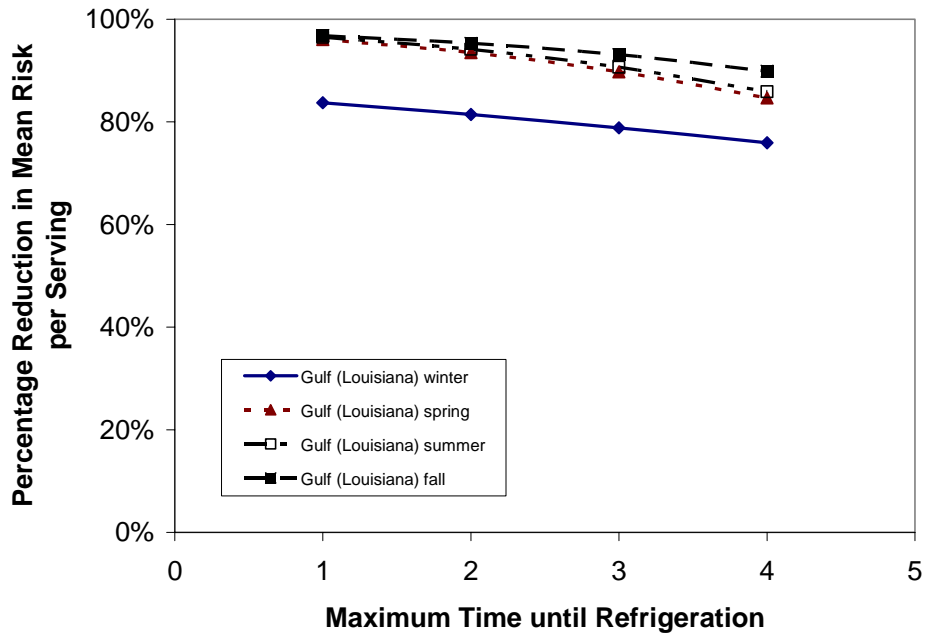
Figure A10-31. Predicted Effect of Maximum Time-to-refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Harvest).
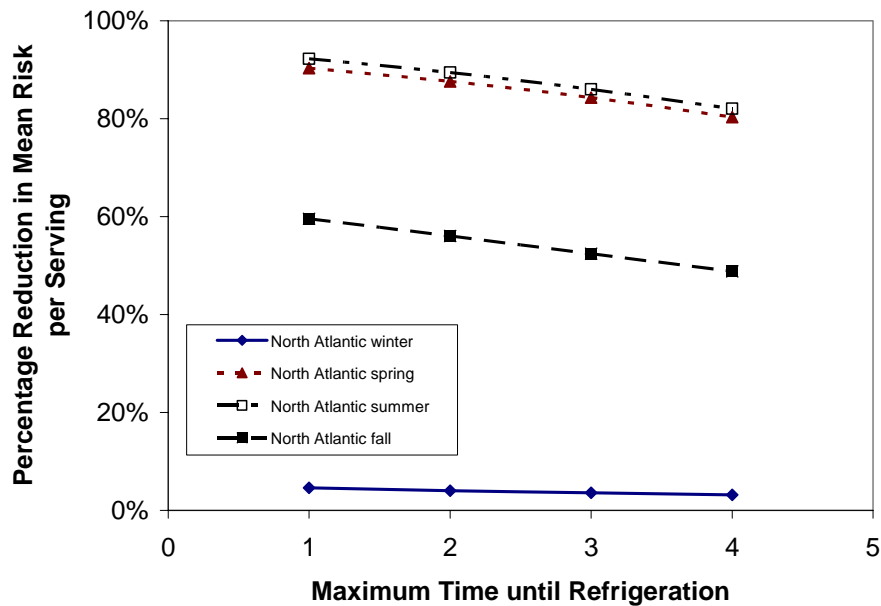
Figure A10-32. Predicted Effect of Maximum Time-to-refrigeration with Rapid (on ice) Cooling of Oyster Shellstock (Northeast Atlantic Harvest).

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
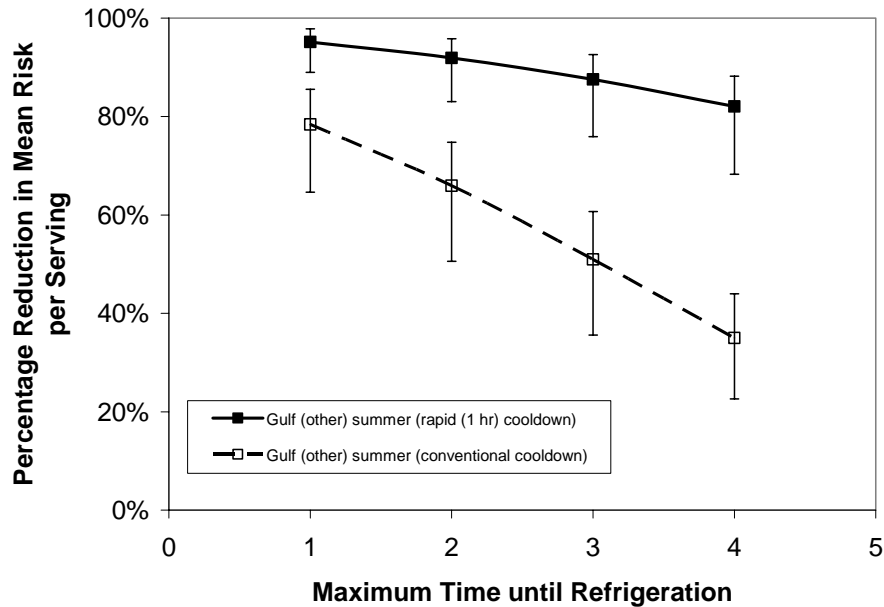
Figure A10-36. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Non-Louisiana Summer Harvest).
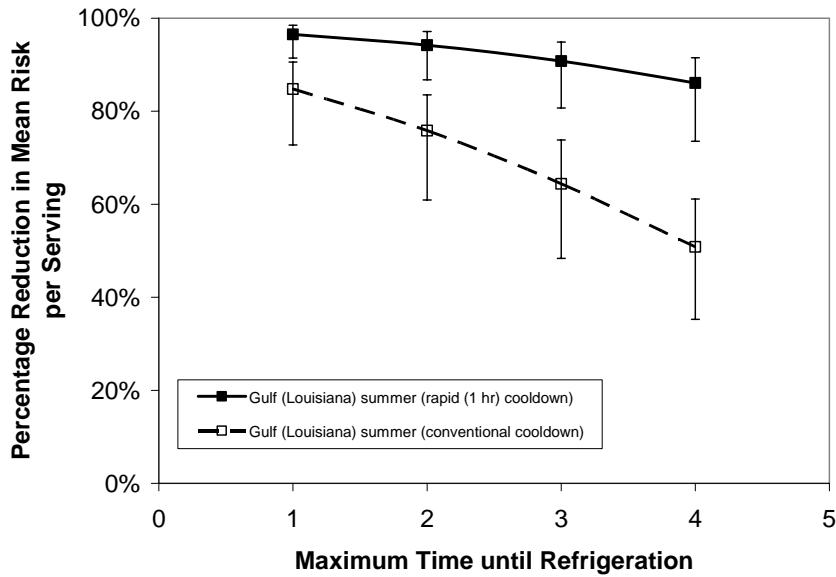
Figure A10-37. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Gulf Coast, Louisiana Suumer Harvest).
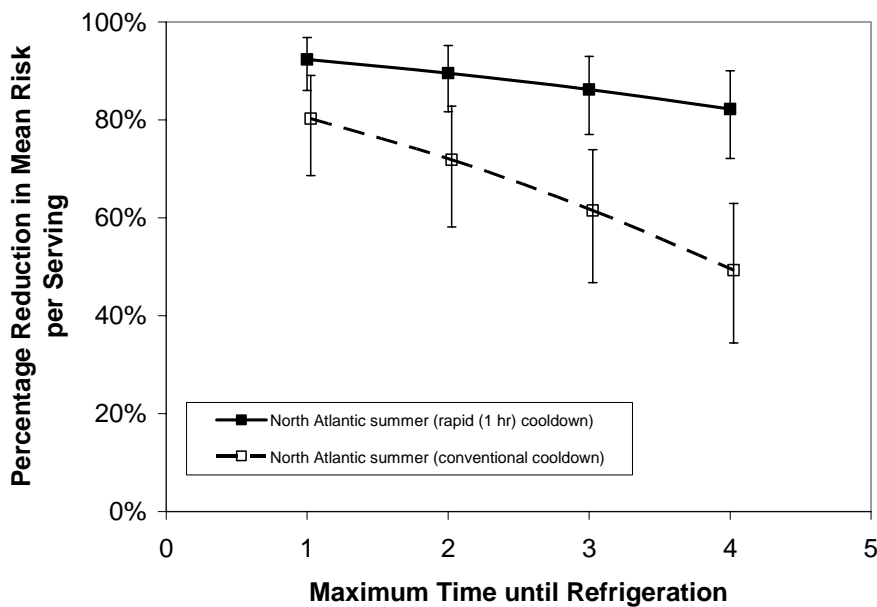
Figure A10-38. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Northeast Atlantic Summer Harvest).
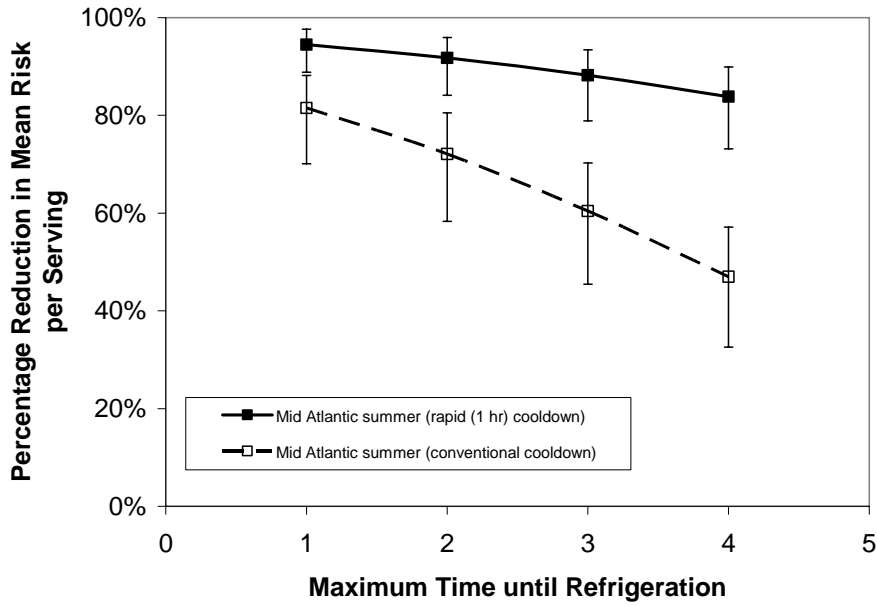
Figure A10-39. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Mid-Atlantic Summer Harvest).
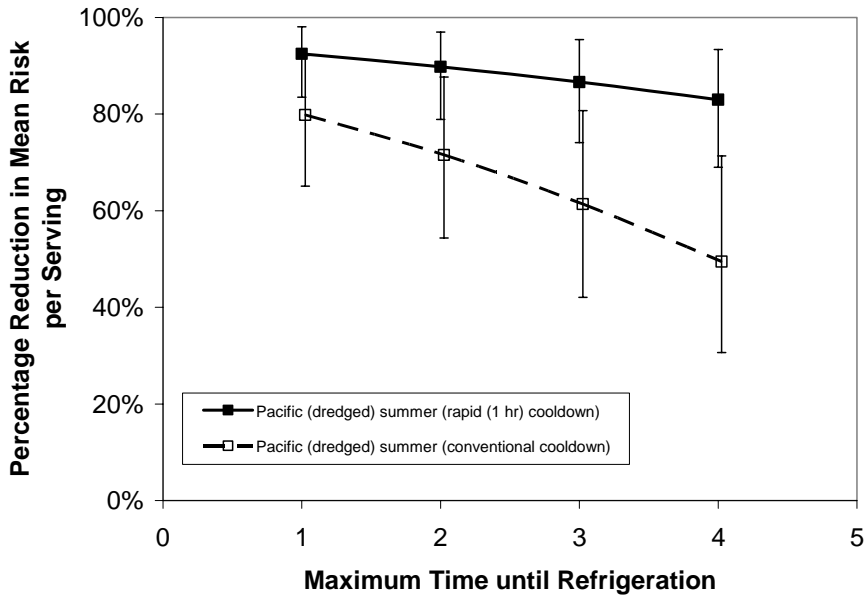
Figure A10-40. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Summer Dredged Harvest).
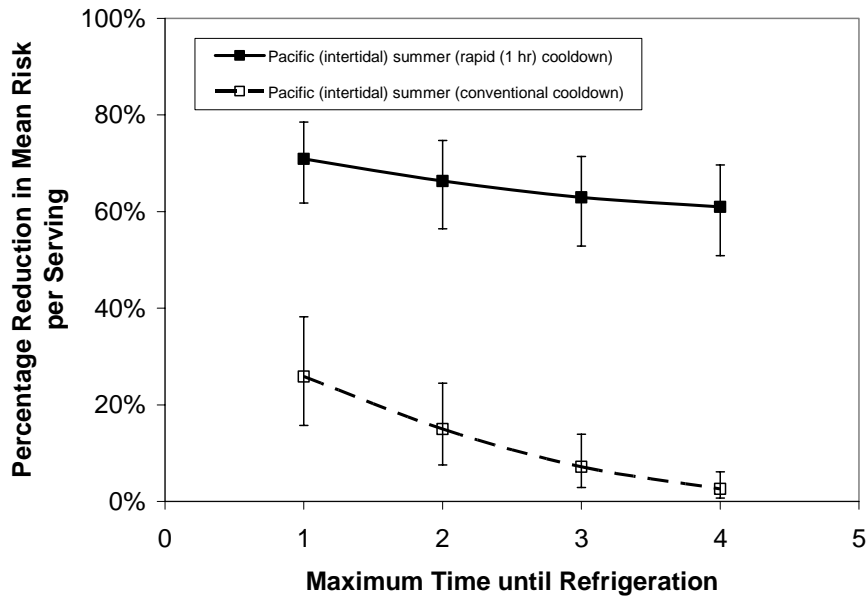
Figure A10-41. Rapid (on ice) Cooling versus Conventional (Air-Circulated) Cooling of Oyster Shellstock (Pacific Northwest Summer Intertidal Harvest).

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%
	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.
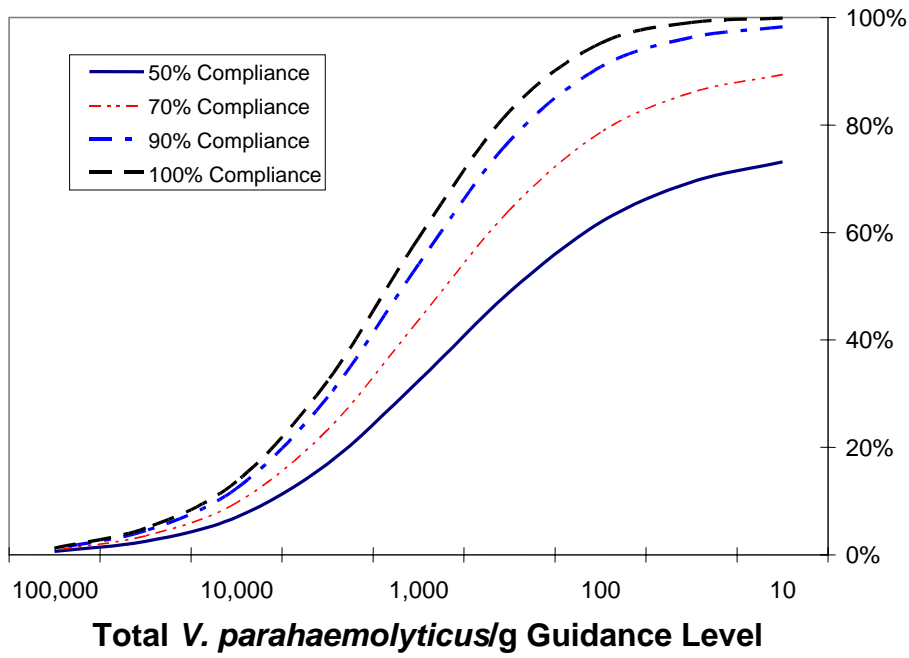
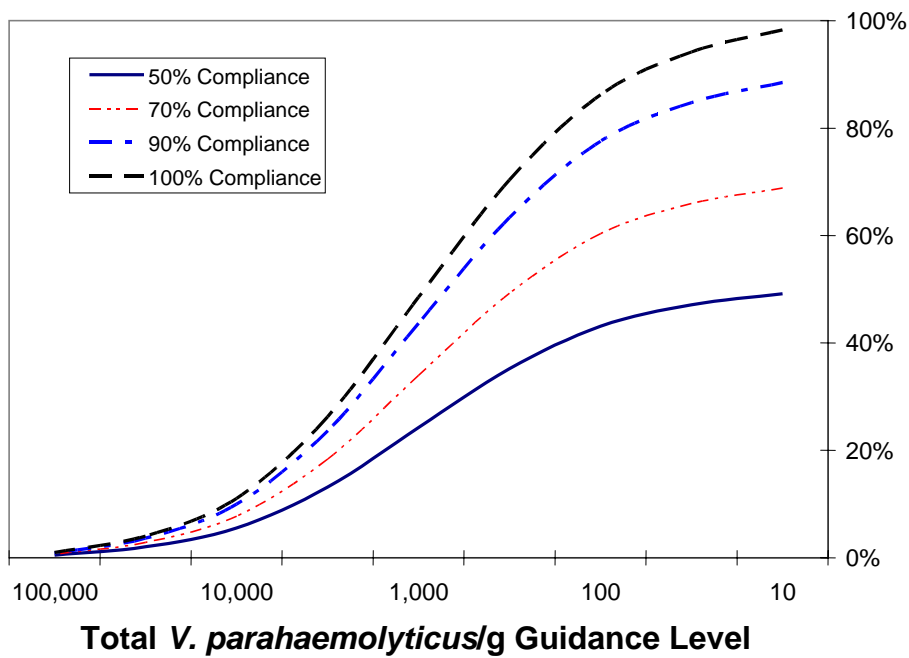**Figure A10-42. Percentage of Illnesses Averted**

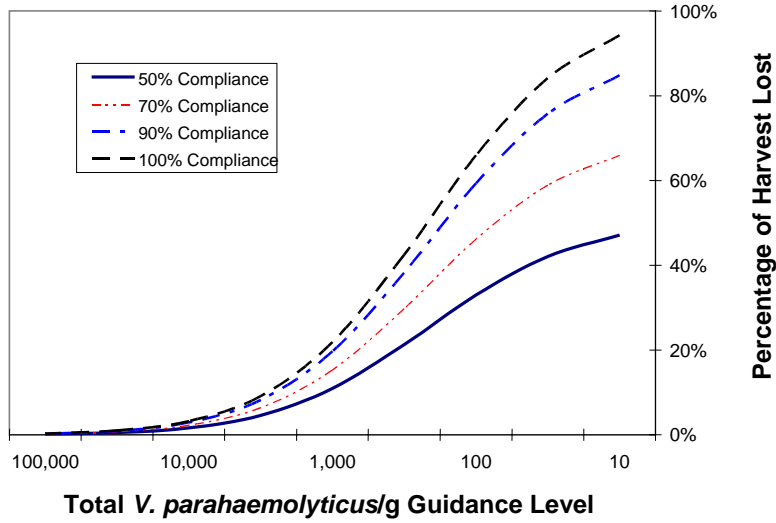**Figure A10-43. Percentage Reduction in Mean Risk per Serving**

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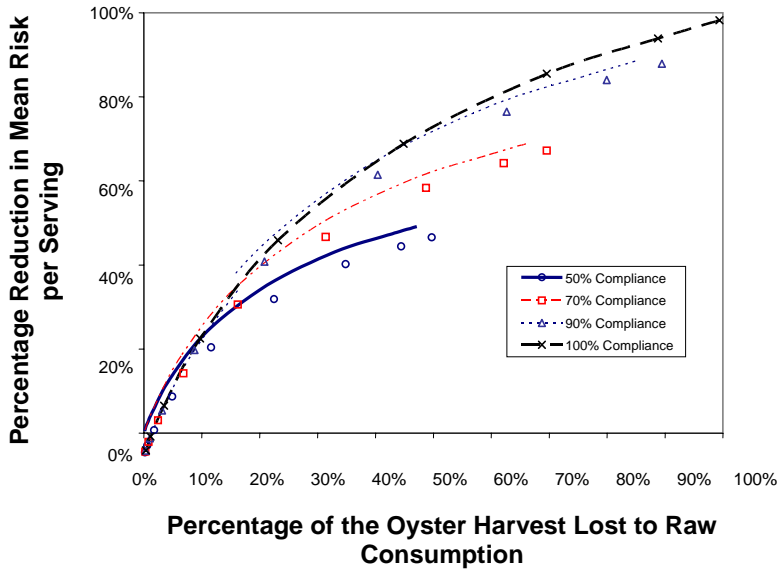

Figure A10-45. Percentage Reduction in Mean Risk per Serving versus Percentage of Harvest Diverted from the "Raw Market or Subjected to Preventive Controls
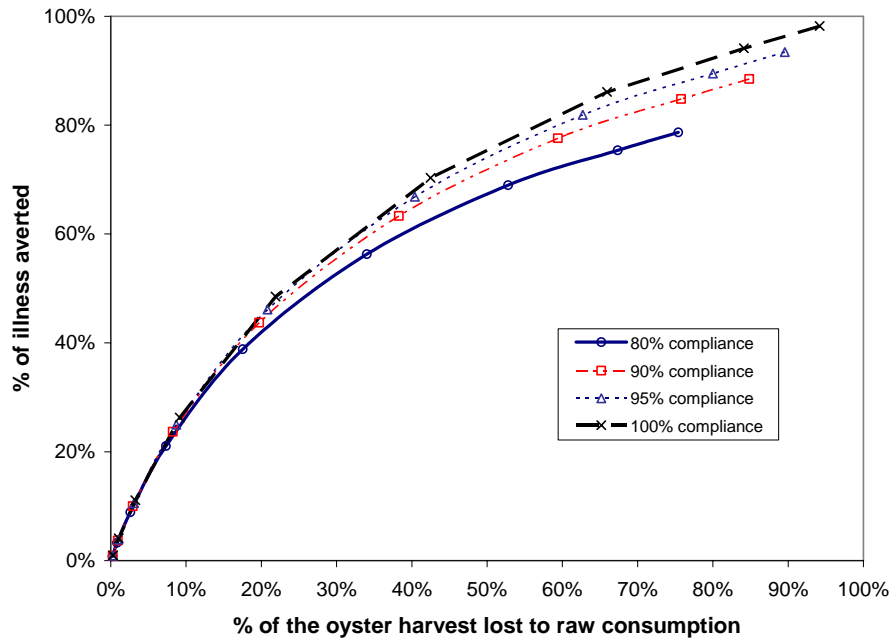
Figure A10-46. Percentage of Illnesses Averted versus Percentage of Harvest Diverted From the "Raw Market" or Subjected to Preventive Controls.

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.
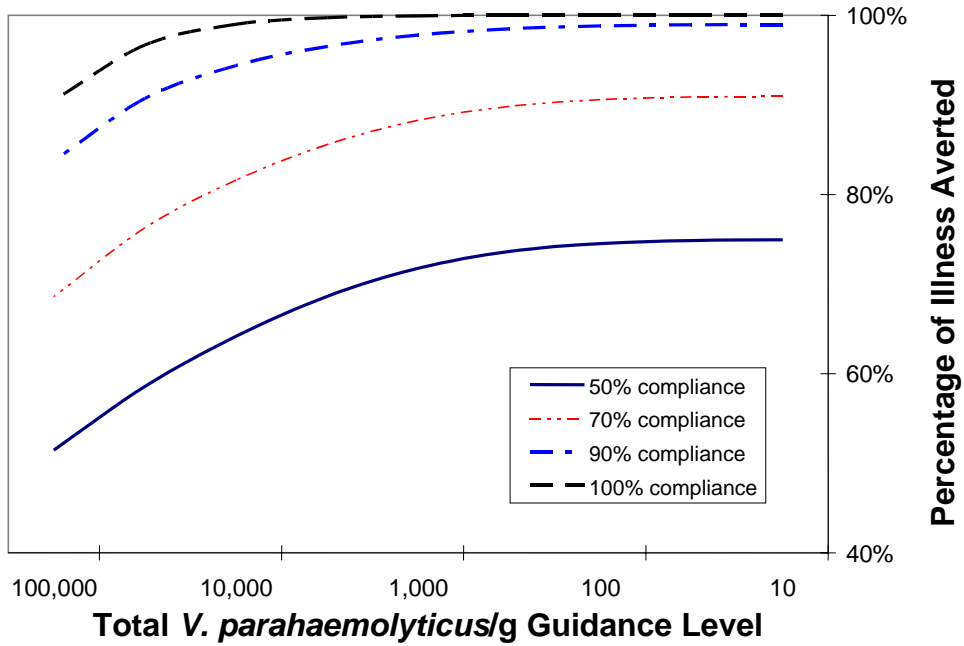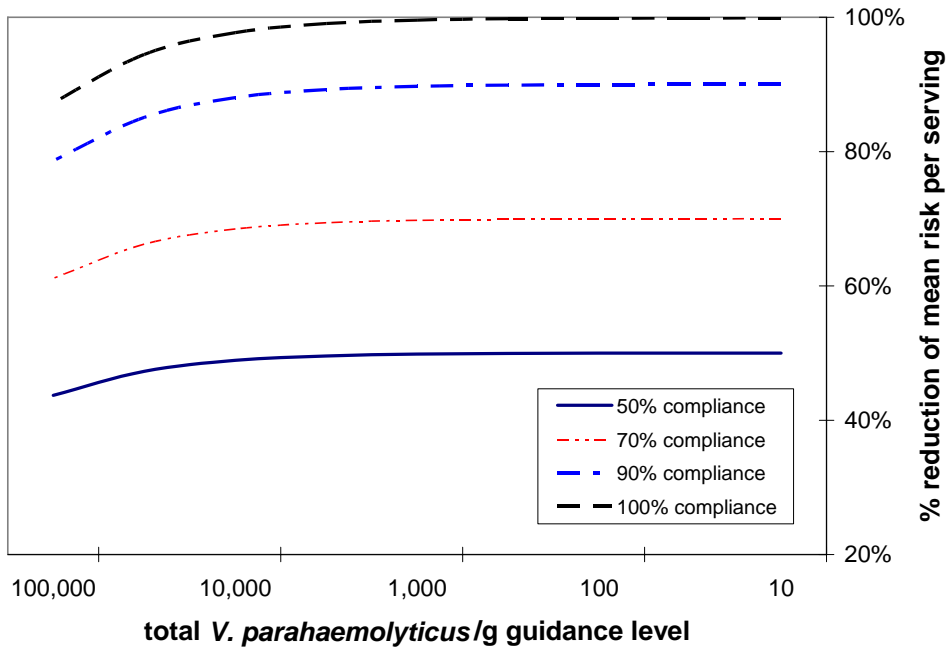
Figure A10-47. Percentage of Illnesses Averted

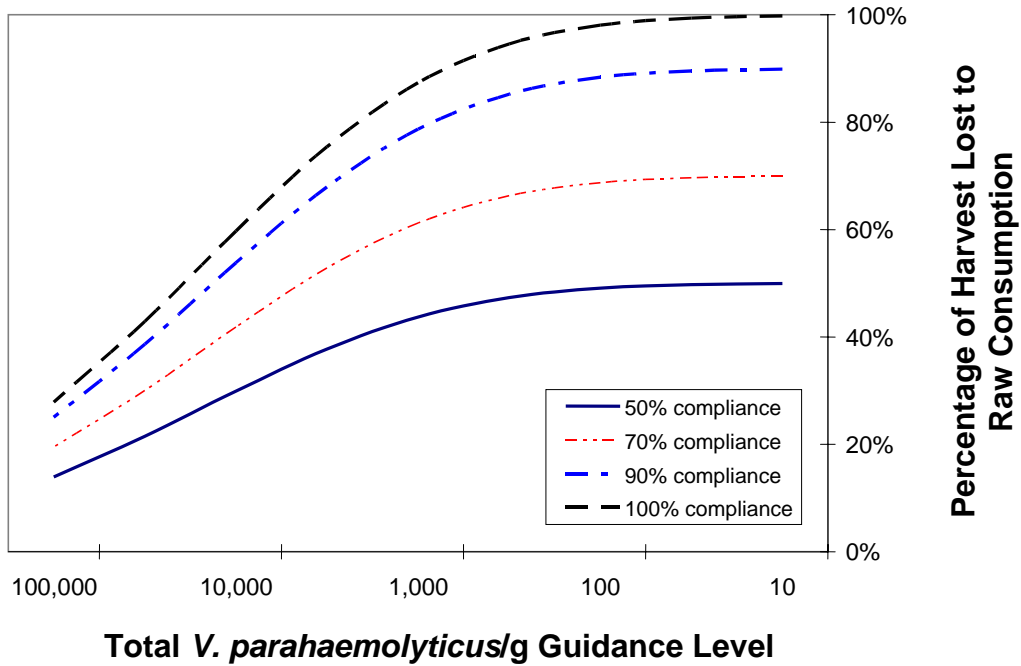**Figure A10-48. Percentage Reduction in Mean Risk per Serving.**

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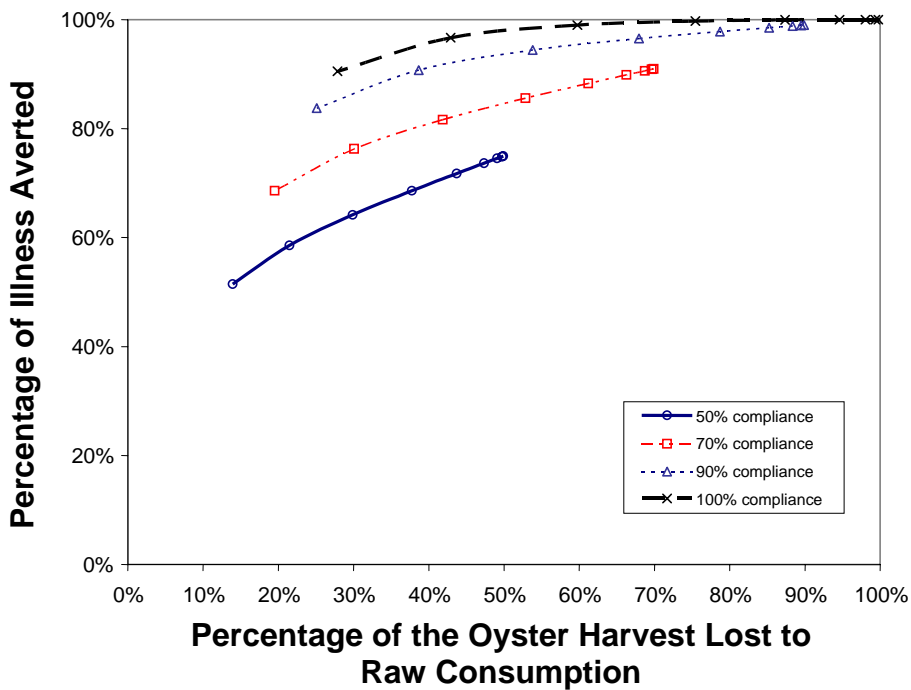

Figure A10-50. Percentage of Illnesses Averted versus Percentage of Harvest Lost to Raw Consumption Market

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".