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Joint FAO/WHO Activities on Risk Assessment of Microbiological Hazards in Foods

- Preliminary Document -

Hazard identification, exposure assessment and hazard characterization of *Vibrio* spp. in seafood

prepared by

John Sumner, M & S Food Consultants, Australia
Angelo DePaola, United States Food and Drug Administration, United States
Ken Osaka, Infectious Disease Surveillance Centre, Japan
I Karunasager, University of Agricultural Sciences, India
Mark Walderhaug, United States Food and Drug Administration, United States
John Bowers, United States Food and Drug Administration, United States

All comments and questions on this preliminary report should be addressed to FAO (sarah.cahill@fao.org) and WHO (toyofukuh@who.ch) and NOT to the authors of the document

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Abbreviations

CDC	Centers for Disease Control and Prevention
cfu	Colony forming unit
g	gram
GCSL	FDA Gulf Coast Seafood Laboratory, Dauphin Island
h	hours
HGMF procedure	Hydrophobic Grid Membrane Filtration procedure
KP+	Kanagawa-positive
min	minute
ml	millilitres
MLE	Maximum likelihood estimates
MPN	Most probable number
NBDC	National Buoy Data Center
NOAA	National Oceanic and Atmospheric Administration
PAC (as in desinfection by PAC coagulation)	?
ppt	Parts per thousand
TDH	thermostable direct hemolysin
TRH	thermostable-related hemolysin
VBNC	Viable but non culturable

1 INTRODUCTION: VIBRIO SPP. IN SEAFOOD

Vibrio spp. are Gram-negative, facultatively anaerobic motile curved rod bacteria with a single polar flagellum. The genus contains twelve species that can cause foodborne illness (Table 1.1), although most of this is caused by *V. cholera*e, *V. parahaemolyticus* or *V. vulnificus* (Oliver and Kaper, 1997, Dalsgaard, 1998). Some species are primarily associated with gastrointestinal illness *(V. cholerae* and *V. parahaemolyticus*) while others can cause non-intestinal illness, such as septicaemia *(V. vulnificus*). In tropical and temperate regions, disease-causing species of *Vibrio* occur naturally in marine, coastal and estuarine (brackish) environments and are most abundant in estuaries. Pathogenic vibrios can also be recovered from freshwater reaches of estuaries (Desmarchelier, 1997). The occurrence of these bacteria does not correlate with numbers of faecal coliforms and depuration of shellfish may not reduce their numbers. Based on data from the United States, there is a positive correlation between water temperature and both the number of human pathogenic vibrios isolated and the number of reported infections, a correlation particularly marked for *V. parahaemolyticus* and *V. vulnificus.*

Table 1.1: *Vibrio* spp. which cause, or are associated with, human infections (after Dalsgaard, 1998)

	Occurrence in human clinical specimens*	
	Intestinal	Non-intestinal
V. cholerae O1	++++	+
V. cholerae non-O1	++	++
V. parahaemolyticus	++++	+
V. fluvialis	++	-
V. furnissii	++	-
V. hollisae	++	-
V. mimicus	++	+
V. metschnikovii	+	+
V. vulnificus	+	+++
V. alginolyticus	-	++
V. carchariae	-	+
V. cincinnatiensis	-	+
V. damsela	-	+

*The symbol (+) refers to the relative frequency of each organism in clinical specimens and (-) indicated that the organism was not found

In Japan (Twedt, 1989; Ministry of Health, Labour and Welfare, Japan, 2000) and eastern Asian countries *V. parahaemolyticus* has been recognised as a major cause of foodborne gastroenteritis. By contrast, in most countries outside of Asia, the reported incidence appears to be low, perhaps reflecting a different mode of seafood consumption. Gastroenteritis caused by this organism is almost exclusively associated with seafood consumed raw or inadequately cooked, or contaminated after cooking. In the

United States prior to 1997 illness was most commonly associated with crabs, oysters, shrimp and lobster (Twedt, 1989; Oliver and Kaper, 1997). Four *V. parahaemolyticus* outbreaks associated with the consumption of raw oysters were reported in the United States in 1997 and 1998 (DePaola *et al.,* 2000). A new *V. parahaemolyticus* clone of O3:K6 serotype emerged in Calcutta in 1996. It has spread throughout Asia and to the United States elevating the status of *V. parahaemolyticus* to pandemic (Matsumoto *et al*., 2000). In Australia, in 1990 and 1992, there were two outbreaks of gastroenteritis caused by *V. parahaemolyticus* in chilled, cooked shrimps imported from Indonesia (Kraa, 1995) and there was also a death in 1992 associated with the consumption of oysters.

V. vulnificus has been associated with primary septicaemia in individuals with chronic pre-existing conditions, following consumption of raw bivalves. This is a serious, often fatal, disease. To date, *V. vulnificus* disease has almost exclusively been associated with oysters (Oliver, 1989; Oliver and Kaper, 1997). Recently, *V. vulnificus* infections have been associated with a variety of raw seafood products in Korea and Japan (Personal Communication, Dr. Yamamoto, Japan). Toxigenic *V. cholerae* O1 and O139 are the causative agents of cholera, a water- and food-borne disease with epidemic and pandemic potential. Non-O1/non-O139 strains may also be pathogenic but are not associated with epidemic disease. Non-O1 strains are generally nontoxigenic, usually cause a milder form of gastroenteritis than O1 and O139 strains, and are usually associated with sporadic cases and small outbreaks rather than epidemics (Desmarchelier, 1997).

Outbreaks of cholera have been associated with consumption of seafood including oysters, crabs and shrimp (Oliver and Kaper, 1997). The largest outbreak was a pandemic in South America in the early 1990s when *V. cholerae* O1 caused more than 400,000 cases and 4,000 deaths, in Peru (Wolfe, 1992). Contaminated water used to prepare food, including the popular, lightly marinated fish *ceviche*, was the cause of the outbreak.

Given the foregoing, it was concluded that four pathogen-product risk assessments should be developed:

- *Vibrio parahaemolyticus* in raw oysters
- *Vibrio parahaemolyticus* in finfish consumed raw
- *Vibrio vulnificus* in raw oysters
- *Vibrio cholerae* in shrimp from developing countries for domestic and export consumption.

Accordingly, the expert drafting group prepared an exposure assessment and hazard characterization for each these pathogen-commodity combinations.

1.1 References

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2 RISK ASSESSMENT OF *VIBRIO PARAHAEMOLYTICUS* IN RAW OYSTERS

2.1 Hazard identification

Vibrio parahaemolyticus is a marine micro-organism occurring in estuarine waters throughout the world. The organism was first identified as a foodborne pathogen in Japan in the 1950s (Fujino *et al*., 1953). By the late 1960s and early 1970s, *V. parahaemolyticus* was recognized as a cause of diarrhoeal disease worldwide, although most common in Asia and the United States. A recent history of seafood consumption is quite a consistent aspect of *Vibrio* infection. Vibrios concentrate in the gut of filter-feeding molluscan shellfish such as oysters, clams, and mussels where they multiply and cohere. Although thorough cooking destroys these organisms, oysters are often eaten raw and, at least in the United States, are the most common food associated with *Vibrio* infection (Hlady, 1997).

2.1.1 Human incidence

In Asia *V. parahaemolyticus* is a common cause of foodborne disease. In general the outbreaks are small in scale, involving fewer than 10 cases, but occur frequently. Prior to 1994, the incidence of *V. parahaemolyticus* infections in Japan had been declining, however, in 1994-95 there were 1,280 reports of infection due to the organism (Anonymous, 1999a) and during this period, *V. parahaemolyticus* food poisonings outnumbered those of *Salmonella* food poisoning. For both years, the majority of the cases occurred in the summer, with the largest number appearing in August. From 1996-1998, there were 496 outbreaks, 1,710 incidents and 24,373 cases of *V. parahaemolyticus* reported. The number of *V. parahaemolyticus* food poisoning cases doubled in 1998 as compared with 1997 and again exceeded the number of *Salmonella* cases (Anonymous, 1999a). As in 1994-1995, outbreaks were more prevalent in the summer with a peak in August and with few outbreaks during winter months. Boiled crabs caused one large-scale outbreak, involving 691 cases. The increased incidence during 1997-1998 has been attributed to an increased incidence of serovar O3:K6;

Between 1986 and 1995 197 outbreaks of foodborne disease were caused by *V. parahaemolyticus* in Taiwan (Pan *et al*., 1997) while in 1997 over 200 outbreaks were reported, including an outbreak of 146 cases acquired from boxed lunches (Anonymous. 1999b).

During 1997 and 1998 there were more than 700 cases of illness due to *Vibrio parahaemolyticu*s in the United States, the majority of which were associated with the consumption of raw oysters. In two of the 1998 outbreaks a serotype of *V. parahaemolyticus,* O3:K6, previously reported only in Asia, emerged as a principal cause of illness for the first time. Subsequent studies on these strains have revealed their pandemic spread. It was suggested that warmer than usual water temperatures were responsible for the outbreaks. In 1999, the United States Food and Drug Administration (FDA) initiated a risk assessment to characterize the public health impact of consuming raw oysters contaminated with *V. parahaemolyticu*s. The FDA Draft Risk Assessment on the Public Health Impacts of *V. parahaemolyticus* in Raw Molluscan Shellfish (FDA-VPRA) was released for public comment in 2001 (Anonymous, 2001).

In Europe few data exist on the incidence of *V. parahaemolyticus* infections, one of the reasons being that such infections are not notifiable. However the current knowledge of the incidence in Europe has been summarized as presented in the recent *Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on Vibrio vulnificus and Vibrio parahaemolyticus in raw and undercooked seafood* issued by the European Commission (Table 2.1) (European Commission, 2001).

2.1.2 Foods implicated

V. parahaemolyticus occurs in a variety of fish and shellfish including clams, shrimp, lobster, crayfish, scallops and crabs as well as oysters. Although oysters are the most common food associated with *Vibrio* infection in some countries (Hlady, 1997), there have been reports of *V. parahaemolyticus* infections associated with the other types of seafood. One such report was a case-controlled study of sporadic *Vibrio* infections in two coastal areas of the states of Louisiana and Texas in the United States conducted from 1992-93, in which crayfish consumption was reported by 50% (5/10) of the persons affected with *V. parahaemolyticus* infection (Bean *et al*., 1998). Outbreaks of *V. parahaemolyticus* gastroenteritis aboard two Caribbean cruise ships were reported in 1974 and 1975 (Lawrence *et al*., 1979). The outbreaks were most likely caused by contamination of cooked seafood by seawater from the ships' seawater fire systems. In 1972, an estimated 50% (600/1,200) of persons who attended a shrimp feast in Louisiana in the United States became ill with *V. parahaemolyticus* gastroenteritis (Barker *et al*., 1974). Samples of uncooked shrimp tested positive for the organism. Three outbreaks occurred in Maryland in the United States in 1971 (Dadisman *et al*., 1972). Steamed crabs were implicated in two of the outbreaks after cross-contamination with live crabs. The third outbreak was associated with crabmeat that had become contaminated before and during canning. Recently, sampling studies in the Adriatic Sea demonstrated the presence of *V. parahaemolyticus* in fish, mussels and clams (Baffone *et al*., 2000). In a recent study conducted mussels from the North-western coast of Spain *V. parahaemolyticus* was isolated from 8% of samples (European Commission, 2001). Also Figure 3.1 in the section on risk assessment of *V. parahaemolyticus* on raw and undercooked finfish summarises the types of seafoods implicated in *V. parahaemolyticus* outbreaks in Japan.

Table 2.1. Available data on the incidence of *Vibrio parahaemolyticus* infections in Europe. (European Commission, 2001)

Country	*Period considered*	*Number of Cases*	*Symptoms*	*Origin of data*
Denmark	1987-1992	13	Wound infection	Hornstrup and Gahrn-Hansen, 1993
		10	Ear infection	
	1980-2000	2	Gastroenteritis	Statens Serum Institut, Copenhagen
England and Wales	1995-1999	115		PHLS, Colindale
France	1995-1998	6	Gastroenteritis	Geneste *et al*., 2000
		1	Septicemia	
	1997	44	Gastroenteritis [1]	Lemoine *et al*., 1999
Northern Ireland	1990-1999	0		CDSC (Communicable Disease Surveillance Centre, NI)
Scotland	1994-1999	6		
Spain	1995-1998	19	Gastroenteritis	Anonymous, 1996 Anonymous, 1998b
Sweden	1992-1997	350	Gastroenteritis [2]	Lindquist *et al*., 2000
Norway	1999	4		Unpublished data

[1] One outbreak associated with seafood imported from Asia.
[2] One outbreak associated with consumption of crayfish imported from China.

2.2 Exposure assessment

The purpose of this exposure assessment is to quantify the exposure of consumers to pathogenic *V. parahaemolyticus* from the consumption of raw oysters.

The approach being taken is to use the United States FDA Draft Risk Assessment on the Public Health Impacts of *V. parahaemolyticus* in Raw Molluscan Shellfish model (FDA-VPRA) (Anonymous, 2001) as the base and further develop it to accommodate data inputs from other countries. The FDA-VPRA contains several key linkages between prevalence of *V. parahaemolyticus* in oysters and temperature, most notably temperature of harvest waters and of oysters throughout the post-harvest-retail-consumption continuum. Temperature profiles in the oyster industry of other countries e.g. New Zealand and Australia indicated the opportunity for growth of pathogenic *V. parahaemolyticus* to potentially dangerous numbers. However, the public health statistics of these countries do not reflect any impact due to this organism in oysters. Accordingly, an exposure assessment will be undertaken on *V. parahaemolyticus* in oysters using data from Australia, Canada, Japan, New Zealand and the United States.

The model incorporates all phases in the harvest - post-harvest - consumption continuum using a modular approach. A schematic representation of the pathway to be modelled is presented in Figure 2.1. Much of the information presented in the following pages is based on the FDA-VPRA. Data from other countries for the exposure assessment have and continue to be obtained via a call for data issued by FAO and WHO. The data are then being analysed for incorporation into the risk assessment model. This process is ongoing and will be completed in 2002.

Thus the objectives of this exposure assessment are;

- to quantify the exposure of consumers to pathogenic *V. parahaemolyticus* from consumption of raw oysters, and
- to take a model that was developed for one particular country scenario and extend it to consumers in other countries that have oyster industries.

2.2.1 Microbial ecology

Vibrio parahaemolyticus is found in the estuarine environment in the tropical to temperate zones. Several studies have been published on the concentration of *V. parahaemolyticus* in shellfish growing areas (Davis & Sizemore. 1982; DePaola *et al*., 1990; Kaneko & Colwell, 1978; Kaysner *et al*., 1990a; Kaysner *et al.*, 1990b; Kaysner & Weagant, 1982: Kelly, 1999; Levine *et al*., 1993). The organism has been isolated from a number of fish species and is associated primarily with the intestinal contents (Nair *et al*., 1980).

There are several pathways by which *V. parahaemolyticus* strains may enter into shellfish growing areas:

- It may be introduced by terrestrial and aquatic animals, some of which may harbour virulent strains and act as intermediate hosts (Sarkar *et al*., 1985).
- It may be introduced through "relaying" shellfish or by release of ballast water. Cargo vessels carry substantial quantities (10^6 litres) of ballast water from the body of water where the voyage originates. This water is retained until the ship is about to load cargo, when it is discharged. Thus, if present, *V. parahaemolyticus* is also released into the loading port. A report that strains of *Vibrio cholerae,* indistinguishable from the Latin American epidemic strain, were found in non-potable water taken from a cargo ship docked in the Gulf of Mexico indicate that ballast water may have been responsible for the spread of an epidemic strain of *V. cholerae* to the Gulf of Mexico (McCarthy & Khambaty, 1994). A similar mechanism could account for the spread of *V. parahaemolyticus*.

Once introduced, a number of factors influence the establishment of *V. parahaemolyticus* including:

- Interactions of environmental conditions;
- Species and physiology of the shellfish;
- Genetics of the micro-organism.

Certain areas may have more favourable environmental conditions that support establishment, survival and growth of the organism such as temperature, salinity, zooplankton, tidal flushing (including low tide exposure of shellfish) and dissolved oxygen (Amako *et al*., 1987; Garay *et al*., 1985; Kaneko & Colwell, 1978; Venkateswaran *et al*., 1990).

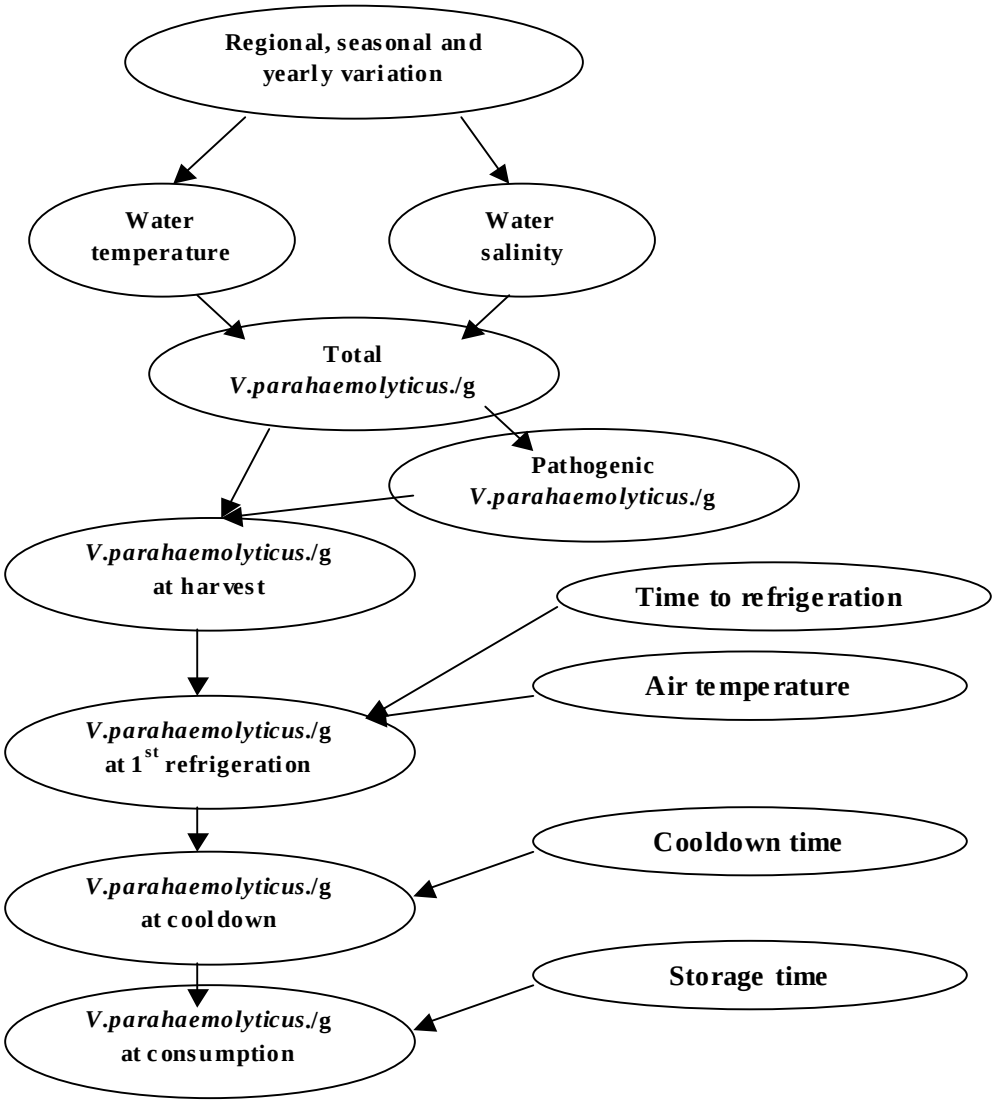


Figure 2.1. A schematic representation of the process pathway to be modeled in the exposure assessment of *V. parahaemolyticus* in oysters.

2.2.2 Growth and survival characteristics

V. parahaemolyticus is a mildly halophilic mesophilic micro-organism and its general growth characteristics are shown in Table 2.2 (ICMSF, 1996). Warmer temperatures and moderate salinity favour the survival and growth of *V. parahaemolyticus* (Covert & Woodburne, 1972; Jackson, 1974; Nair *et al*., 1980; Zhu *et al*., 1992). A correlation exists between *V. parahaemolyticus* infections and environmental temperatures with most of the shellfish-borne illnesses caused by this organism occurring in the warmer months. This has been observed in the United States, Asia and Europe (Daniels *et al*., 2000; Geneste *et al*., 2000).

In the United States the Centers for Disease Control and Prevention (CDC) randomly selected 9% (7/76) of the Texas Department of Healths' monitoring sites for environmental conditions in Galveston Bay, and compared water temperature and salinity levels before and during the 1998 outbreak, with environmental data recorded over the previous five years. It was found that in May 1998 the water temperature was 27^{o}C compared with 24^{o}C for the previous five years. In June, water temperatures were 29^{o}C, compared with 28^{o}C for the previous five years. In addition, low rainfall during April (1.2cm) and May (<0.1cm) preceding the outbreak resulted in markedly increased salinity levels (18.3 ppt compared with 8.4 ppt for the previous five Mays and 21 ppt *versus* 9.1 ppt for the previous five Junes). Elevated temperatures have also been associated with the 1997 outbreak on the West Coast on the United States (CDC, 1998).

Table 2.2. Growth characteristics of *Vibrio parahaemolyticus* (ICMSF, 1996)

	Optimum	**Range**
Temperature (^{o}C)	37	5-43
pH	7.8 – 8.6	4.8 –11
NaCl (%)	3	0.5 - 10
Water activity (Aw)	0.981	0.940 – 0.996
Atmosphere	Aerobic	Aerobic - anaerobic

Although outbreaks of foodborne disease associated with *V. parahaemolyticus* are less commonly reported in Europe there have also been a number of studies that indicate the importance of temperature in the survival and growth of *Vibrio*. In a two year study undertaken in Italy on seawater and molluscs from the Adriatic sea it was found that *Vibrio* strains were most prevalent during the summer months (Croci *et al*., 2001). In another study conducted in Norwegian waters *V. parahaemolyticus* was only detected in July and August (Gjerde and Bøe, 1981).

2.2.2.1 Growth rate

Growth of *V. parahaemolyticus* can be rapid, for example, doubling times of 27 minutes have been reported in crabmeat at both 20 and 30 ^{o}C (Liston, 1974). Growth rates in a range of seafoods and tryptic soy broth with 2.5% salt (NaCl) have been recorded and summarized (ICMSF, 1996). These data indicate that moderate populations of 10^2 - 10^3 organisms / g on seafood can increase to > 10^5 organisms / g in two to three hours at ambient temperatures of between 20^{o} and 35^{o}C (ICMSF, 1996).

Miles *et al.* (1997) modelled the growth rate of *V. parahaemolyticus* based on studies of four strains at different temperatures and water activity. For each combination of temperature and water activity bacterial growth was modelled using the Gompertz function, a sigmoid growth curve with a growth rate (slope) monotonically increasing to a maximum before falling to zero as the bacterial population reaches a steady state. The maximal rate of growth (μ_m) is the most relevant summary of the fit because the growth rate approaches its maximum rapidly and does not decline significantly until steady-state is reached.

A secondary model was used to estimate the effect of environmental parameters on the maximal growth rate. This model was assumed to be of the square root type:

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

where

μ_m = maximal growth rate ($\log_{10}$ per minute)

a_w = water activity

T = temperature (in degree Kelvin)

Based on the data from the fastest growing strain the estimates of the parameters were:

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 $a_{w,max}$ describe the range of temperature and water activity over which growth can occur. The authors validated their model by comparing predicted growth with observed rates in eight other studies of growth in broth model systems obtained from the literature.

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 favourable 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. Accordingly, this parameter was set at the optimal value of 0.985 predicted for the broth model system where the predicted growth rate in broth at 26°C is 0.84 $\log_{10}$ per hour. This is four times greater than the rate of growth observed for *V. parahaemolyticus* in oysters held at 26° C (Gooch *et al*., 1999). Based on this observation, the predicted growth rate in oysters at temperatures other than 26° C was obtained by dividing the predicted rate in broth by four.

This assumes that the growth rate in oysters is a constant fraction of the growth rate in broth at all temperatures. This assumption in the risk assessment is accounted for as an uncertainty parameter varying according to a triangle distribution in the range of 3 to 5 with a mean of 4. This evaluates the sensitivity of the conclusions to the magnitude of the relative growth rate in oysters *versus* broth model but does not fully address the uncertainty in so far as it is conceivable that the relative growth rate could be temperature-dependent.

The use of the Gompertz function by Miles *et al.* (1997) to model bacterial growth in broth is appropriate; after transfer of an inoculum to different medium or environmental conditions there is a demonstrable lag phase (Colwell *et al*., 1985). However, the Gompertz is not an appropriate model for growth of *V. parahaemolyticus* in oysters after harvesting because changes in the environment are gradual and do not induce a lag phase. Consequently, for oysters, the extent of growth occurring over time at a

given average temperature and predicted maximal growth rate is assumed to follow a simple three-phase loglinear model with no lag phase (Buchanan *et al*., 1997). This model is of the form:

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

where $N(t)$ refers to the bacterial density at a given time (t) post harvest, A is the logarithm of the maximum attainable density of *V. parahaemolyticus* in oysters, and the parameter μ_m is a function of ambient temperature as described above.

At 26° C, the density of *V. parahaemolyticus* in oysters was observed to approach a plateau of approximately 6.0 $\log_{10}$ per gram after 24 hours (Gooch *et al*., 1999). We have assumed this value for the maximal density (A) at all temperatures. Figure 2.2 shows predictions of the $\log_{10}$ increase in *V. parahaemolyticus* density from an initial level of 1,000/g as a function of time for three ambient temperatures (20, 26 and 32° C).

Ideally, the average temperature used to determine the parameter μ_m in the above equation is the temperature of oyster meat of shellstock. Clearly the temperature of oyster meat depends on the temperature of both the air and water at the time of harvest. Temperature of the oyster meat after harvest will gradually equilibrate with the temperature of the air, which has been used as a surrogate for oyster meat temperature. In the present study, ambient air temperature data recorded at noon from the near-shore National Buoy Data Centre (NBDC) buoys in various coastal regions of the United States have been used.

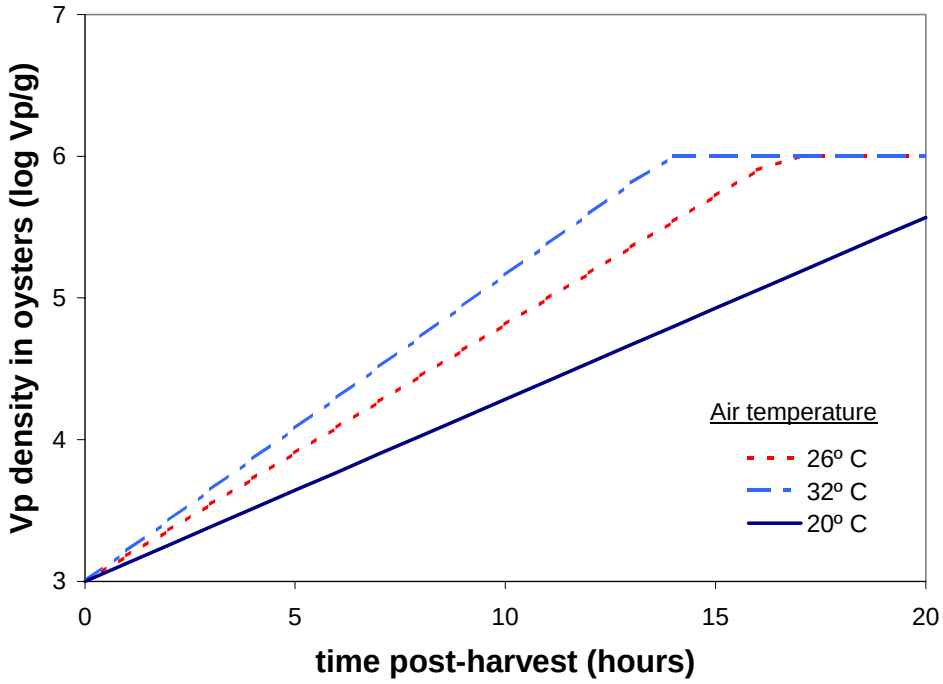


Figure 2.2. Predicted loglinear growth of *V. parahaemolyticus* (Vp) from an initial density of 1,000 (3 $\log_{10}$) *V. parahaemolyticus* /g as a function of ambient air temperature.

2.2.2.2 Death and inactivation

Although the ecology of *V. parahaemolyticus* has been studied (Joseph *et al*., 1983; Kaneko & Colwell, 1978), little is known about the growth and survival of *V. parahaemolyticus* in shellstock oysters (Cook & Ruple, 1989). By contrast, post harvest growth of *V. vulnificus* in oyster shellstock (Cook, 1994; Cook, 1997a) and the effectiveness of various mitigation strategies for reducing *V. vulnificus* have been studied more extensively (Cook & Ruple, 1992; Eyles & Davey, 1984; Motes & DePaola, 1996; Richards, 1988; Son & Fleet, 1980). These include depuration, relaying, refrigerated storage and mild heat treatment.

The United States National Shellfish Sanitation Program (NSSP) time/temperature matrix for the control of *V. vulnificus* requires oyster harvesters from any state which has had two or more confirmed cases of *V. vulnificus* to refrigerate oysters within 10 hours after harvest during summer months, depending on water temperature. This provides approximately a 10-fold reduction in *V. parahaemolyticus* growth relative to refrigeration within 20 h.

2.2.3 Prevalence in water, sediment and shellfish

Prevalence of *V. parahaemolyticus* is associated with the presence of particulates, zooplankton and other chitin sources (Kaneko and Colwell, 1978; NACMCF, 1992; Venkateswaran *et al*., 1990). Several studies have shown that *Vibrio* spp. are capable of surviving and multiplying within certain protozoa such as amoeba (Barker and Brown, 1994). It has also been reported that *V. parahaemolyticus* "over-winters" in the sediment and is absent from the water column and oysters during the winter months (Joseph *et al*., 1983; Kaysner *et al*., 1989; United States Department of Health and Human Services Food and Drug Administration, 1995). During the summer, shellfish often have levels *of V. parahaemolyticus* from ten to one hundred fold greater than those in the water *(*DePaola *et al*., 1990; Kaysner *et al*., 1990a*)* indicating that sediment should be monitored during the winter and shellfish meat during the summer. Under extreme environmental conditions, *Vibrio* species, including *V. parahaemolyticus*, may enter a "viable but non-culturable" (VBNC) phase in marine waters and could be missed by traditional cultural methods (Bates *et al*., 2000; Colwell *et al*., 1985; Oliver, 1995; Xu *et al*., 1982). This issue remains controversial. Methods such as gene probes developed by the United States FDA are capable of detecting most virulent strains and could be useful in monitoring programs (Gooch *et al*., 1999).

Microorganisms are incorporated into shellfish by filter feeding and factors which favour active filter feeding increase the uptake of the pathogen (Murphree & Tamplin, 1991). Shellfish species and physiology (e.g. sexual maturity, immune function, metabolic state) can affect survival and growth of disease-causing *Vibrio* spp. within shellfish (Fisher and DiNuzzo, 1991; Kothary *et al*., 1997; LaPeyre and Volety, 1999; Ordas *et al*., 1998; Volety *et al*., 1999). There also appears to be seasonal differences in the oyster cellular defence system with the bactericidal activity of haemocytes (oyster blood cells) being greater in summer than in winter (Genthner *et al*., 1999).

The oyster parasite *Perkinsus marinus* also plays a role in the affinity of bacteria for oyster tissue and in the ability of oyster haemocytes to kill the internalized organisms (Kothary *et al*., 1997; LaPeyre and Volety, 1999; Tall *et al*., 1999). Factors, such as spawning or adverse environmental conditions (such as tributyltin oxide, polycyclic aromatic hydrocarbons, wood preservative leachates) reduce or stop filter feeding in shellfish. Selective feeding (e.g. new nutrient sources) may prevent or delay assimilation of *V. parahaemolyticus* into these shellfish by affecting oyster physiology and oyster-bacterial interactions (Sujatha *et al*., 1996; Weinstein, 1995; Wendt *et al*., 1996).

It is not known whether virulent and non-virulent strains are similarly affected by environmental and other factors. The presence of the urease gene may provide a competitive environmental advantage over other strains by allowing access to a wider range of nutrients (Abbott *et al*., 1989). Urease-positive

strains have been identified as a predominant cause of *Vibri*o-associated gastroenteritis on the West Coast of the United States and in Mexico (Abbott *et al*., 1989). The presence of a pathogenicity island (a physical grouping of virulence-related genes) in *V. parahaemolyticus* may foster rapid microevolution, promote growth and survival and result in transmission of factors (including virulence) to other strains (horizontal gene transfer) (Frischer *et al*., 1990; Ichige *et al*., 1989; Iida *et al*., 1998). Bacteriophages may also genetically alter vibrios (Baross *et al*., 1978; Ichige *et al*., 1989).

The distribution and variation in numbers of virulent *V. parahaemolyticus* in oysters and among oyster growing areas may need to be determined before harvest because many of the described factors may have contributed to higher concentrations of virulent strains in certain areas. During the 1998 outbreaks in the United States, shellfish harvested from the Hood Canal area of Washington State in the Pacific Northwest were responsible for 67% (32/48) of the illnesses in that state (Thieren, 1999). In the Gulf Coast area of the United States two thirds (20/30) of the harvest sites were implicated while in the Atlantic Northeast, only one harvest area, Oyster Bay Harbour, was implicated in the outbreak in that region (CDC, 1999).

2.2.4 Consumption of oysters

Anyone who consumes shellfish raw is "at risk" for infection by *V. parahaemolyticus* and the characteristics of the host are addressed in more detail in section 2.3.1.2. Intake data for molluscan shellfish are available from a number of governmental and non-governmental sources, however, there is a scarcity of such consumption data as noted recently in the European Union (European Commission, 2001). Also, because raw shellfish is not a commonly consumed food in many countries, for example in the United States approximately 10- 20% of the population will consume shellfish raw at least once during a year, some of the data are available are typically based on very few eaters reporting consumption. The United States Department of Agriculture (USDA) Continuing Survey of Food Intake by Individuals (CFSII) (USDA, 1989-1992) and the food frequency survey conducted by the Market Research Corporation of America (MRCA) (Degner, 1998) suggest that in the United States raw oysters are consumed on average approximately once every 6 weeks. The mean amount of raw oysters consumed at a single serving is 110g, approximately one-half dozen raw large oysters (TAS, 1995). The distribution of shellfish intake will be derived from food intake surveys, food frequency surveys, and from reported landings of shellfish and industry estimates of the percentage of shellfish consumed raw. In Asian countries, for example Japan, consumption of raw seafood can be more frequent as reported in section 3.2.4.3.

2.2.5 Modelling exposure to V. parahaemolyticus

2.2.5.1 Approaches

The solicitation and assembly of information and scientific data on *V. parahaemolyticus* from many sources is being undertaken to produce a thorough, up-to-date compilation of data from around the world. This process is ongoing therefore the following section is currently based primarily on data from the United States and the FDA-VPRA (Anonymous, 2001). Various gaps in the data and current knowledge have been identified (section 2.5) and some of these have to be filled before the model can be extended to meet the needs of a variety of countries.

This information available was used in the construction of a mathematical model to produce results on the risk of illness incurred by eating raw oysters containing pathogenic *V. parahaemolyticus*. Three factors were proposed to model exposure:

- Level of pathogenic *V. parahaemolyticus* in seafood at harvest

- Effect of post harvest handling and processing
- Ability of the organism to multiply to an infective dose

As a result, the exposure assessment was divided into separate modules, which corresponded to different stages leading potentially to consumer exposure: the harvest and post harvest, retail and consumption modules.

The *Harvest Module* estimates the prevalence of pathogenic *V. parahaemolyticus* at time of harvest. The *Post Harvest Module* determines the role of post harvest processing and handling on the numbers of pathogenic *V. parahaemolyticus* at consumption.

As this section is currently based on the model developed in the United States FDA-VPRA (Anonymous, 2001) a similar approach and structure is being used. It also involves the collection of data from other countries, if possible on a regional and seasonal basis and then incorporating such data into the model. In the development of FDA-VPRA model, because of harvesting and temperature differences, the United States harvest areas were divided into five regions, and each region was divided into four seasons. Differences existing in oyster harvesting practices and climates in the United States were sufficiently significant to identify five separate geographic regions (Northeast Atlantic, Mid-Atlantic, Pacific Northwest, Louisiana Gulf Coast and the remainder of the Gulf Coast) for each season, for consideration in modelling each of the modules. Factors influencing the risk of illness posed by *V. parahaemolyticus* were identified and incorporated into each module as appropriate. Integration of the various parameters comprising these modules into a quantitative risk assessment model will provide a more comprehensive understanding of the relative importance and interactions among the factors influencing risk.

2.2.5.2 Assumptions

While providing a framework for understanding the relationship of risk to various parameters, the development of the risk assessment model necessarily requires certain assumptions to fill the data gaps. In the development of the United States *Vibrio* risk assessment the assumptions incorporated in the model were reviewed by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1998) at a public meeting in September 1999. In the current risk assessment the work undertaken to date including the assumptions made have been reviewed by a group of experts at a joint FAO/WHO expert consultation on risk assessment of microbiological hazards in foods that was convened in July 2001 (FAO/WHO, 2001). Such a review step ensures that the assumptions are the best that can be made based on current knowledge and also facilitates transparency of the risk assessment process.

Based on the information currently available, for the *Harvest Module*, it was assumed that the presence of the thermostable direct hemolysin (TDH) gene be used as the basis for pathogenicity. It is not currently known what average numbers of TDH-positive strains exist in shellfish, nationally or regionally (see also section 3.1.3 on TDH). The estimates made in the *V. parahaemolyticus* risk assessment, based on the observed frequency of TDH-positive isolates, were the best possible from the data currently available. However, since it is currently not known how this frequency may vary from one year to the next, a two-fold up or down triangle distribution was assumed. Also, within a given year, there is uncertainty about the variance of the percentage pathogenic *V. parahaemolyticus* in one composite of oysters to the next. For example, for the United States coastal area, with the exception of the Pacific Coast (where the range was 2% to 4%), the percentage pathogenic *V. parahaemolyticus* in a given year ranged from 0.1% to 0.3%. However, these estimates are based on older data and may not be predictive of future years, given that the frequency of percentage pathogenic *V. parahaemolyticus* may be changing as new outbreak strains emerge or re-emerge, such as the emergence of O3:K6 or recurrence of known outbreak strains such as O4:K12. It has also been noted that the proportion of pathogenic strains occurring can vary from region to region, for example these strains tend to occur with greater frequency in Asia than in the United States (FAO/WHO,

2001) and so this assumption may have to be revised when applying the model to other countries or regions.

For the *Post Harvest Module*, several assumptions were made based on the knowledge of the current post harvest practices and information available in the United States but again these assumptions may have to be revised when information on the practices in other countries becomes available. The time oysters are harvested to the time they are refrigerated was based on the current NSSP requirement (ISSC & FDA, 1997) put into effect in 1997 in the United States. The extent of growth that occurs during the period from harvest until oysters are first placed under refrigeration is determined by three factors:

- Growth rate of *V. parahaemolyticus* as a function of temperature
- Temperature of oyster meat after harvest
- Length of time held unrefrigerated.

The growth rate of pathogenic *V. parahaemolyticus* in oysters was assumed to be one fourth that in broth culture at all temperatures (see section 2.2.2.1). Also, since the *V. parahaemolyticus* organisms do not change their growth environment after harvest (within the oyster meat), it was assumed that lag time was negligible and was therefore omitted from the growth model. Regarding growth rates, preliminary studies at the Gulf Coast Seafood Laboratory (GCSL) in the United States showed no significant difference between pathogenic and non-pathogenic strains of *V. parahaemolyticus.*

Since data on cooling rates of commercial oyster shellstock has not been located to date, the time for oysters to cool after being placed under refrigeration was assumed to be quite variable. Cooling rates are dependent on the efficiency of the cooler, the quantity of oysters to be cooled and their arrangement in the cooler. A uniform distribution between 1 and 10 hours was used to model this parameter. This was based on preliminary experiments carried out at GCSL in the United States for the time it took a single shell oyster at 30°C placed into a 3°C cooler to reach that temperature, and the time it took for 24 oysters in an uninsulated plastic container at 26° C to reach 3° C.

For the sake of simplicity of the model, it was assumed that consumption patterns were the same for both the sensitive and otherwise healthy population, for all regions. It was assumed that all virulent/pathogenic strains of *V. parahaemolyticus* are equally virulent with the same dose-response as those strains fed to human volunteers in earlier studies. This assumption was based on personal communication with Dr. Nishibuchi, Kyoto University, Japan who stated that due to lack of information, it is not known whether there are differences in virulence among different strains.

2.2.5.3 Harvest module

Although a number of factors have been identified as potentially affecting the numbers of pathogenic *V. parahaemolyticus* in oysters at time of harvest, there are not sufficient quantitative data available to incorporate all of these factors into a predictive model. Incorporation of an environmental factor into the simulation as a predictor of *V. parahaemolyticus* numbers at harvest requires both the relationship between *V. parahaemolyticus* numbers to the parameter of interest and the regional and temporal variation of the parameter within the environment to be identified. Moreover, due to the relatively low prevalence of pathogenic *V. parahaemolyticus* and limitations of current methods of detection, the distribution of pathogenic *V. parahaemolyticus* is not well understood. A critical issue in the development of the *Harvest Module* simulation is the use of the estimated distribution of total *V. parahaemolyticus* numbers to bridge this data gap and derive an estimate of the distribution of pathogenic *V. parahaemolyticus* numbers in oysters at harvest. Figure 2.3 illustrates the parameters considered in modelling the *Harvest Module*.

The main factors that have been identified as potentially affecting the numbers of pathogenic *V. parahaemolyticus* in oysters at the time of harvest are water temperature and salinity and this issue is addressed in more detail below. The preliminary modelling demonstrated that the parameter, water

salinity is not as strong a determinant of *V. parahaemolyticus* numbers as water temperature, and therefore is represented as a dotted bubble in Figure 2.3.

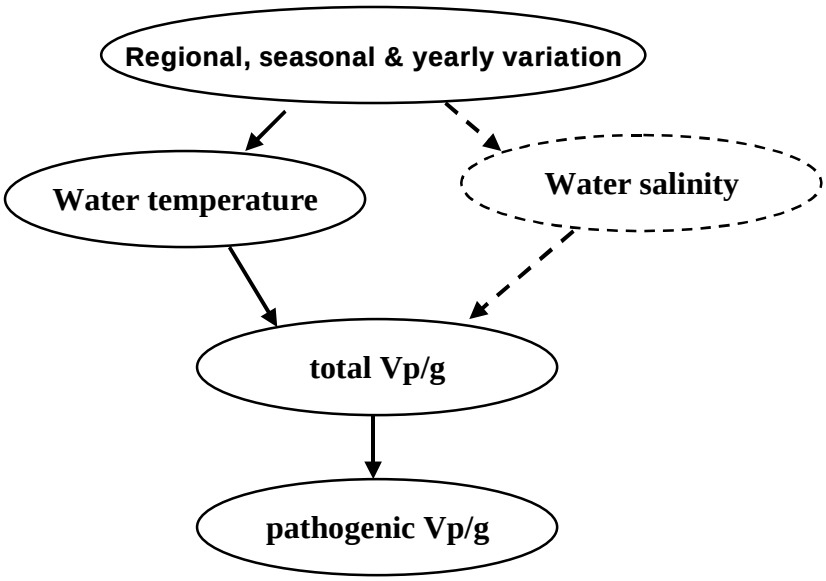


Figure 2.3: Schematic depiction of the *Harvest Module* of the *V. parahaemolyticus* (Vp) risk assessment model.

2.2.5.3.1 Effect of water temperature and salinity on prevalence

A number of studies have been carried out on the prevalence of *V. parahaemolyticus,* but many of these report only presence or absence *V. parahaemolyticus*, which are of limited value for quantitative risk assessment (Hanrihan *et al*., 1995, Kelly and Stroh, 1988a; Kiiyukia *et al*., 1989). Some studies which did measure *V. parahaemolyticus* numbers in oysters were also limited because samples were either obtained from a single estuary (Chan *et al*., 1989; Hanrihan *et al*., 1995; Kaysner *et al*., 1990b; Kelly and Stroh, 1988a; Kelly & Stroh 1988b; Kiiyukia *et al*., 1989; Tepedino, 1982), were not seasonal (Chan *et al*., 1989; Kaysner *et al*., 1990b; Tepedino, 1982), or did not report salinity and temperature (Chan *et al*., 1989; Tepedino, 1982). A number of there are summarised in Table 2.3.

The limitations to the methodology currently used in the enumeration of *V. parahaemolyticus* have been acknowledged. In a study that evaluated four methods for enumerating *V. parahaemolyticus* in natural seawater and oysters, it was found that there was considerable variability between methods for *V. parahaemolyticus* recoveries, with highest recoveries being obtained using filtration through a hydrophobic grid membrane (DePaola *et al.,* 1988). In a subsequent study DePaola *et al.* (1990) used the hydrophobic grid membrane filtration (HGMF) procedure developed by Watkins *et al*. (1976) and later revised by Entis & Boleszczuk (1983). When all suspect colonies are tested for confirmation, the precision of the HGMF method has been shown to be greater than the three tube most probable number (MPN) procedure (Entis & Boleszczuk, 1983; Watkins *et al*., 1976).

The most informative study currently available was found to be that on the enumeration of *V. parahaemolyticus* (hydrophobic grid method) in seawater and oyster samples collected from May 1984 through April 1985 from shellfish growing areas in the Pacific, Gulf and Atlantic Coasts of the United

States. The study was undertaken on 65 paired samples of oyster and water from these areas to generate data relating *V. parahaemolyticus* numbers in oysters (and water) *versus* water temperature and salinity (DePaola *et al*, 1990). The study noted that seasonal and geographical distributions of *V. parahaemolyticus* were related to water temperature with the highest numbers of the organism found in samples collected in the spring and summer from the United States Gulf Coast (DePaola *et al*., 1990). As this is the most comprehensive regional/seasonal study available it was considered to be most appropriate for the purpose of the recently released quantitative risk assessment of *V. parahaemolyticus* illness from consumption of United States oysters - FDA-VPRA (Anonymous, 2001).

Table 2.3. Surveys on the numbers and prevalence of *V. parahaemolyticus* in oysters, sediment and water

Location	**Time period**	**Samples taken**	**No of V. *parahaemolyticus***	**Prevalence**	**Environmental conditions**	**Reference**
British Columbia, Canada	July – August March - April	Cultivated oyster Natural oyster Cultivated oyster Natural oyster	-	21% 44% nd nd	Estuarine waters	Kelly and Stroh, 1988a
British Columbia, Canada	Summer	Estuarine water	70 cfu/mL	11-33% of water samples	Estuarine water warm	*Kelly and Stroh, 1988b
Willapa Bay, Washington State, United States	August	Water Sediment Oyster	0.5 - 3.0 MPN/g 1.6 - 5.4 MPN/g 1.5 - 4.0 MPN/g	-	Salinity 23.6 – 30.5ppt Temperature 15.5 –22.6^{o}C	Kaysner *et al.* 1990b
Long Island, United States	October to June	Oysters	3.6-23 MPN/g.	33%	-	Tepedino 1982
Prince Edward Island, Canada	All year	Oysters Mussels	-	6.7% 4.7%	-	Hariharan *et al*. 1995
Hong Kong	June through October	oysters (harvest) mussels (market) clams (market)	3.4 x 10^4 4.6 x 10^4 6.5 x 10^3/g	-	-	Chan *et al.* 1989
Japan		Water Sediments Market oyster	Not-	+ + 2/8	-	Kiiyukia *et al.* 1989
Hiroshima Bay, Japan	July 1987 to June 1988	Oysters	10^3 – 10^1/100g	69% (May to October)	Temperature ranged from 19.3-22.0°C	Ogawa *et al*. 1989

nd: none detected

Reported an association with *V. parahaemolyticus* illness and *V. parahaemolyticus* density in the estuarine waters of British Columbia.

While it is currently recommended to consider all available data as a potential source of information for exposure assessment it is also recognized that heterogeneity among studies make it difficult to combine data sets. For example, in the FDA-VPRA (Anonymous, 2001), including the data from other studies of the United States Atlantic (Tepedino, 1982) and Pacific Coasts (Kaysner *et al*., 1990b) would increase the sample size. However, these studies employed different methodology than that used by DePaola *et al*. (1990) and their inclusion could bias comparisons. Specifically, due to differences in method error associated with various analytical methods, statistical analysis of pooled data must account

for the differences in variation of observed measurements according to the analytical methods used. Although this may be readily accomplished, the precision of estimating trends is not necessarily increased due to the necessity of estimating multiple sources of variation. Furthermore, lack of precise estimates of method error makes it difficult to estimate the population variation of *V. parahaemolyticus* numbers (i.e. true variation in the absence of method error). For consistency in the FDA-VPRA risk assessment only data from DePaola *et al.* (1990) were used in developing the harvest module. Consequently, it is the use of this data that is described here. However, in meeting the second objective of this risk assessment, data from other countries will be considered in the next stage to see if the model can be applied to other country scenarios.

In the study of DePaola *et al.* (1990) distributions of *V. parahaemolyticus* numbers in water and oyster samples were positively skewed. This is consistent with the almost universal observation that microbial populations in foods are lognormally distributed. Therefore, the logarithm of the density, being more normally distributed, was regressed against temperature and salinity. *V. parahaemolyticus* was only detected in 31% oyster samples (19/61). Some of these samples are likely to have been false-negatives due to limitations of the method used (hydrophobic grid method). In order to avoid upward bias of predicted numbers at low temperatures the estimate of the regression line of $\log_{10}$ total *V. parahaemolyticus*/g oyster meat was obtained by the censored or Tobit regression method. The Tobit regression is a maximum likelihood procedure with likelihood reflecting both the probability of obtaining a nondetectable outcome at a given temperature as well as the probability distribution of observable numbers given that a sample has detectable cells of *V. parahaemolyticus*. The effect of this likelihood structure is to weight the influence of nondetection on estimated trends differently from samples with quantifiable numbers. The influence of nondetection is based on the probability of the number of cells in a sample falling below a fixed limit of detection rather than the assumption that a nondetectable measurement corresponds to an observed and quantifiable number at the limit of detection or one-half the limit of detection as is commonly assumed.

In a reanalysis of the paper of DePaola *et al.* (1990), the effect of temperature on *V. parahaemolyticus* numbers was found to be approximately linear over the range of water temperatures. The presence of a quadratic effect in temperature was not evident (not significant). With regard to salinity, a quadratic effect was found to be significant, suggesting that *V. parahaemolyticus* numbers increase with increasing salinity up to an optimal level and decrease thereafter. There was no significant interaction between temperature and salinity evident based on the data. Consequently, the best fitting model obtained was of the form

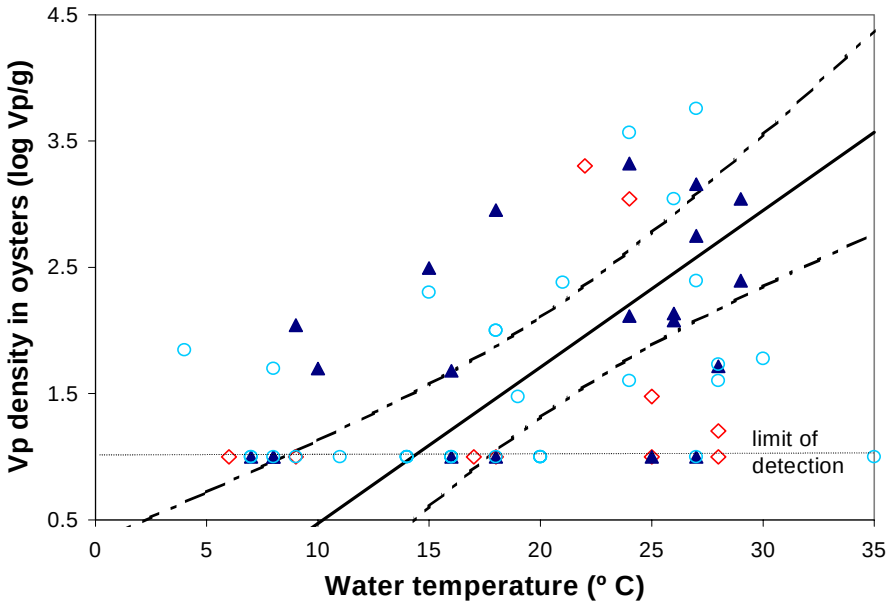
$$\log(Vp/g) = \alpha + \beta * TEMP + \gamma_1 * SAL + \gamma_2 * SAL^2 + \varepsilon$$

where TEMP denotes temperature in °C; SAL denotes salinity in parts per thousand (ppt); α, β, γ_1, and γ_2 are regression parameters for temperature and salinity effects on mean $\log_{10}$ numbers, 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 resulting parameter estimates were

$$\alpha = -2.6$$
$$\beta = 0.12$$
$$\gamma_1 = 0.18$$
$$\gamma_2 = -0.004$$
$$\sigma^2 = 1.0$$

The estimated relationships between total *V. parahaemolyticus* numbers in oysters *versus* water temperature and salinity are shown in Figures 2.4 and 2.5, respectively. Both salinity and temperature effects were significant based on the regression. The variation of observed values about the predicted mean regression line shown in Figure 2.4 is attributable to the effects of salinity as well as the variation about the mean due to population variation and method error. This regression line gives the predicted mean numbers *versus* temperature at a predicted optimal salinity of 22 ppt. Similarly the variation of the observed data about the regression curve (parabola) for salinity effect shown in Figure 2.5 is partially



attributable to differences in water temperature in addition to population and method error variation about the mean.

Figure 2.4: Observed $\log_{10}$ *V. parahaemolyticus* (Vp) numbers in oysters *versus* water temperature at different salinities (<10 ppt (◇), 10 to 20 ppt (▲) and >20 ppt (○)) in comparison to model predicted effect of temperature on mean $\log_{10}$ numbers (solid line) and 95% confidence limits (dashed lines) at salinity of 22 ppt.

Salinity of <5 ppt is known to be detrimental to the survival of *V. parahaemolyticus*. However, the influence of salinity within a range of moderate environmental salinities (i.e. 5-35 ppt) is not as clear. Based on the regression analysis, a quadratic relationship for *V. parahaemolyticus* numbers *versus* salinity within the 5-35 ppt range is consistent with the data from DePaola *et al*. (1990). However, this projected effect of salinity is not as strong as that of temperature. Within a broad range around the optimal salinity of 22 ppt, the results of the regression suggest that the differences in salinity actually encountered in oyster harvesting have relatively little effect on the *V. parahaemolyticus* population (Figure 2.6).

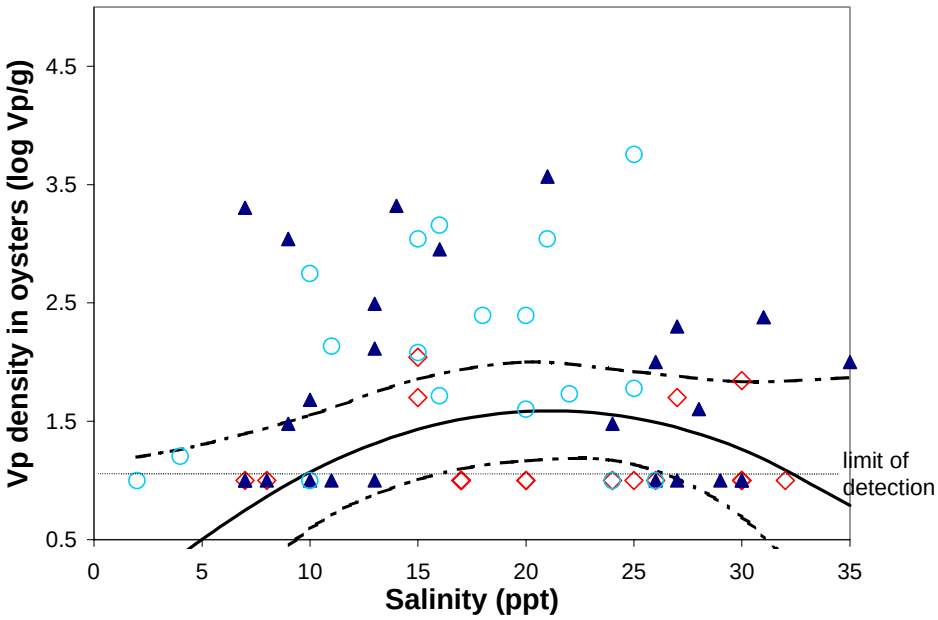


Figure 2.5: Observed $\log_{10}$ *V. parahaemolyticus* (Vp) numbers in oysters *versus* salinity at different temperatures (<15°C (◇), 15 to 25°C (▲), and >25°C (○)) in comparison to model predicted effect of salinity on mean $\log_{10}$ density (solid line) and 95% confidence limits (dashed lines) at temperature of 19°C.

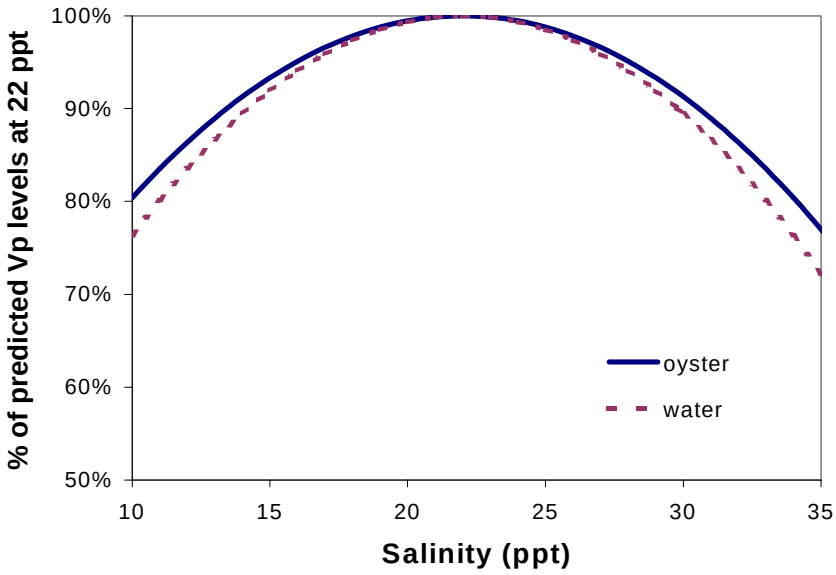


Figure 2.6: Effect of salinity on predicted mean $\log_{10}$ *V. parahaemolyticus* (Vp) numbers in oysters and water relative to predicted numbers at optimal salinity (22 ppt).

Clearly, in order to predict the distribution of *V. parahaemolyticus* numbers at harvest, based on the regression and the projected influence of water temperature and salinity in the environment, representative distributions of water temperature and salinity need to be estimated. Based on near-shore buoy data available from the National Buoy Data Center in the United States, regional and seasonal distributions of water temperature were available for the FDA-VPRA (Anonymous, 2001). However, representative data concerning the variation of salinity in shellfish growing areas were not identified. Consequently the effect of salinity was not incorporated into the present simulation for the United States.

Two considerations suggest that neglecting the effect of salinity does not adversely affect the predictive value of a model based on temperature alone. First, as shown in Figure 2.6, predicted mean *V. parahaemolyticus* numbers vary by less than 10% from the optimal (maximum) number as salinity varies from 15-30 ppt. Secondly, measurements of oyster liquor salinity at the retail level, which are strongly correlated with the salinity of harvest waters (FDA, 2000), suggest that oysters may be harvested from the more saline areas of the estuaries year round. In one survey (n=249) conducted year round with samples obtained from all regions of the United States the mean oyster liquor salinity was found to be 24 ppt (SD = 6.5 ppt) (ISSC & FDA, 1997). These two considerations suggest that the effect of variation of salinity on predicted distributions of *V. parahaemolyticus* numbers is minor. Variations in salinity between 15 and 30 ppt would increase the variance of the predicted distribution by only a small amount.

Ignoring the effect of variations in salinity in the simulation can be accomplished in either of two ways. Either salinity can be fixed to a mean value (i.e. 22 ppt) in the regression relationship derived above or the prediction of *V. parahaemolyticus* numbers can be based on a regression analysis of the data from DePaola *et al*. (1990) with water temperature as the only effect in the model.

With water temperature as the only effect the regression equation is:

$$\log(Vp/g) = \alpha + \beta * TEMP + \varepsilon$$

where TEMP denotes the temperature in °C, α and β are regression parameters for temperature effect on mean $\log_{10}$ numbers, 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.

Parameter estimates obtained based on the Tobit estimation method are

$$\alpha = -1.03$$
$$\beta = 0.12$$
$$\sigma^2 = 1.1$$

Based on the data of DePaola *et al*. (1990), the estimate of the variance about the mean (σ^2) is an inflated estimate of *V. parahaemolyticus* population variation due to method error. An estimate of population variation about the mean is obtained by subtracting an estimate of the method error. Because a Tobit estimation method is used, standard R^2 statistics cannot be used to characterize the correlation of the model to the data, and a pseudo-R^2 analysis was used. The pseudo-R^2 maximum likelihood R^2 (Maddala's) value of 0.47 was obtained (Long and Freese, 2000). In the study of DePaola *et al.* (1990) enumeration of *V. parahaemolyticus*, following the HGMP procedure, was based on testing of five suspect colonies and consequently was not as precise as possible and overall method error associated with estimating *V. parahaemolyticus* numbers may have been more comparable with that of a three tube MPN procedure. An estimate of the method error variance of the three tube MPN procedure is 0.35 (DePaola *et al*., 2000) and this value was considered a reasonable estimate of the method error for the methodology used in the study of DePaola *et al*. (1990).

The predicted mean log *V. parahaemolyticus* level *versus* temperature for the temperature-only regression is shown in Figure 2.7. Clearly, this relationship is comparable with that which would be obtained by fixing the salinity to a near optimal value (22 ppt) in the prediction equation based on both water temperature and salinity. The temperature-only regression was used to model the relationship between temperature and density of total *V. parahaemolyticus* at time of harvest.

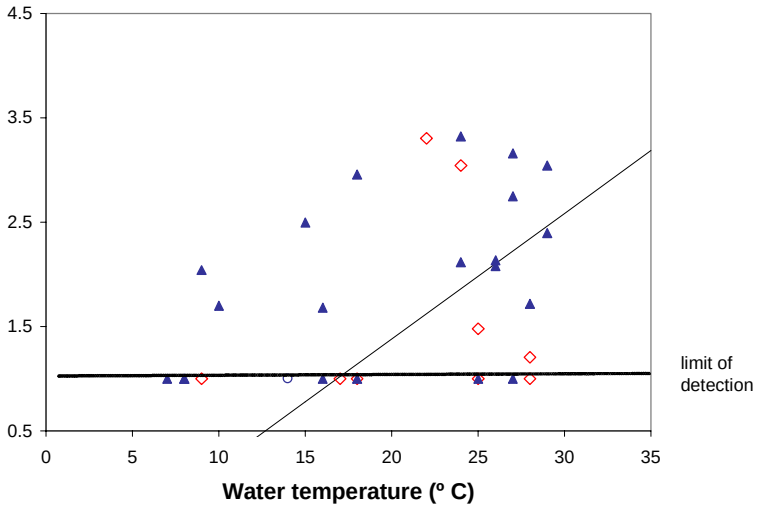


Figure 2.7: Observed $\log_{10}$ *V. parahaemolyticus* (Vp) numbers in oysters *versus* water temperature at different salinities (<10 ppt (◇), 10 to 20 ppt (▲) and >20 ppt (○)) in comparison to predicted $\log_{10}$ numbers (solid line) and 95% confidence limits (dashed lines) based on temperature only regression model.

2.2.5.3.2 Water Temperature Distributions

In the United States FDA-VPRA (Anonymous, 2001), regional and seasonal distributions of water temperatures were developed based on accumulated records from coastal water buoys (National Buoy Data Center data). Seasons were defined by calendar month: winter (January-March), spring (April-June), summer (July-September) and fall (October-December). For each region and season a shallow water buoy was selected as representing the water temperature distribution for oyster harvest areas within that region/season combination. The available database for most buoys has hourly water temperatures from 1984 up to the present time, with occasional data gaps due to instrumentation malfunction. The correlation between water temperature and the ambient air temperature that oysters are subject to after they are harvested was accounted for by selecting buoys for which air temperature records were also available.

Because oyster harvesting in the United States outside of the Pacific Coast region commences early in the morning and ends mid or late afternoon, the daily water temperature recorded at noon was considered to represent an average daily temperature. The distribution of these "average" temperatures within a given region and season varies from year-to-year with wider variations occurring during the transitional seasons of spring and fall.

Within a given year, the distribution of the noon water temperature was found to be unimodal within a given range. This empirical distribution is adequately approximated as a normal distribution provided that no weight is given to implausible values outside the historical range of values that may be

expected. Differences in these distributions from one year to the next are evident in the buoy data. This year-to-year variation in the water temperature distributions has been characterized by calculating the central tendency and variation in both the mean and standard deviation of these distributions. The buoys selected for the study, and the summary statistics calculated, are shown in Table 2.4.

In Table 2.4, μ and σ denote the mean and standard deviation of the distribution of water temperatures within any particular year for different regions and seasons. The extent of year to year variation of these distributions is summarised by the mean and the variance of the parameters μ and σ. The mean and variance of these parameters are denoted in the table as mean(μ), variance(μ), mean(σ) and variance(σ), respectively. The correlation between μ and σ is denoted by corr(μ, σ). A positive correlation between parameters μ and σ summarises the observation that when the mean water temperature is higher than normal the variation in temperatures from one day to the next is generally greater than that observed when the mean temperature is lower than normal. Similarly, a negative correlation summarises the observation that temperatures are less variable when the mean water temperature is higher than normal. The regional and seasonal nature of the FDA-VPRA seems to indicate that this model would be applicable for use in other regions and countries. However, this means that data similar to that which is accessible in the United States needs to be available in other countries wishing to apply this model.

In the United States data on water temperature are also available from the Storage and Retrieval of U.S. Waterways Parametric Data (EPA STORET) (http://www.epa.gov/OWOW/STORET/) and the National Estuarine Reserve Sites (NERR) program (http://inlet.geol.sc.edu/cdmoweb/home.html) but these temperature measurements tend to be more specific to estuaries as opposed to open waterways. Comparing data from these various sources in the United States suggests that shallow water estuaries may be slightly warmer than open coastal waters but the difference is not substantial (i.e. ~1ºC difference on average).

An additional consideration is the availability of enough long-term historical data to determine the extent of year-to-year variation. In the United States, data are available from most NDBC buoys from 1988 to the present, the NERR program from 1995 while EPA STORET has data dating back to 1964, although not all of this is readily accessible. Also, STORET records do not necessarily correspond to fixed locations, as is the case for NBDC and NERR. The availability of such data from other countries is another point that must be considered in adapting this model for use outside the United States. Additional data on water temperature (and salinity) measurements specific to oyster harvesting areas were made available to the United States risk assessment team by State agencies in Texas, Alabama, New York, and Connecticut. Water temperatures provided were not substantially different from the NBDC data selected for each region. This is noteworthy in relation to data availability as it indicates that there may be more than one source for certain types of data.

2.2.5.3.3 Prediction of the distribution of pathogenic V. parahaemolyticus numbers

Estimates of the percentage of total *V. parahaemolyticus* isolates that have been found to be pathogenic in several studies are presented in Table 2.5. The estimate based on studies by Kaysner *et al.* applies to the United States Pacific Northwest (Kaysner *et al.*, 1990b; Kaysner and Weagant, 1982) with the other estimates in the table being appropriate for all other areas of that country. The estimates seem to suggest that the average prevalence of *V. parahaemolyticus* that are pathogenic, relative to total *V. parahaemolyticus* varied from one region to another. On the United States West Coast it is approximately 3% compared with 0.2-0.3% for the Gulf Coast and other areas of the country. However, because of the relatively small sample size and the small number exhibiting pathogenicity in these studies (Table 2.5) there is still uncertainty with regard to the average percentage of *V. parahaemolyticus* isolates that are pathogenic.

Table 2.4: Summary statistics of the year-to-year variation in the mean and standard deviation of noon water temperature distributions for different regions and seasons in the United States

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

Source of data: National Buoy Data Center (NBDC) http://www.seaboard.ndbc.noaa.gov/Maps/Wrldmap.shtml and Washington State shellfish specialist N. Therien, personal communication (1999)

NBDC measures surface water temperature (sensors are generally 1.0 to 1.5 meter deep)

[a] μ and σ denote mean and standard deviation of within region/season temperature distribution, respectively; mean(), variance(), and corr() denote the mean, variance and correlation between the parameters μ and σ across different years For example the NBDC buoy located at Dauphin Island, Alabama (Gulf Coast) has recorded water and air temperatures from 1987 to the present time. For the spring season (April-June), the noon water temperature was found to be 24.5°C with an inter-year variance of 0.98°C (standard deviation 0.99°C). Similarly, for the standard deviation of the within year temperature distributions, the central tendency across different years was an average of 3°C [mean(σ)] with a variance of 0.27°C [variance(σ)]. The correlation between μ and σ [corr(μ, σ)] was -0.55 indicating that the day-to-day temperatures were less variable when the overall mean temperature was higher than that of a typical year.

[b]There were no near-shore NBDC buoys recording water temperatures which represented oyster growing areas and consequently, for this region, seasonal and year-to-year variations in water temperature distributions were developed based on compiled data from WA State shellfish specialists (Washington State Department of Health) from 1988-99. These water temperature data were recorded in association with collection of samples for monitoring of vibrios and faecal coliforms and therefore represent temperatures for oyster growing areas. Averages of water temperature were substituted when multiple

measurements were recorded for any given day. Year-to-year variations in the water temperature distributions for the Pacific Coast were developed in the same manner as that for the other regions.

Table 2.5: Estimates of pathogenic *V. parahaemolyticus* as a percentage of total *V. parahaemolyticus*

Number oyster samples [(a)]	Number (%) positive for pathogenic Vp	Number isolates tested	Number (%) pathogenic	Source in the United States
193	8 TDH+ (4.1%)	32 33	9 TDH+ (0.3%)	ISSC/FDA retail study (unpublished) (FDA, 2000)
153	Not done[b]	2218	4 KP+ (0.18%)	Galveston Bay , Texas (Thompson *et al*., 1976)
25	4 TDH (16%)	308	10 TDH (3.2%)	Grays Harbour, Washington (Kaysner *et al.,* 1990b) Puget Sound, Washington (Kaysner & Weagant, 1982)
96	3 TDH (3.1%)	Not done	10, 140, 10 cfu/g in three samples (0.3%)	FDA study of Texas outbreak Galveston Bay (DePaola, 1999)

[a] Oyster samples (12-oyster composites) containing detectable pathogenic *V. parahaemolyticus* TDH+ (thermostable direct haemolysin-positive, a toxin produced by *V. parahaemolyticus* that lyses red blood cells in Wagatsuma agar) or KP+ (Kanagawa-positive)

KP+ and TDH+ are used interchangeably in defining pathogenicity of *V. parahaemolyticus*

[b] Oyster, water, & sediment samples tested for KP+

Prevalence may vary from one year to the next. Even if an average percentage were known with certainty this information, together with the estimated distributions of total *V. parahaemolyticus* numbers, is not sufficient to identify the distribution of pathogenic *V. parahaemolyticus* numbers. It is likely that the density of pathogenic strains is spatially and temporally clustered in the environment to some degree. The average number of isolates that are pathogenic does not identify the extent of this clustering.

To account for the probable spatial and temporal clustering of pathogenic strains relative to total *V. parahaemolyticus* numbers a beta-binomial distribution was assumed for the number of pathogenic *V. parahaemolyticus* at the time of harvest. Under a beta-binomial distribution the percentage of total *V. parahaemolyticus* that are pathogenic varies from one sample of oysters (e.g. 12 oyster composite) to the next. Given the occurrence of outbreaks, at least in the United States, this appears to be a reasonable assumption but cannot be validated directly since extensive quantitative surveys of pathogenic *V. parahaemolyticus* numbers are not available. Specifically, based on the number of total *V. parahaemolyticus* (Vp_{total}), within a given composite, the number of pathogenic *V. parahaemolyticus* (Vp_{path}) present was assumed to be distributed as a binomial random variable with Vp_{total} trials (size parameter) and a probability of success (p) distributed as a beta random variable. The distribution of the probability parameter p is called a mixing distribution and the variation of this parameter across composites of oysters induces a clustering of pathogenic strains relative to the total number of *V. parahaemolyticus* cells.

Formally this beta-binomial model is expressed as:

$$Vp_{path} \mid (Vp_{total} = n) \quad \sim \quad B(n, p) \bigwedge_{p} Beta(\alpha, \beta)$$

The notation here indicates that the distribution of the number of pathogenic *V. parahaemolyticus* present is conditional on the number of total *V. parahaemolyticus* present (n). The mean and variance of this conditional distribution are:

$$E[Vp_{path} \mid Vp_{total} = n] = \frac{\alpha}{\alpha + \beta} * n$$

$$Var[Vp_{path} \mid Vp_{total} = n] = n * \left[\frac{\alpha * \beta}{(\alpha + \beta)^2} \left(1 + \frac{1}{\alpha + \beta + 1} (n - 1) \right) \right]$$

$$= n * \left[\frac{\alpha * \beta}{(\alpha + \beta)^2} \left(1 + \phi * (n - 1) \right) \right]$$

where E[X] and Var[X] denote the mean and variance, respectively. The parameter ϕ is called the overdispersion parameter. The parameters α and β of mixing distribution in the beta-binomial can be expressed in terms of the average percentage of isolates which are pathogenic (*P*), which is the mean of the mixing distribution, and the dispersion parameter ϕ:

$$\alpha = \frac{P * (1 - \phi)}{\phi}$$

$$\beta = \frac{(1 - P) * (1 - \phi)}{\phi}$$

From Table 2.5, best estimates of the parameter *P* are 0.03 for the United States West Coast and 0.002 for other regions of that country. The information is more limited with respect to the value of the shape parameter ϕ. This parameter pertains to the variation of frequency of pathogenic *V. parahaemolyticus* across different oyster samples or composites. Based on the data on frequency of pathogenic isolates, Bayes estimates of the parameters α and β are:

$$\hat{\alpha} = r + 1$$

$$\hat{\beta} = n - r + 1$$

where r is the number of pathogenic isolates and n is the total number of isolates. These estimates differ for the United States West Coast *versus* other regions of that country in the same manner as does average percentage pathogenic (*P*). An estimate of the dispersion parameter is:

$$\hat{\phi} = \frac{1}{\hat{\alpha} + \hat{\beta} + 1} = \frac{1}{n + 2}$$

Based on the data shown in Table 2.5, estimates of the dispersion parameter are 0.0032 for the United States West Coast and 0.00045 for the other regions of the country.

Because the prevalence of pathogenicity is uncertain and may vary from year to year, *P* was evaluated as an uncertainty parameter in the Monte Carlo simulations. The uncertainty was modelled as a triangle distribution with a different mean and range for the Pacific Northwest than for other regions of the United States. For the Pacific Northwest the average percentage pathogenic was estimated to be 3% and the minimum and maximum of the distribution was taken to be 2% and 4%, respectively. For all other

regions of the country the average percentage pathogenic was estimated to be 0.2% and the corresponding minimum and maximum of the distribution was 0.1% and 0.3%, respectively. Uncertainty with regard to the shape parameter ϕ was not evaluated.

The Monte Carlo simulation of the distribution of pathogenic *V. parahaemolyticus* present in oysters was performed as follows. For each region and season the mean and standard deviation of water temperature distributions were sampled based on the bivariate normal distributions given in Table 2.4. Each random sample from these distributions represents a distribution of water temperature (i.e. for different years). Given a water temperature distribution, the distribution of total *V. parahaemolyticus* numbers in composites of 12 oysters at harvest was simulated by:

- Sampling from the distribution of water temperature
- Using the regression relationship to calculate a mean density corresponding to each sampled water temperature
- Perturbing the calculated means by a random normal deviate corresponding to the estimate of population variation of the numbers.

The distribution of pathogenic *V. parahaemolyticus* numbers was derived from that of total *V. parahaemolyticus* assuming a beta-binomial model for the extent of clustering of pathogenic relative to total counts. Multiple simulations were run with different values of average percentage of isolates pathogenic in order to evaluate the uncertainty with regard to this parameter.

2.2.5.4 Post harvest

The *Post Harvest Module* describes the effects of typical industry practices including transportation, handling and processing, as well as distribution, storage and retail on *V. parahaemolyticus* numbers in oysters harvested from various locations and seasons. Factors considered as possible influences on the numbers of pathogenic *V. parahaemolyticus* at consumption include:

- Ambient air temperatures at time of harvest
- Time from harvest until oysters are placed under refrigeration
- Time it takes oysters to cool once under refrigeration
- Length of refrigeration time until consumption

The purpose of modelling the *Post Harvest Module* is to simulate the effects of typical industry practices on the numbers of *V. parahaemolyticus* in oysters from harvest to consumption for various locations and seasons. The module can also be used to simulate the effect of intervention strategies. The inputs to the module are the regional and seasonal distributions of total and pathogenic *V. parahaemolyticus* at harvest. The output of the module is a series of predicted distributions of the total and pathogenic numbers at time of consumption. The final steps to be addressed in the exposure assessment are the storage and retail conditions of the product, storage after retail and finally preparation and consumption. Some of these factors are included at the end of the flowchart presented in Figure 2.8. Prior to consumption, temperature of storage is probably among the most critical factors to be considered. A diagrammatic representation of the parameters modelled in this section is presented in Figure 2.8. The baseline prediction is the distribution of numbers of *V. parahaemolyticus* (in 12 oyster composites), assuming current industry practices in the United States.

The principal assumption used to develop the relationships between numbers at harvest and at consumption is that the growth and survival of pathogenic *V. parahaemolyticus* is the same as total *V. parahaemolyticus*. Although no definitive studies of the growth characteristics of pathogenic

V. parahaemolyticus are available, preliminary data suggest that there is little difference between growth characteristics of pathogenic *versus* nonpathogenic strains (DePaola, 1999). Furthermore, observation of the growth of total *V. parahaemolyticus* in oysters is limited to only one temperature (26°C). To bridge this data gap a model of *V. parahaemolyticus* growth in broth developed by Miles *et al*. (1997) was used (see section 2.2.2.1). The predictions of this model have been adjusted to predict the growth rate in oysters, which is less than that of broth model systems possibly due to the influence of competing microflora.

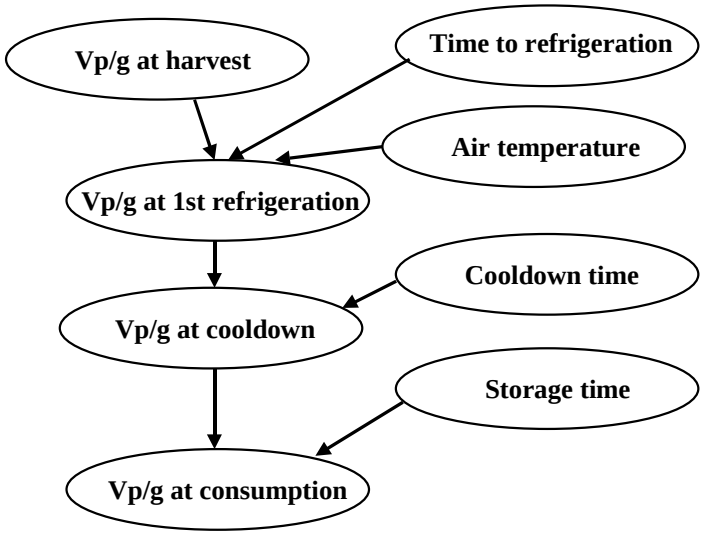


Figure 2.8. Schematic depiction of the Post Harvest Module of the *V. parahaemolyticus* (Vp) risk assessment model.

2.2.5.4.1 Growth of V. parahaemolyticus from harvest to first refrigeration

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

- Growth rate of *V. parahaemolyticus* as a function of temperature
- Temperature of oyster meat following harvest
- Length of time product is unrefrigerated

2.2.5.4.2 Distribution of ambient air temperature

Examination of water and air temperatures obtained from the NOAA/NBDC database in the United States showed a strong correlation between water and air temperature. This correlation has been incorporated into the risk simulation by modelling the distribution of the difference in water *versus* air temperatures based on the normal distribution within any given region and season. These distributions are then used to predict the air temperature that oysters would be subjected to depending on the water temperature at the time of harvest.

In the process of simulating the distribution of total and pathogenic *V. parahaemolyticus* at harvest by the Monte Carlo method, the water temperature associated with any given outcome is

retained. A corresponding air temperature is predicted by sampling from the appropriate distribution for the difference in air *versus* water temperature. This difference is then added to the water temperature to derive a corresponding air temperature. The distributions of difference in air *versus* water temperature were obtained by pooling the data available for each near-shore buoy across all available years. The mean and variance of these distributions are shown in Table 2.6.

Table 2.6. Means and standard deviations of the distribution of the difference between recorded air and water temperatures at midday (°C) in the United States

Region in the United States	Mean (standard deviation) distribution differences between air and water temperature			
	Winter (Jan-March)	Spring (April-June)	Summer (July-Sept)	Fall (Oct-Dec)
Northeast Atlantic (Ambrose buoy, NY harbour)	-2.6 (5.0)	2.2 (3.2)	0.52 (2.7)	-3.2 (4.2)
Mid-Atlantic (Thomas Point Lighthouse buoy, Chesapeake Bay, MD)	-0.25 (4.0)	0.54 (2.9)	-1.4 (2.1)	-2.1 (3.1)
Gulf Coast (Dauphin Island, AL buoy)	-1.07 (3.3)	-1.24 (1.63)	-1.66 (1.33)	-1.62 (3.3)
Pacific Northwest (3 years of data from NOAA buoy on north end of Puget Sound, WA)	-1.6 (1.8)	1.3 (1.3)	1.3 (1.5)	-0.8 (2.0)

Source of data: http://www.seaboard.nbdc.noaa.gov/Maps/Wrldmap.shtml

2.2.5.4.3 Distribution of time oysters are left unrefrigerated

The distribution of the length of time that oysters are held unrefrigerated can be developed by using the distribution of length of working day, with the assumption that oysters are harvested uniformly from the start of the harvest up to one hour prior to conclusion of harvesting, when they are landed and placed in cold storage. Table 2.7 shows the minimum, maximum and mean duration of oyster harvesting that we have projected for the different regions and seasons in the United States. In the risk simulation Beta-PERT distributions were used based on these parameters to simulate the variation in the duration of harvesting. A Beta-PERT distribution is a translated and scaled Beta distribution with specified moments. It is commonly used for the purpose of simulating parameter variation within a defined range in Monte Carlo simulations. Figure 2.9 shows the probability density of the Beta-PERT distribution with minimum of 2, maximum of 11 and mean of 8 hours.

The parameters for these distributions were developed from a 1997 GCSL survey in the United States that included statistics on the length of harvest (Cook, 1997b). The study was conducted in several Gulf Coast states during the fall of two successive years; one season prior to initiation of the NSSP time to refrigeration requirements (for states whose product has been confirmed as the source of two or more *V. vulnificus* illnesses) and then the following year after implementation. According to this survey remote growing areas results in a longer harvesting process. In the United States this reflects the situation in Louisiana while the practices of Florida and Texas were considered to be representative of other regions and, in the absence of conflicting information, the longer times were assumed for the other regions throughout the year.

For the United States Gulf Coast States it was assumed that harvesting is done more quickly in the spring, summer and fall due to the NSSP time to refrigeration requirements and is generally

longer in the winter when cooler water conditions prevail. Other areas of the United States were treated separately for various reasons such as the longer duration of harvest year round in Louisiana, the fact that oysters are generally harvested during intertidal periods and so the length of time held unrefrigerated is substantially less on the West Coast and reports from the Pacific Coast Shellfish Growers Association (PCSGA) that Pacific oysters are placed under refrigeration within four hours.

Table 2.7. Minimum, maximum and most likely duration of oyster harvest (length of harvesting operation) for different regions and seasons

Location in the United States	Duration of harvest (hours)			
	Winter (Jan-March)	**Spring (April-June)**	**Summer (July-Sept)**	**Fall (Oct-Dec)**
Northeast Atlantic (assumed same as pre-NSSP Control plan in Gulf- TX (ISSC & FDA, 1997)	max = 11 min = 2 most likely = 8	max = 11 min = 2 most likely = 8	max = 11 min = 2 most likely = 8	max = 11 min = 2 most likely = 8
Mid-Atlantic (assumed same as pre-NSSP Control plan in Gulf- TX (ISSC & FDA, 1997)	max = 11 min = 2 most likely = 8	max = 11 min = 2 most likely = 8	max = 11 min = 2 most likely = 8	max = 11 min = 2 most likely = 8
Gulf Coast - LA (50% of harvest) (pre-NSSP Control plan in LA in winter; ICP otherwise (ISSC & FDA, 1997)	max = 13 min = 7 most likely = 12	max = 11 min = 5 most likely = 9	max = 11 min = 5 most likely = 9	max = 13 min = 7 most likely = 12
Gulf Coast - FL, AL, TX (50% of harvest) (assumed same as pre-NSSP Control plan in Gulf- TX in winter, NSSP Control otherwise (ISSC & FDA, 1997)	max = 11 min = 2 most likely = 8	max = 10 min = 3 most likely = 7	max = 10 min = 3 mean = 7	max = 10 min = 3 most likely = 7
Pacific Northwest (Watkins, 2000)	max = 4 min = 1 most likely = 3	max = 4 min = 1 most likely = 3	max = 4 min = 1 most likely = 3	max = 4 min = 1 most likely = 3

Source of data: ISSC & FDA (ed.) 1997 National Shellfish (ISSC & FDA, 1997)
Washington State Shellfish experts and Washington State Department of Health (Watkins, 2000)

As indicated, harvesting of oysters was assumed to occur uniformly from start of harvest, up to one hour prior to end of harvest operation. The distribution of the duration of time oysters were held unrefrigerated was simulated by first sampling from the distribution for duration of harvest operation and then sampling from a uniform distribution with a minimum of one hour and maximum corresponding to the randomly selected duration of harvest. Because they are harvested over the length of harvesting operations, the mean time that oysters remain unrefrigerated is almost always shorter than the maximum length of duration of harvesting.

Overall, the extent of growth occurring prior to time of first refrigeration (i.e. time at which oysters are first placed in refrigerated storage) was simulated by:

- Sampling air temperature corresponding to the water temperature at harvest;
- Sampling duration of harvest;
- Sampling the length of time unrefrigerated given a particular duration of harvest; and
- Calculating the extent of growth expected for the given duration of time unrefrigerated.

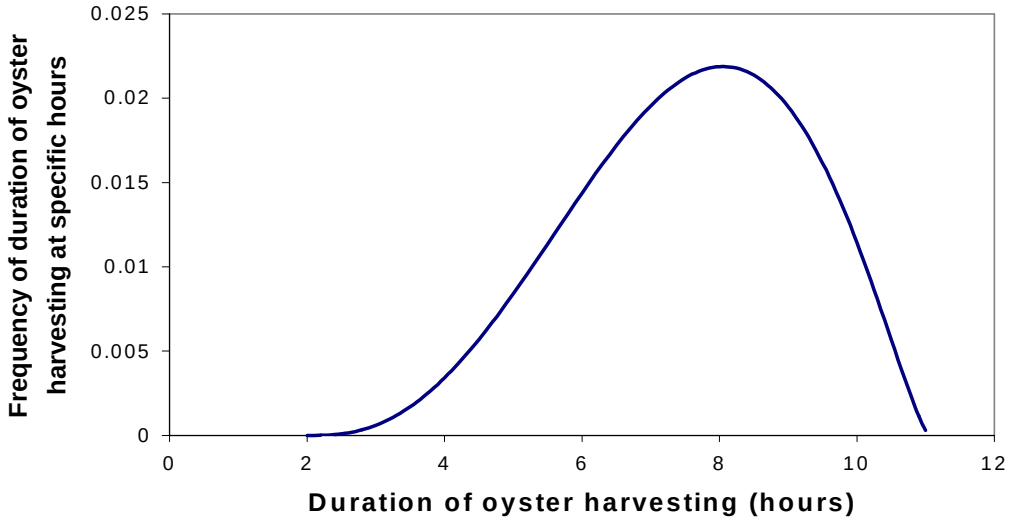


Figure 2.9. Beta-PERT probability density distribution for the duration of harvesting operations during the winter season (Mid-Atlantic, Northeast Atlantic, Gulf Coast, excluding Louisiana) (FDA, 2000)

2.2.5.4.4 Growth of V. parahaemolyticus during cooling

V. parahaemolyticus will continue to grow in oysters after they are placed under refrigeration until the temperature of the oyster tissues falls below a certain threshold (e.g. 10°C). The time it takes for oysters to cool once under refrigeration is assumed to vary according to the efficiency of the chilling medium, the quantity of oysters to be cooled and their arrangement in the cool room. Data on cooling rates of commercial oyster shellstock could not be located. In the United States preliminary GCSL experiments with a single in-shell oyster at 30°C, in which a temperature probe was inserted into its tissue, indicated a cooling rate of approximately 0.5°C/min when placed into a 3°C cooler (DePaola, 1999). However, 24 oysters in an uninsulated plastic container required approximately 7 hours to cool from 26°C to 3°C. These data suggest considerable uncertainty for cooling times after oysters are refrigerated and it was concluded that a uniform distribution between 1 and 10 hours would be appropriate to describe the current state of knowledge.

As oysters cool down to storage temperatures it is reasonable to expect that the growth rate of *V. parahaemolyticus* slows with declining temperature. At the start of the cooling period, when oysters are first placed under refrigeration, the growth rate is still equal to the initial rate as determined by ambient air temperature. At the end of the cooling period, when oysters have reached storage temperatures, it was assumed that there is no further growth and that numbers will decline slowly thereafter (Gooch *et al*., 1999). Implicitly, this assumes that there is no appreciable temperature abuse after oysters have been placed in cold storage. As the rate at which oysters cool during cold storage is not known, it was assumed that during the period of cooling, the growth rate of *V. parahaemolyticus* falls uniformly to zero.

A discrete approximation of the extent of growth that may occur during cooling was simulated by first sampling from a discrete random uniform distribution between 1 and 10 hours (duration of cooling). The extent of growth during each hour of the cooling period was then estimated

from the average growth rate during that hour. The average growth rates were dependent upon the growth rate of *V. parahaemolyticus* in oysters left unrefrigerated (i.e. as determined by the ambient air temperature for a given oyster lot) and the duration of cooling. The total excess growth was the sum of these values over the cooling period subject to the restriction that the maximum density of 6.0 $\log_{10}$ per gram could not be exceeded. These calculations are illustrated in the Table 2.8, where, for example, it takes k hours for a particular oyster lot to reach cooler temperature.

Table 2.8. Discrete approximation of variation in the growth rate of *V. parahaemolyticus* during a cooling period of k hours

Hour of the cooling period	**Average growth rate ($\log_{10}$/hr) during the hour of cooling**
1	$\frac{(k+1)-1}{k}\mu_m$
2	$\frac{(k+1)-2}{k}\mu_m$
3	$\frac{(k+1)-3}{k}\mu_m$
...	...
k	$\frac{(k+1)-k}{k}\mu_m$
k+1	0

The total excess growth is the sum of the growth over the k hours:

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

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

$$= \mu_m * \frac{k+1}{2}$$

Since the cooling time k is a random variable with a mean of 5.5 hours, the average extent of growth is $3.25*\mu_m$, where μ_m is the maximal growth rate determined by ambient air temperature at time of harvest. Thus, for an initial growth rate of 0.19 $\log_{10}$ per hour (i.e. at 26° C), the average growth occurring during cooling is approximately 0.6 $\log_{10}$, or almost two generations.

2.2.5.4.5 Die-off of V. parahaemolyticus during cold storage

Gooch *et al.* (1999) showed that, in oysters, *V. parahaemolyticus* declined 0.003 $\log_{10}$ per hour when stored 14-17 days at 3°C. This die-off rate was assumed to be typical of all refrigerated oysters. Error may be introduced because commercial oysters are typically stored at higher temperatures (5-10°C). Die-off may have been overestimated because chill-stressed *V. parahaemolyticus* may not be recovered by the methods used in the study. One of the enumeration methods employed a repair step in a medium containing magnesium, which has been shown to increase recovery of chill-stressed cells. This method did not give higher *V. parahaemolyticus* counts after refrigeration than did the other methods that were used to calculate die-off. Therefore, the effect of chill-stress on die-off rate was assumed to be negligible.

Data from the ISSC/FDA retail study for the time between harvest and sample collection were assumed to be a reliable estimate for the refrigerated storage time to consumption (Cook, 1997). Summary statistics on the storage time for samples obtained during the study are shown in Table 2.9. A small degree of error may be introduced by assuming that these data are representative of storage time in so far as samples were generally collected on Monday or Tuesday and most servings are consumed in restaurants on weekends. Since this was a year long nationwide survey, the mean of 7.7 days and range of 1-21 days was assumed to be representative of all seasons and regions. In the simulation, we used a Beta-PERT distribution based on the overall mean, minimum, maximum and mode in order to obtain a smooth representation of the variation in the duration of storage time.

Table 2.9. Summary statistics of the distribution of storage times (time under refrigeration in days) of oysters samples obtained during the ISSC/FDA retail study

Storage Time	Consumed locally (within the same region of harvest)	Non local (transported outside region of harvest)	Overall
Minimum	1	2	1
Maximum	20	21	21
Mean	6.3	9.9	7.7
Most likely	6	5	6

Source of data: (FDA, 2000)

The predicted numbers of *V. parahaemolyticus* at time of consumption were therefore simulated by randomly sampling from the distribution of storage times and multiplying by a die-off rate of 0.003 $\log_{10}$ per hour. The resulting distribution was then subtracted from the predicted distribution of *V. parahaemolyticus* numbers in oysters initially reaching cooler (no growth) temperatures.

2.2.5.4.6 Consumption

Food surveys and oyster landing statistics provide a basis for estimating the extent of exposure in the population. Distributions of ingested dose were developed by considering the probabilistic variation of number and meat weight of oysters in a serving in addition to the expected variation of the numbers of pathogenic *V. parahaemolyticus* determined in the *Harvest* and *Post Harvest Modules*.

2.2.6 Mitigation Strategies

This model can be used to demonstrate the effect of mitigation strategies. For example, the effects of three possible post harvest mitigations can be evaluated in the Monte Carlo simulations:

- Reduced time to refrigeration (rapid cooling)
- Heat treatment
- Freezing/frozen storage

Mitigation of rapid cooling can be was modelled by assuming that oysters will be cooled to no growth temperatures immediately following harvest by icing or otherwise cooling oyster shellstock aboard ship. In the simulation it is assumed that the time unrefrigerated is zero (i.e. a degenerate distribution or constant). However, some growth is still projected to occur during cooling as described above.

The effects of heat treatment and of freezing/cold storage can be evaluated by adjusting the simulated output of the baseline simulation (no mitigation) downward by factors of 4.5 $\log_{10}$ (the lowest level which caused a substantial reduction in illness after mild heat treatment) and 2 $\log_{10}$, respectively. Thus, random sequences of values for total and pathogenic numbers produced in the course of Monte Carlo simulation were divided by 31,623 and 100, respectively. The implicit assumption is that the effect of treatment on $\log_{10}$ *V. parahaemolyticus* numbers is uniform with no induced change in the variance of $\log_{10}$ numbers.

The effects of these mitigations on the probability of illness will be shown in the Risk Characterization Section when completed.

2.3 Hazard characterization

Dose-response relationships can be developed from epidemiological investigations of outbreaks and sporadic case series, human feeding trials or animal models of *V. parahaemolyticus* and related (surrogate) pathogens. In Japan, for example, human trials showed an increase in the number of illnesses with increasing numbers of pathogenic *V. parahaemolyticus*. Different dose-response models have been compared for the purpose of extrapolating risk of illness estimated on the basis of human feeding trials at high levels of exposure to the lower levels of exposure associated with consumption of raw oysters (Anonymous, 2001). However, consideration of United States CDC estimates of annual illness suggests that the dose-response under conditions of population exposure is different from that observed in human volunteer studies. In other words, direct extrapolation of the dose-response under conditions of exposure in the feeding trials is not supported by the epidemiological data. The human feeding trials were conducted under conditions of concurrent antacid administration. Due to possible food matrix effects of the oyster, dose-response was shifted by 1 $\log_{10}$ from that based on published clinical trials. Preliminary data have shown that this shift is "supported" by consideration of the CDC numbers of *V. parahaemolyticus* infection.

2.3.1 Description of the pathogen, host, and food matrix factors and how these impact the disease outcome.

2.3.1.1 Characteristics of the pathogen

2.3.1.1.1 Infectivity, virulence/pathogenicity

Infection by *V. parahaemolyticus* is characterized by an acute gastroenteritis usually within 4-30 hours after exposure. Most cases of *V. parahaemolyticus* infections resolve themselves without medical intervention, however; on rare occasions, infection can lead septicaemia and death. Not all strains of

V. parahaemolyticus cause illness - most illness causing strains are associated with the presence of a virulence factor, thermostable direct haemolysin (TDH) and possibly another virulence factor, the TDH related haemolysin (TRH).

2.3.1.1.2 Genetic factors (e.g. antimicrobial resistance and virulence factors).

The full number and role of virulence factors for *Vibrio* spp. is unknown and is the subject of continuing research. While a complete characterization of the virulence of each pathogenic *Vibrio* spp. strain and its associated virulence factors is not practical, several of the leading virulence factors have been characterized.

The virulence of *V. parahaemolyticus* appears to be largely attributable to thermostable direct haemolysin (*tdh* $^{+}$) (Miyamoto, *et al*., 1969). Strains of *V. parahaemolyticus* expressing this toxin lyse red blood cells on Wagatsuma agar and are also called Kanagawa positive (KP+). TDH+ and KP+ both indicate the presence of the toxin that is coded for by *tdh*$^{+}$. The tdh^{+} allele is seldom found in environmental isolates of *V. parahaemolyticus*, but is frequently found in clinical isolates. Another genetic factor that may play a role in the virulence of *V. parahaemolyticus* is *trh*$^{+}$, an allele that codes for the TDH related haemolysin (Honda *et al*., 1988).

2.3.1.2 Characteristics of the host

2.3.1.2.1 Immune-status

The host immune system is the critical host defence mechanism against *Vibrio* spp. infection. The immunocompromised are at special risk for both infection and for more severe sequelae associated with that infection. In Japan cases of *V. parahaemolyticus* bacteraemia have reported among patients who were all immunosuppressed, especially with leukaemia and cirrhosis (Ng *et al.*, 1999).

2.3.1.2.2 Age, sex and ethnic group

The vehicle of infection under consideration in this risk assessment for *V. parahaemolyticus* is raw seafood. The consumption patterns for raw oysters the United States have been estimated for age, sex, and ethnic group (Desenclos *et al*., 1991 and Timbo *et al*., 1995).

Anyone who consumes shellfish raw is “at risk” for infection by *V. parahaemolyticus*. A FDA telephone survey in the United States completed in 1993 and repeated in 1998 showed that consumption of raw shellfish is not uniformly distributed (Levy & Fein, 1999). A higher percentage of men consume raw oysters than women (16% *versus* 7%) and raw shellfish consumption is higher for those living along the coastline of the United States than for those living inland (22% *versus* 13%). The trend, as evidenced in the 1998 FDA survey, is toward lowered consumption of raw shellfish. This may be the result of education efforts by the FDA concerning risks associated with the consumption of raw or undercooked protein foods such as beef, chicken, eggs and shellfish. Raw shellfish consumption is highest among those with the highest education levels and the trend toward reduction in raw shellfish consumption over the last 5 years is smallest in this group.

Japanese data on the age and sex group also suggests that every age group is susceptible to infection of *V. parahaemolyticus* (Anonymous, 1998b*).*

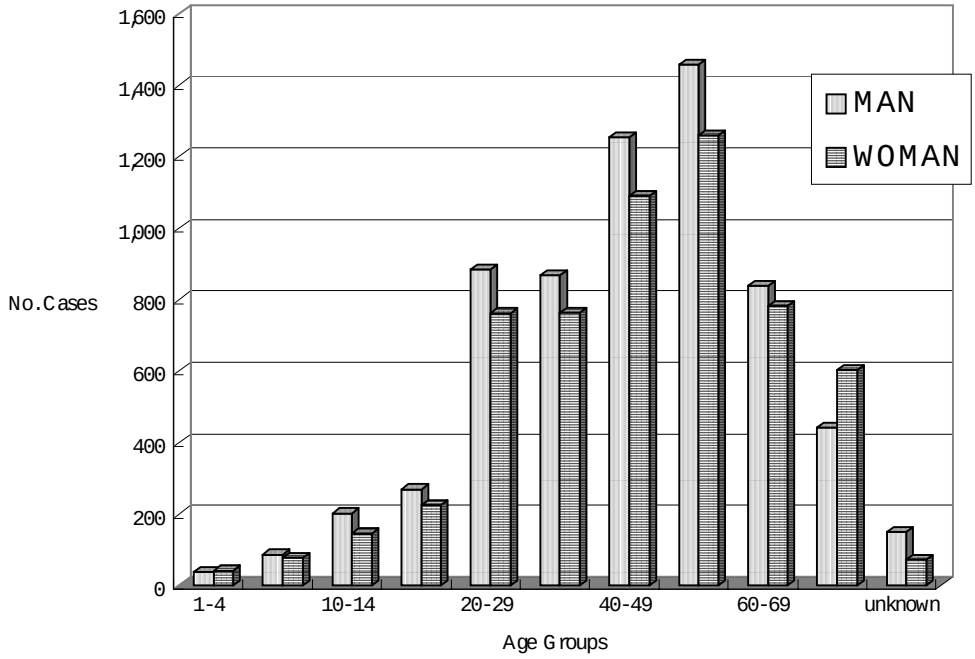


Figure 2.10: Age distribution of patients infected with *V. parahaemolyticus*, 1998 Japan

2.3.1.2.3 Health behaviours

All *Vibrio* spp. are relatively susceptible to inactivation by cooking. Most of the risk associated with the relevant strains of *Vibrio* spp. in food comes from the consumption of raw seafood or from cross contamination of other foods by raw seafood or contaminated water.

The time of year of consumption was considered in the risk assessment, as most infections occur during warm months. That is, a person consuming raw oysters in July is at higher risk than a person consuming the same amount in December. The location of harvest is also important. In the United States for example most landings of oysters occur in the Gulf of Mexico, particularly off the coast of Louisiana where the water temperature in the summer can reach the high twenties (^{o}C) and with increases in salinity this results in environmental conditions that favour the survival and growth of *V. parahaemolyticus*.

2.3.1.2.4 Physiologic status

There are no known measures of physiologic status relating to susceptibility to *V. parahaemolyticus* illness, however, analysis of epidemiological data indicate that pre-existing illnesses may predispose individuals with gastrointestinal illness to proceed to septicaemia (Anonymous, 2001).

In the United States the average annual incidence of raw oyster-associated illness from any *Vibrio* species among adults (>17 years of age) who consume oysters raw was estimated to be 10.1/1,000,000 (95% CI: 8.3-11.9). The annual incidence of fatal infections from any *Vibrio* species was estimated to be 1.6/1,000,000 adults who consume oysters (95% CI: 1.3-1.9). In two epidemiological studies (Hlady, 1997; Klontz, 1990), *V. parahaemolyticus* accounted for 77/339 reported *Vibrio* infections (Table 2.10). Of those 77 persons, 68 reported gastroenteritis and 9 had septicaemia. Twenty-nine persons were hospitalised for gastroenteritis with no deaths reported. Eight patients were hospitalised for septicaemia

and four of those patients died. Patients with septicaemia had underlying illness including, but not limited to cancer, liver disease, alcoholism and *diabetes mellitus* (Hlady, 1997; Klontz, 1990).

Table 2.10: Clinical syndromes of raw oyster-associated *Vibrio* infections in Florida, 1981-1994

***Vibrio* Species**	**Total Cases**	**Gastroenteritis**	**Septicaemia**
V. vulnificus	95	13	82
V. parahaemolyticus	77	68	9
V. cholera Non-O1	74	8	66
V. hollisae	38	35	3
V. mimicus	29	29	0
V. fluvialis	19	19	0

Source of data: (Hlady, 1997; Klontz, 1990).

Hlady and Klontz (1996) reported that, of patients with infections, 25% had pre-existing liver disease or alcoholism. These included 75% of the septicaemia patients and 4% of the gastroenteritis patients. Of the remaining septicaemia patients, 9 reported having a history of at least one of the following: malignancy, renal disease, peptic ulcer disease, gastrointestinal surgery, diabetes, antacid medication and pernicious anaemia. Among the gastroenteritis patients, 74% had none of the above pre-existing medical conditions or had insufficient information to classify. Thus, while the prevalence of underlying illness was high in the septicaemia patients the majority of patients with raw-oyster associated *Vibrio* gastroenteritis had no underlying conditions.

During the first year of *Vibrio* surveillance in the United States (1989), *V. parahaemolyticus* accounted for 27/85 reported *Vibrio* illnesses characterized by gastroenteritis or septicaemia (Levine *et al*., 1993); *V. parahaemolyticus* was the most prevalent of the *Vibrio* species reported. Twelve of the 27 persons with *V. parahaemolyticus* were known to have eaten raw oysters. One person had septicaemia while the remaining 26 persons had gastroenteritis. Oyster-associated infections occurred throughout the year with a peak in October.

Based upon CDC surveillance data on *V. parahaemolyticus* from 1988-1997 in Alabama, Florida, Louisiana and Texas, the six most common underlying medical conditions associated with infection include diabetes, peptic ulcer, heart disease, gastric surgery, liver disease and immunodeficiency (Angulo & Evans, 1999). For gastroenteritis, 24% of respondents reported one or more of these six conditions compared with 71% of respondents who had sepsis (Table 2.11).

Table 2.11: Underlying conditions in patients treated for *V. parahaemolyticus* infections

Underlying medical condition	**Type of infection**	
	Gastroenterititis (263 cases)	**Septicaemia (20 cases)**
Diabetes,	7%	17%
Peptic ulcer,	6%	18%
Heart disease,	6%	12%
Gastric surgery,	4%	12%
Liver disease	-	63%
Alcoholism	3%	14%
Immunodeficiency	3%	18%
Haematological	2%	13%

disease		
Malignancy	2%	11%
Renal disease	1%	12%

2.3.1.2.5 Genetic factors

There are no known human genetic factors that appear to be related to the susceptibility of individuals to *V. parahaemolyticus* illness.

2.3.1.3 Characteristics of the food matrix

2.3.1.3.1 Fat and salt content

Fat and salt content are probably not relevant in the determination of risk with respect to *Vibrio* spp. While the fat content of a matrix may be relevant with respect to the increase of effective dose of pathogens through protection of *Vibrio* spp. in micelles during gastric passage, there is insufficient evidence to model the degree of increased survival.

2.3.1.3.2 pH and water activity

Vibrio spp. appear to be relatively sensitive to both low pH and dehydration. Because of the nature of most foods associated with the unintended consumption of *Vibrio* spp., pH and water activity are probably not relevant in modelling survival of *Vibrio* spp. in raw seafood, however these parameters may be relevant in modelling the growth of *Vibrio* spp. in other foods as the result of cross contamination.

2.3.2 Public Health Outcomes

2.3.2.1 Manifestations of disease

Gastroenteritis due to *V. parahaemolyticus* infection is usually a self-limiting illness of moderate severity and short duration (Barker, 1974; Barker & Gangarosa, 1974; Levine *et al*., 1993). However, severe cases requiring hospitalisation have been reported. A summary of clinical features associated with *V. parahaemolyticus* gastroenteritis infection is presented in Table 2.11 (Barker & Gangarosa, 1974; Levine *et al*., 1993). Symptoms include explosive watery diarrhoea, nausea, vomiting, abdominal cramps, and less frequently headache, fever and chills. On rare occasions, septicaemia, an illness characterized by fever or hypotension and the isolation of the micro-organism from the blood, can occur. In these cases, subsequent symptoms can include swollen, painful extremities with haemorrhagic bullae (Hlady, 1997; Klontz, 1990). Duration of illness can range from 2 hours to 10 days (Barker & Gangarosa, 1974; Barker *et al*., 1974).

Table 2.11: Clinical symptoms associated with gastroenteritis caused by *V. parahaemolyticus*

Symptoms	Incidence of symptoms (%)	
	Median	**Range**
Diarrhoea	98	80-100
Abdominal cramps	82	68-100
Nausea	71	40-100

Vomiting	52	17-79
Headache	42	13-56
Fever	27	21-33
Chills	24	4-56

Source of data: (Barker & Gangarosa, 1974; Levine *et al*., 1993)

An outbreak is defined as the occurrence of two or more cases of a similar illness resulting from the ingestion of a common food. The incubation period ranges from 12-96 hours with a median of approximately 15-24 hours. The number of raw oysters consumed ranges from 1-109 (median of 12). However, the duration of consumption is not known. The typical prevalence of symptoms for cases with gastroenteritis parallels those that were identified during the Pacific Northwest outbreak in the United states in 1997. These symptoms included diarrhoea (99%), abdominal cramps (88%), nausea (52%), vomiting (39%), fever (33%) and bloody diarrhoea (12%). Some of the outbreaks associated with *V. parahaemolyticus* that have occurred in the United States are listed in Table 2.12.

Although *V. parahaemolyticus* outbreaks are less frequent in occurrence, sporadic cases are not infrequent, as further described below.

Table 2.12. Outbreaks associated with *V. parahaemolyticus* that have occurred in the North America

No. of persons ill	Location	Year	Food implicated	Reference
-	Maryland, United States	1971	contaminated steamed crabs	Dadisman *et al.,* 1972
40 outbreaks	15 states in the United States and Guam territories	1973 - 1998	seafood or cross-contamination from raw or undercooked seafood	Daniels *et al*., 2000
209 persons	United States	1997	oysters from California, Oregon, Washington, US and British Columbia, Canada	CDC, 1998
6 culture-confirmed cases	North America	1981	-	Nolan, 1984
416	Texas	1998	raw oysters harvested from Galveston Bay, US	Daniels *et al*., 2000
23 culture-confirmed cases	United States	1998 (May-December with a peak in July-August)	raw shellfish	CDC, 1999)

Several case reports have been published that outline clinical presentations and outcomes of patients with *V. parahaemolyticus*. One such describes a 35-year-old woman who sought medical attention for abdominal pain after she had consumed raw fish (Tamura *et al*., 1993). She presented with gastrointestinal symptoms, redness on lower extremities, fever, polyarthritis and weakness. *V. parahaemolyticus* was isolated in the stool culture. She was diagnosed as having reactive arthritis induced by *V. parahaemolyticus* infection.

Another clinical case report describes a 31-year-old female with a history of alcohol abuse, hepatitis C virus infection, and cirrhosis (Hally *et al*., 1995). She presented with diarrhoea, weakness, leg pain and urine retention. The patient had ingested raw oysters and steamed shrimp 72 hours prior to admission. *V. parahaemolyticus* was isolated from blood samples. The patient suffered cardiac arrest and died six days after presentation.

A suspected case of a laboratory-associated infection was reported in 1972 (Sanyal *et al*., 1973). One day prior to the development of diarrhoeal disease the laboratory worker had been handling *V. parahaemolyticus* strains for the first time. The illness was associated with severe upper abdominal pain, bloody stools, nausea and fever. Weakness and abdominal discomfort continued for two days beyond the onset of illness. No other source of *V. parahaemolyticus* could be identified, and it was believed that the infection was caused by a relatively small inoculum (Sanyal *et al*., 1973).

A case series is a study of sporadic cases over a period**.** Sporadic cases of *V. parahaemolyticus* infections, while commonly reported by many states in the United States, are primarily reported by Gulf Coast states. Most *V. parahaemolyticus* infections present clinically as gastroenteritis, which has a low case fatality rate. Life threatening septicaemia can occur, especially in patients with underlying medical conditions. The case series has a range of infection throughout the year, with a peak in September to October. A case series of *Vibrio* infections related to raw oyster consumption was reported in Florida from 1981-1994 (Hlady, 1997).

2.3.2.2 Rational for the biological end points modelled

Gastrointestinal illness is modelled as endpoint, corresponding to that measured in the human volunteer studies. Since most gastrointestinal illnesses are not reported, epidemiological data used in validating the model will have to be scaled to account for underreporting.

2.3.3 Dose-response relationship

2.3.3.1 Summary of available data

2.3.3.1.1 Probability of illness given exposure.

Human volunteer studies are available to estimate the probability of illness given exposure. Sanyal and Sen (1973), Takikawa (1958) and Aiso (1963) have conducted dose-response investigations.

2.3.3.1.2 Probability of sequellae given illness.

In the United States, approximately 5% of culture-confirmed cases of *V. parahaemolyticus* progress to septicaemia (Angulo and Evans, 1999).

2.3.3.1.3 Probability of secondary and tertiary transmission.

There are few, if any, reports of secondary or tertiary transmissions of illnesses caused by *V. parahaemolyticus*.

2.3.3.1.4 Probability of death given illness.

Based on then United States statistics, around twenty percent of patients who are septicaemia with *V. parahaemolyticus* die (Angulo and Evans, 1999).

2.3.3.2 Dose-response model

2.3.3.2.1 Sources of data used

Figure 2.11, taken from the FDA-VPRA (Anonymous, 2001) shows the maximum likelihood fit of the Beta-Poisson to the available feeding trial data. Due to the small number of subjects exposed during these studies, there is considerable uncertainty about the best estimate of the dose-response. Several statistical methods for characterizing the uncertainty of the dose-response parameters are available, including likelihood ratio-based confidence regions and bootstrapping techniques (parametric or non-parametric).

The results of the non-parametric bootstrap analysis of the *V. parahaemolyticus* data are shown in Table 2.12. There is one entry in the table for each possible (realized or unrealised) outcome of the study. For each of these possible outcomes a fit of the Beta-Poisson dose-response function was obtained by the maximum likelihood procedure and the resulting estimates of alpha and beta are shown (as well as the log of the ID_{50} of the fit obtained). The non-parametric bootstrap estimates of probability weight associated with each possible outcome and summary fit is denoted by "likelihood of resample". An analysis of deviance indicates that a good fit of the Beta-Poisson was obtained for most of the outcomes and those that are not adequately fit by the Beta-Poisson model ($p<0.05$) are relatively unlikely.

For the purpose of the present assessment, the dose-response uncertainty was characterized using a nonparametric bootstrap procedure. If the true probabilities (risk) of illness at each study dose level were known then the likelihood of the observed and alternative (possible but unrealised) outcomes would also be known. The likelihood of alternative outcomes (i.e., if the studies were to be replicated) could then be used to characterize the uncertainty of the parameter values for any dose-response function used to estimate (i.e., interpolate/extrapolate) the overall dose-response. Given that the true probability of illness at each study dose level is unknown, bootstrap procedures substitute an estimate for these probabilities and, for the non-parametric bootstrap, that estimate is the observed frequency of illness.

Table 2.12 can be used to characterize the effect of parameter uncertainty in subsequent analysis by probability weighted selection of the alpha and beta parameters for the dose-response segment of a Monte Carlo simulation run. For clarity, these alternate dose-response parameters are not shown on the graph.

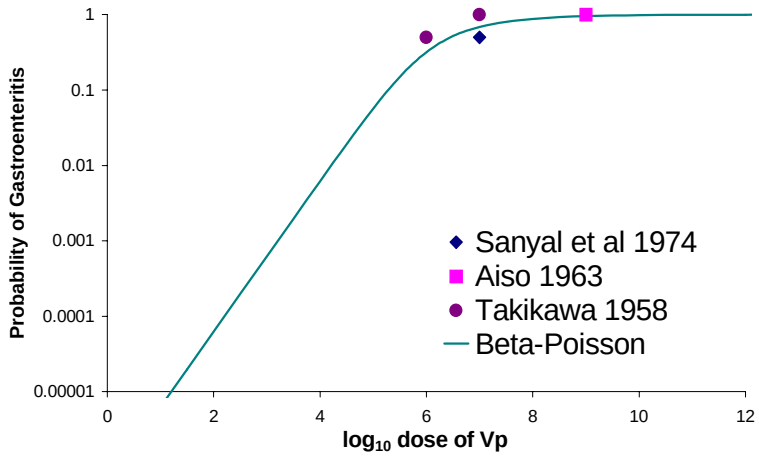


Figure 2.11. Beta-Poisson dose-response curve for *Vibrio parahaemolyticus*

Table 2.12. Uncertainty of Beta Poisson dose-response for *V. parahaemolyticus*: Maximum likelihood estimates (MLEs) of parameters and nonparametric probability (based on the observed data)) associated with bootstrap resamples.

	Possible resample					MLEs of parameters		Likelihood of resample	MLE of Log ID50	Deviance of fit to resample	p-value of fit to resample
ID	x1	x2	x3	x4	x5	alpha	beta				
1*	0	0	0	0	4	1.47E+06	3.53E+14	0.00034	8.22	0.6450	0.8861
2*	0	0	0	1	4	1.26E+07	7.20E+14	0.00412	7.60	0.0857	0.9935
3*	0	0	0	2	4	636.53	1.65E+10	0.02058	7.26	0.1901	0.9792
4*	0	0	0	3	4	35.81	5.42E+08	0.05487	7.03	0.3262	0.9550
5*	0	0	0	4	4	20.84	1.99E+08	0.08230	6.83	0.5204	0.9144
6*	0	0	0	5	4	14.87	8.78E+07	0.06584	6.62	0.8557	0.8361
7*	0	0	0	6	4	10.58	2.99E+07	0.02195	6.31	2.2562	0.5210
8	0	0	1	0	4	3.89	2.28E+08	0.00069	7.65	7.4536	0.0588
9	0	0	1	0	4	1.31	2.93E+07	0.00823	7.31	4.4426	0.2175
10	0	0	1	0	4	0.52	3.61E+06	0.04115	7.00	2.9538	0.3988
11	0	0	1	0	4	0.47	1.50E+06	0.10974	6.70	1.7571	0.6243
12	0	0	1	0	4	0.60	1.31E+06	0.16461	6.46	0.9994	0.8014
13	0	0	1	0	4	1.00	1.80E+06	0.13169	6.26	0.6272	0.8902
14*	0	0	1	0	4	8.59	1.30E+07	0.04390	6.04	0.6242	0.8909
15	0	0	2	0	4	0.15	2.33E+05	0.00034	7.32	15.9553	0.0012
16	0	0	2	1	4	0.19	2.29E+05	0.00412	6.90	10.6999	0.0135
17	0	0	2	2	4	0.25	2.36E+05	0.02058	6.57	7.9684	0.0467
18	0	0	2	3	4	0.32	2.57E+05	0.05487	6.30	6.0785	0.1079
19	0	0	2	4	4	0.43	3.04E+05	0.08230	6.08	4.6970	0.1954
20	0	0	2	5	4	0.69	4.34E+05	0.06584	5.88	3.6564	0.3010
21*	0	0	2	6	4	6.92	4.49E+06	0.02195	5.68	2.3697	0.4993

* unconverged estimates

2.3.3.2.2 Assumptions

The primary assumptions are:

- healthy volunteers responses to oral challenge is representative of the general population.
- the virulence of the pathogens or susceptibility of the host does not vary
- the Beta-Poisson dose-response model is reasonable for use in characterizing risk of illness when consuming *Vibrio* spp.

While these assumptions are not representative of what we believe is the nature of the human dose-response relationship, they form the basis of the first iteration of the hazard characterization. As more information becomes available, these assumptions will be revised to better reflect our understanding of dose-response relationships.

2.3.3.2.3 Goodness of fit of the distribution

The goodness of fit of the distributions for all of the *Vibrio* spp. are uncharacterised as a family of dose-response parameters are used to represent the parameter uncertainty.

2.3.3.2.4 Uncertainty and variability in the estimates

This analysis incorporates both uncertainty and variability in the estimates. Since the dose-response estimates are based upon curves fitted to human volunteer data, there is uncertainty as to whether the parameters that give the best fit are the "true" parameters of the dose-response curve. To account for this uncertainty, a Monte Carlo simulation model can be set up to probabilistically select from the group of plausible dose-response parameters generated in this analysis. While it is not modelled in this analysis, there is variability in the virulence of the pathogens and in the susceptibility of the host. Further research is needed to provide data for assessing and modelling variability in pathogen and in host. A key uncertainty in this hazard characterization is the effect of the food matrix on the dose-response relationship. Both the experience of the United Stated risk assessment on *V. parahaemolyticus* (Anonymous, 2001) and evidence from the studies of Cash, *et al*. (1974) have indicated that some food matrices may shift the dose-response curve to the left indicating that a higher dose of the pathogen is required to cause illness. Future human volunteer studies may help to resolve this question and provide data that will allow scaling factors to be applied to predict the risk for specific pathogens consumed with specific food matrices.

2.4 Risk characterization

to be completed in 2002

2.5 Gaps in the data

Deficiencies of the current research with respect to risk assessment were identified in order to suggest future research or further data gathering to reduce uncertainties. They areas are as follows;

- Incidence/frequency of pathogenic *V. parahaemolyticus* in water and shellfish.
- Factors that affect incidence of pathogenic *V. parahaemolyticus* in the environment.
- Role of oyster physiology and immune status in levels of *V. parahaemolyticus*. There is a need to correlate the number of *V. parahaemolyticus* with the percentage oysters diseased.
- More research on the potential virulence factors of pathogenic strains other than TDH, e.g. urease, enterotoxins. *V. parahaemolyticus* strains that do not produce TDH, TRH, or urease have recently been found to induce fluid accumulation in suckling mice and diarrhoea 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.
- Growth rate of *V. parahaemolyticus* within oysters at temperatures other than 26° C; including the issue of potential differences in the growth rate of pathogenic strains *versus* total *V. parahaemolyticus* populations.

- Rates of hydrographic flushing (water turnover) in shellfish harvest areas based on levels of freshwater flows, tidal changes, winds, depth of harvesting area and how these factors may influence pathogenic *V. parahaemolyticus* numbers.
- Consumer handling of oysters.
- Improved global public health surveillance of *V. parahaemolyticus* to identify new epidemic strains as they emerge.

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3 RISK ASSESSMENT OF *VIBRIO PARAHAEMOLYTICUS* IN RAW AND UNDERCOOKED FINFISH

3.1 Hazard identification

3.1.1 Human Incidence

With the globalization of Japanese cuisine and the increased practice of eating raw fish and shellfish, there is increased possibility of *Vibrio parahaemolyticus* infection. While consumption of raw oysters has been a major cause of *V. parahaemolyticus* infections in the United States (Anonymous, 1998 & 1999) *V. parahaemolyticus* infections from oysters has accounted for <5% of all outbreaks in Japan (Anonymous, 2000a) where consumption of a variety of raw fish and shellfish other than raw oyster have been implicated (Figure 3.1). The practice of not selling cultured oysters during the summer season may also contribute to the low level of implication of raw oyster on *V. parahaemolyticus* infection in the region.

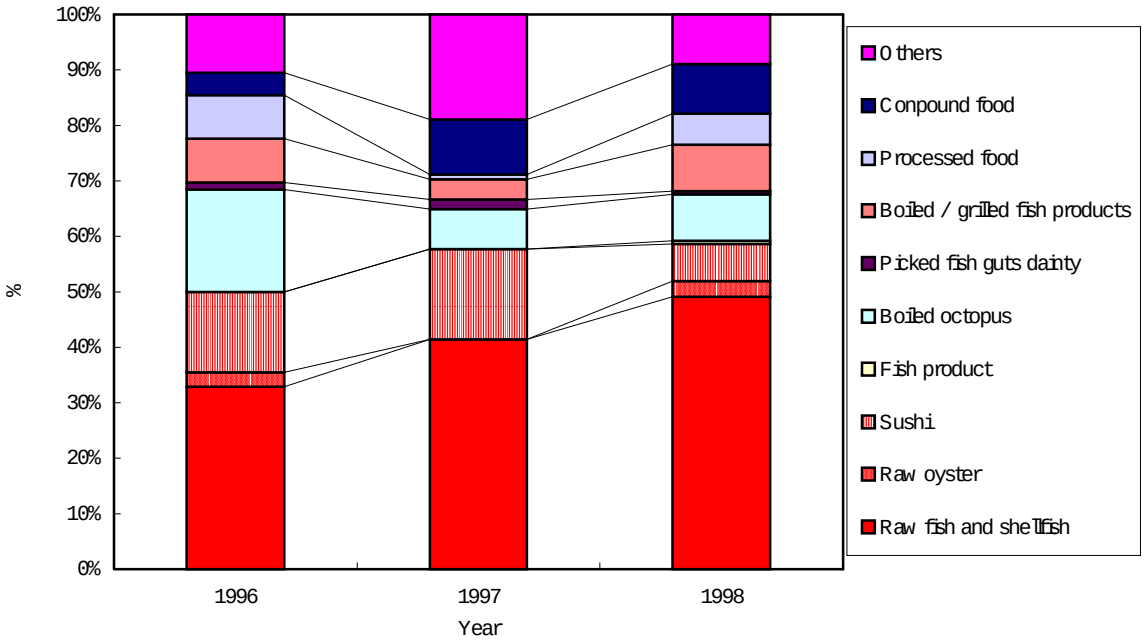

Figure 3.1. Foods incriminated in *V. parahaemolyticus* outbreaks in Japan (1996-98)

Outbreaks of foodborne disease due to *V. parahaemolyticus* associated with fish and shellfish other than oysters have been reported in several countries. Sporadic infections and outbreaks due to *V. parahaemolyticus* associated with clam, molluscan shellfish, crayfish, lobster and shrimp have been reported in the United States (Daniels *et al.,* 2000, Bean *et al.*, 1998). There was an epidemiological case-control study in Guam where seafood consumption was thought to be important in *V. parahaemolyticus* infection (Haddock *et al.*, 1994). Fish–balls were associated with an outbreak of food poisoning due to *V. parahaemolyticus* in Thailand (Tangkanakul *et al.*, 2000). Fish, shellfish and raw oyster were incriminated in a *V. parahaemolyticus* outbreak in Spain (Molero *et al.*, 1989). Furthermore, there was a report that undercooked white crabmeat served on an aeroplane on an international flight caused *V. parahaemolyticus* gastroenteritis (Peffers *et al.*, 1973).

In Japan, there are typically 500-800 *V. parahaemolyticus* outbreaks affecting around 10,000 people in each year. Implicated foods include raw fish *Sashimi* (responsible for 26% of outbreaks), followed by raw fish with rice – *Sushi* (23%), shellfish (16%) and cooked seafood (12%) (Anonymous, 2000a).

3.1.2 Prevalence in food

Although limited information is available on foods associated with *V. parahaemolyticus* outbreaks in other countries, there are several reports on high prevalence of the organism in a variety of seafood.

Wong *et al.,* (1999) recovered *V. parahaemolyticus* from 315 (45.9%) seafood samples from Asian countries. The incidence of *V. parahaemolyticus* in shrimp, crab, snail, lobster, sand crab, fish and crawfish was 75.8%, 73.3%, 44.3%, 44.1%, 32.5%, 29.3% and 21.1%, respectively; recovery from products from Hong Kong and Thailand was markedly higher than those from Indonesia and Vietnam.

Matte *et al.* (1994) examined mussels (*Perna perna*) harvested at three different stations on the coast of Ubatuba, in the State of Sao Paulo, Brazil, for *Vibrio spp*. over a 1-year period. The ranges of most probable number (MPN/100g) were: *Vibrio alginolyticus* (<3-24,000), *V. parahaemolyticus* (<3-24,000), *V. fluvialis* (<3-1100), *V. cholerae* non-O1 (<3-23), *V. furnissii* (<3-30), *V. mimicus* (<3-9) and *V. vulnificus* (<3-3).

Chan *et al.*, (1989) reported summer prevalence of *V. parahaemolyticus* and other halophilic vibrios in seafood from Hong Kong markets. Halophilic vibrios were isolated from all seven types of seafood examined and comprised 9.1%, 8% and 6.1% of contaminating aerobic heterotrophic bacteria from mussels, clams and oysters, respectively.

El-Sahn *et al.* (1982) examined samples of seawater, sediment invertebrates around Alexandria, Egypt. Average counts (per 100 mL or 100 g) of *V. parahaemolyticus* were as follows: seawater, 36; sea urchins, 349; sediment, 436; wedge shells, 534; clams, 1872. Samples collected in the summer months contained higher numbers of *V. parahaemolyticus* than winter samples.

Chowdhury *et al.*, (2001) reported the prevalence of *V. parahaemolyticus* in the imported seafood sampled at Kansai international airport during 1998-2000, where 65/593 bacteria in 65 samples (14%) of 6 species (average 8.9 bacteria in one sample) were found to be positive. Of those species tested, tuna was reported to be the highest prevalence in fish.

3.1.3 Virulence factors

Some *V. parahaemolyticus* induce beta-type haemolysis when grown on a special blood agar, the so-called as Kanagawa Phenomenon (KP) and this has been used as an indicator for pathogenic *Vibrio* for many years. KP is caused by thermostable direct hemolysin (TDH) produced extracellularly by *V. parahaemolyticus.* However, some KP-negative strains isolated from clinical sources were shown to produce a TDH-related hemolysin (TRH) but not TDH. Recently, strains capable of producing TDH, TRH or both have been considered as pathogenic strains. While the prevalence of pathogenic strains in seafood or environmental samples was relatively low, prevalence of these strains in clinical samples was high (Wagatsuma, 1974; Matte *et al.,* 1994; Wong *et al.*, 1992, 1999; Fang *et al.* 1987; Chowdhury, 2001). In undertaking exposure assessment, data on exposure to pathogenic strains is critical, however, as presently there are insufficient data on pathogenic strains at each module, the current assessment focuses on total numbers of *V. parahaemolyticus*.

3.2 Exposure assessment

From the foregoing it can be seen that there is a potential for raw fish and shellfish, other than oysters, to cause *V. parahaemolyticus* infection. In assessing exposure to *V. parahaemolyticus*, four modules were developed at the following stages: pre-harvest, harvest, post-harvest and preparation and consumption.

Table 3.2. The prevalence of pathogenic strains in food (or environmental) samples

Report	Sample	% Pathogenic Vp	TDH/TRH/KP
Wagatsuma (1974)	Sea water/mud/oyster	0.35%(47/13, 345)	KP
Matte G.R. *et al*., (1994)	Mussels (*Perna perna*)	0.51%	KP
Wong H.C. *et al*., (1992)	Seafood	1.65% (2/121)	TDH
Fang S.W. *et al*. (1987)	Seafood	0% (0/182)	KP
Wong H.C. *et al*., (1999)	Imported Seafood	0% (0/ NC)	TDH/TRH
Chowdhury N.R. *et al*., (2001)	Imported seafood (raw)	1.96% (14/715)	TDH/TRH
Chowdhury N.R. *et al*., (2001)	Imported seafood (frozen)	0.84% (5/598)	TDH/TRH

3.2.1 Pre Harvest module

A schematic representation of the pre-harvest module is presented in Figure 3.2. A number of factors influence the prevalence of *V. parahaemolyticus* in seawater and fish and these are outlined below.

3.2.1.1 Seawater temperature and salinity

The incidence of *V. parahaemolyticus* in seawater is correlated with seawater temperature where a temperature of 14-20°C is considered a threshold for the organism to leave the VBNC state and begin proliferation (Kaneko *et al.*, 1975).

As indicated in section two most of the modelling work on *V. parahaemolyticus* has been undertaken in the United States as part of the FDA-VPRA on raw oysters (Anonymous, 2001). In addition to that Ogawa *et al.,* (1989) reported on the ecology of *V. parahaemolyticus* in Hiroshima Bay and developed the equation where:

Y = concentration of *V parahaemolyticus*

Xa = seawater temperature in °Celsius

Xb = salinity of water

the relationship between seawater temperature and density of *V. parahaemolyticus* is:

$\text{Log } 10(Y) = 1.203(Xa)-0.934$ ($r=0.506$, $P<0.01$)

the relationship between salinity and density of *V. parahaemolyticus* is:

$\log_{10}(y) = -0.674(X)+3.448$ ($r=-0.694$)

the relationship between seawater temperature, salinity and density of *V. parahaemolyticus* is:

$\text{Log}_{10}(Y) = 2.00+0.05(Xa)-0.584(Xb)$ ($P<0.001$).

and the relationship between the concentration of *V. parahaemolyticus* in raw oyster and *V. parahaemolyticus* in seawater is:

Yr = *V. parahaemolyticus* No/100g

Xs = *V. parahaemolyticus* No/100mL

$\text{Log10}(Yr) = 0.900\text{log10}(Xs)+1.232$ ($r=0.663$ $P<0.05$)

The concentration of *V. parahaemolyticus* in raw oyster was found to be 11.1 times greater than that in seawater. In addition, several factors were found to influence the density of *V. parahaemolyticus.*

3.2.1.2 Tide

Kumazawa *et al.* (1999) reported that thick accumulation of muddy sediments on the riverbed and stagnation of brackish water at low tide seemed to be essential for *V. parahaemolyticus* to survive in neritid gastropods including *C. retropictu*.

Ogasawara (2000) observed that the area where water flow was very slow and turbid there were higher numbers of *V. parahaemolyticus* and longer periods of contamination.

3.2.1.3 Plankton and other factors

Colwell *et al.*, (1974) reported the association of *V. parahaemolyticus* with zooplankton and demonstrated that one of the factors determining the distribution of the organism was its adsorption onto chitin. Watkins *et al.* (1985) reported that numbers of *V. parahaemolyticus* were greatest in the near-surface waters of contaminated areas and decreased sharply with both the distance from the sources of faecal pollution and the depth of the water column. A positive association with the amount of particulate matter in the water, and specifically with its zooplankton content, also was reported. In addition Sarkar *et al.*, (1985) reported that the incidence and counts of *V. parahaemolyticus* were consistently higher in samples with plankton than water and sediment samples without plankton. The highest recovery rate of this halophile from fish was invariably from faecal samples.

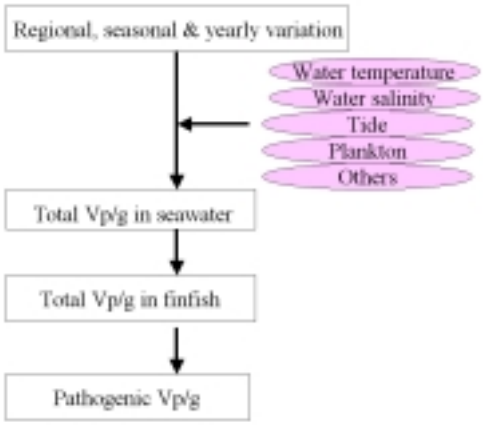

Figure 3.2. Schematic representation of the pre-harvest module of the exposure assessment of *V. parahaemolyticus* in raw fish

3.2.2 Harvest module

3.2.2.1 Species of fish and shellfish and handling and storage aboard vessels

A variety of fish species are harvested commercially using methods of fishing. Fish caught near to the coast are caught by purse seine, trawl and fixed nets. Tuna and large deep-sea fish are caught by long line. Some species (Greater amberjack, Red sea bream) are farmed in coastal seawater. It has been noted that the type of seafood and the waters (coastal, deep-sea, farmed) in which they are caught are factors in their contamination with *V. parahaemolyticus*. For example, Sakazaki *et al.* (1975) reported the

prevalence of *V. parahaemolyticus* in several kinds of fish and shellfish and concluded that shellfish were contaminated more than fish and that fish with scales were contaminated more than those without.

Yamazaki *et al.* (1996) reported the prevalence of *V. parahaemolyticus* in various kinds of fish and concluded shore fish were more likely to be contaminated with *V. parahaemolyticus* than fish caught in deep-sea. Vessels for long line or deep-sea fishing are relatively large and usually have refrigeration systems onboard. In addition, tuna and other species of fish caught by longline are usually found well away from the coast, in waters where *V. parahaemolyticus* is less prevalent, thereby reducing the opportunity for contamination. By contrast, vessels for coastal fishing are relatively small, trips are usually short (several hours) and some the vessels have no refrigeration onboard. The possibility of contamination and growth of *V. parahaemolyticus* of fish during coastal fishing is therefore greater than during deep-sea fishing. Also aquacultured fish are more likely to be contaminated with *V. parahaemolyticus* with reports that cultured fish such as Greater amberjack and Red sea bream were contaminated with *V. parahaemolyticus* (Yamazaki *et al.,* 1996).

There have also been some studies on the numbers of *V. parahaemolyticus* in fish and shellfish. Kou *et al.*, (1996) immersed oysters, clams and turban shellfish in seawater containing *V. parahaemolyticus* (dose $5x10^4$/mL) for 24 hours and recovered 10^6 cells per gram of meat. Shiozawa *et al.* (1998) undertook a study involving monthly testing of the density of *V. parahaemolyticus* on horse mackerel, clam and round clam and found that both clam types were highly contaminated and at a level about 2 logs higher than horse mackerel and seawater. A study on the relationship between seawater concentration and fish (horse mackerel) concentration indicated that as well as fish being less contaminated with *V. parahaemolyticus* than raw oyster, the cell counts were ten times lower (no./g) than those of seawater (no./mL) (Shiozawa *et al*., 1998).

There are few data available on the potential or risk of fish being contaminated with *V. parahaemolyticus* while on the fishing vessels. In some cases gilling and evisceration is carried immediately after the fish is caught (ICMSF, 1988). This is done by hand with a sharp knife and releases the intestines, including the digestive tract, which are a reservoir of potential spoilage bacteria and a potential source of *V. parahaemolyticus*. In some of the larger fishing vessels processing may be mechanised. So while the risk associated with this step is not understood it is also recognized that processing fish on the fishing vessels is not overly common.

Taking the abovementioned factors into account a framework for the harvest component of the exposure assessment was developed (Figure 3.3).

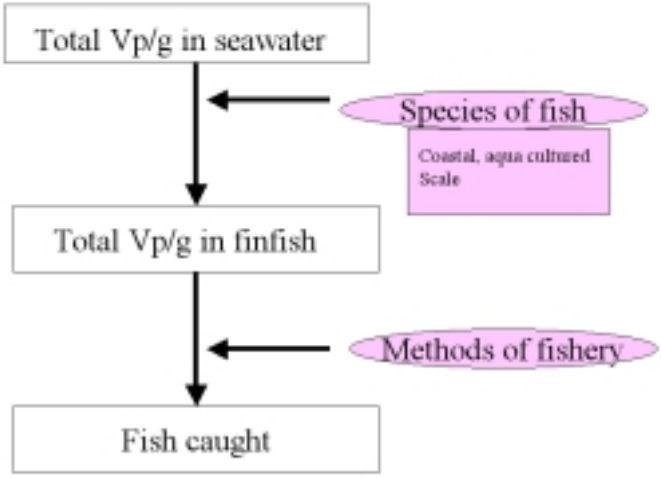

Figure 3.3. Schematic representation of the harvest module of the exposure assessment of *V. parahaemolyticus* in raw fish

3.2.3 Post harvest module

Once the fish have been harvested there is still some risk of contamination. However at this point the potential for growth of *V. parahaemolyticus* must also be considered and this can be influenced by a number of factors such as temperature, pH etc. as discussed in the following section. Taking these factors into account a framework for the post-harvest module was developed and this is presented in Figure 3.4.

3.2.3.1 Water as a source of contamination

During and after landing at the port there is the potential for the fish catch to be contaminated with *V. parahaemolyticus*. The primary source of contamination is coastal water containing *V. parahaemolyticus*, which may be used;

- during selling of the fish at the market (washing, spraying etc.),
- to fill boxes and containers in which live fish are kept, and
- for the transportation of fish

In a recent study it was found that water used during the process of landing, selling and transportation of fish was highly contaminated (Yamai *et al.,* 2001). The study found that 73% of water samples were contaminated with *V. parahaemolyticus* and 38% had 10^3-10^5 MPN/100mL. Some water was disinfected by PAC coagulation and filtration, chlorination and UV irradiation before use and then analysed again after use. After PAC coagulation and filtration, 71% of water samples were contaminated but with low levels while only 8% of those water samples that were disinfected in some way were contaminated.

3.2.3.2 Potential for growth

3.2.3.2.1 Temperature

In a study on the survival of *V. parahaemolyticus* in oyster meat homogenates at various temperatures (4 °C, 0°C, -18°C and -24°C) and numbers of bacterial (10^2, 10^4, 10^5 and 10^7 /mL) it was determined that the time to total inactivation depended on both the initial number of microorganisms and the temperature, with more rapid inactivation at lower temperatures (Muntada *et al.*, 1995). Oliver *et al.*, (1981) measured survival of both *V. vulnificus* and *V. parahaemolyticus* in oyster homogenates held at 4°C. While the former organism underwent a rapid decrease in viability not attributable to either cold shock or the oyster homogenate alone but to a combination of the two, no such decline was observed with *V. parahaemolyticus*.

The minimum temperature for growth of *V. parahaemolyticus* has been observed to be 8.3°C, while the maximum temperature for growth was observed at 45.3°C, the optimum occurring between 37-39°C (Miles *et al*., 1997). This was based on a study of the growth rates of four strains of *V. parahaemolyticus* in a model broth system. The results for the fastest growing strain, based on 77 combinations of temperature and water activity (aw) using NaCl as the humectant, were summarized in the form of a predictive mathematical model (Miles *et al*., 1997). Kou *et al*. (1996) measured the growth of *V. parahaemolyticus* at 10°C and 25°C in Round clam and Turban shellfish. While *V. parahaemolyticus* did not grow appreciably in the unshucked shellfish, it increased by one log ^{o} in 6 hours at 25C in the round clam meat. On horse mackerel it has been reported that *V. parahaemolyticus* did not grow significantly over the first 4 hours, but increased 1-3 logs in 8 hours at 25°C (Watanabe *et al.*, 1994).

Iwashita *et al.*, (1991) investigated *V. parahaemolyticus* contamination at each step in the transporting – processing continuum in 1988-1990 in the Yamanashi prefecture in Japan. Many wholesalers sell fish and shellfish for raw eating within 3 days of stocking. The temperatures of the refrigerators in these facilities were less than 5°C with the exception of at one large retailer. *V. parahaemolyticus* density in round clam at each step of the transportation chain from the local fishery market to the retail sector was also monitored over several years and it was concluded that if each step of transportation was kept below 10 C^{o} there was no increase of *V. parahaemolyticus* (Iwashita *et al.*, 1991).

3.2.3.2.2 pH

Growth of *V. parahaemolyticus* has been demonstrated to grow at pH levels of between 5 and 11 with some strains growing at pH 4.8 (Beuchat *et al.* 1973). Being halophilic *V. parahaemolyticus* will grow in NaCl concentrations of 1-7% (Twedt *et al.*, 1969). It has been reported that when 3 x 10^3 cells of *V. parahaemolyticus* in seawater were suspended in distilled water they were all killed within five minutes (Uryu *et al.,* 1961; Lee, 1972). However, there have been reports of the presence of *V. parahaemolyticus* in non-marine environments (Yasunaga, 1970; Catterjee & Neogy 1972).

3.2.3.2.3 Chlorination and other potential inhibitors

Chlorination is traditionally used to control *V. parahaemolyticus.* However, Liu *et al.*, (1994) reported that *Vibrio* spp. absorbed onto chitin particles are resistant to certain level of heating and chlorination.

Other possible bacteriostatic and bacteriocidal agents have been reported such as glycerine (Chun *et al.*, 1972), high pressure (Baross *et al.*, 1975), irradiation (Hackney *et al.*, 1988), basil and sage essential oils (Koga *et al.*, 1999) and mild heating (Beuchat, 1976). However, many of these have little practical potential for use in the control of *V. parahaemolyticus* during the harvesting and post-harvesting steps of fish to be consumed raw.

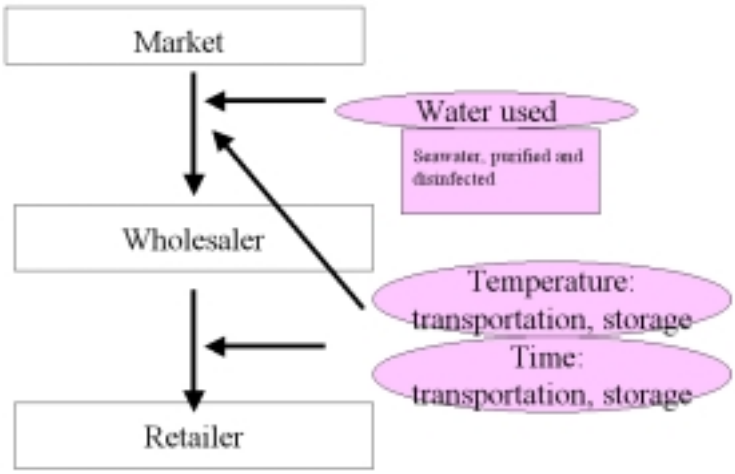

Figure 3.4. Schematic representation of the post-harvest module of the exposure assessment of *V. parahaemolyticus* in raw fish

3.2.4 Preparation and consumption module (home, restaurants and hotels)

A ten-year summary of outbreaks due to *V. parahaemolyticus* in Japan revealed that outbreaks occurred in restaurants (48%), in Japanese traditional hotels (18%), in catering establishments and from lunch boxes (12%), homes (12%) and from other venues (10%) (Anonymous, 2000). This highlighted the importance of considering different types of food preparation facilities in this module. The factors that affect prevalence and numbers of *V. parahaemolyticus* in fish were also identified and are further addressed below. The framework for this module is outlined in Figure 3.5.

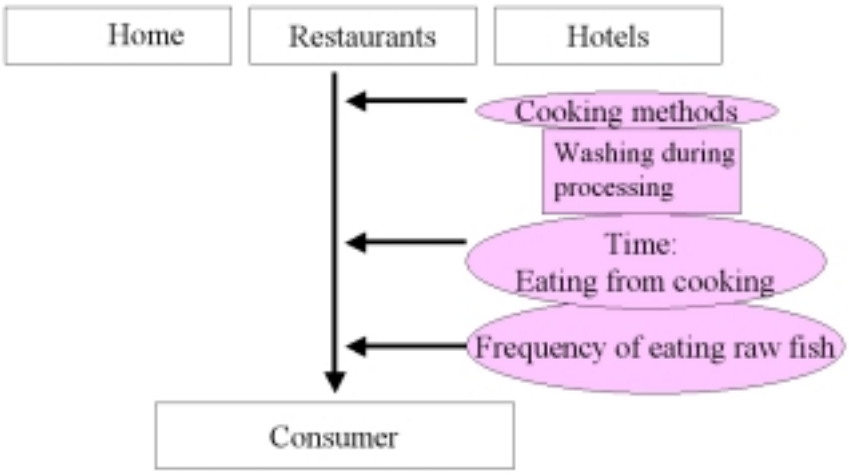

Figure 3.5. Schematic representation of the preparation and consumption module of the exposure assessment of *V. parahaemolyticus* in raw fish

3.2.4.1 Preparation of *Sashimi* (raw fish)

Watanabe *et al.* (1994) studied different preparation methods and tested the effectiveness of washing fish (horse mackerel) with tap water. Washing fish with clean water reduced the level of *V. parahaemolyticus* by $10^2/cm^2$ on the fish surface, but did not reduce the numbers in gills and viscera. Washing with clean water during the process of making sashimi was also important, with unwashed fish fillets found to be more highly contaminated with *V. parahaemolyticus.*

3.2.4.2 Timeframe between preparation and eating

There are currently no definitive data on the time that elapses between the preparation and eating of *Sashimi*. At the consumer level preparation seems simple, just dishing up, and as raw fish is best eaten fresh, the most likely scenario is that the fish is usually consumed within one hour in the home. By contrast, due to the scale of production it is not unusual for large restaurants and hotels to prepare sashimi several hours prior to consumption. But, since raw fish (*Sashimi*) is always refrigerated, the length of time to consumption does not seem to be critical.

3.2.4.3 Frequency of eating raw fish

Data from the National nutrition survey conducted in Japan in 1995 on about 5000 households and 15000 individuals on one day of November are summarised in Figure 3.6.

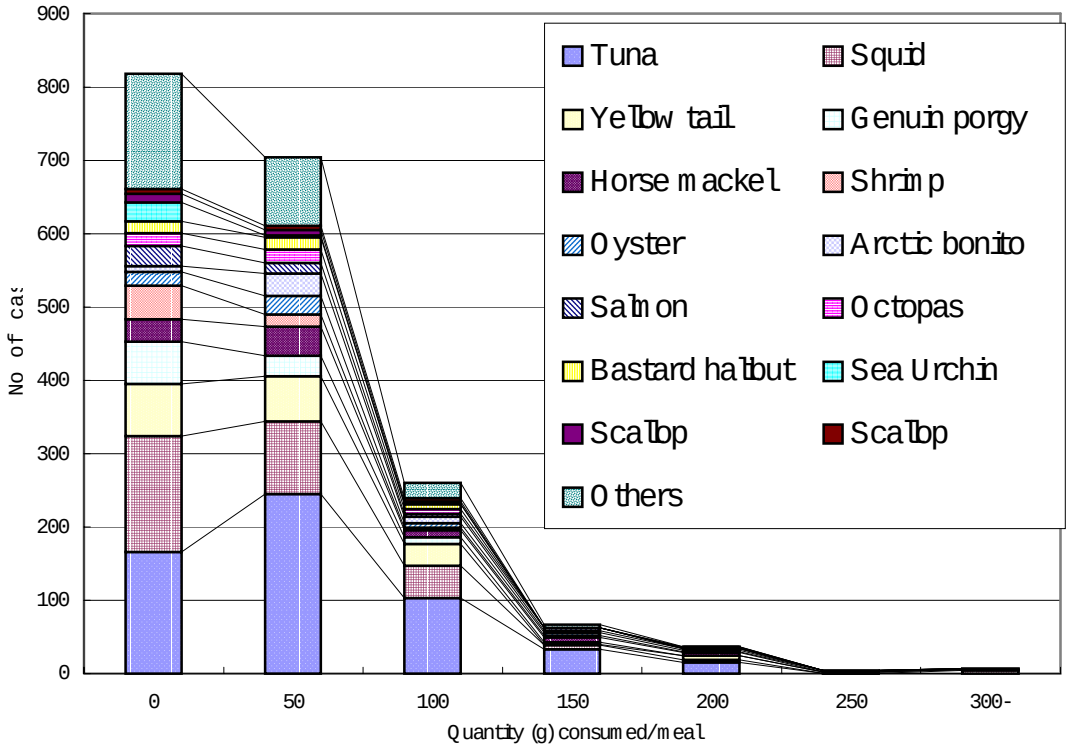

Figure 3.6. Survey of consumption of raw fish and shellfish in Japan in 1995

The survey found that most people ate less than 100 g per meal when consuming raw fish and shellfish. There were additional data on the frequency of eating raw fish such as *Sushi* and *Sashimi.* Interestingly, one household in Japan purchased around 45–50kg of fresh fish and shellfish and 2.2-3kg Sashimi per annum (Anonymous, 2000).

The frequency of eating sushi from the national nutrition survey, which investigates the frequency of eating many kinds of food out of home, is 5.9% per one meal out of home and the frequency of eating out was 16.8% (breakfast 2.7%, lunch 40.5%, dinner 7.3%) (Anonymous 1998).

3.3 Hazard Characterization

Please refer to section 2.3

3.4 Risk Characterization

To be completed in 2002

3.5 Gaps in the data

- Data on pathogenic *V. parahaemolyticus* - The collection of background data on the prevalence of pathogenic strains is necessary to model the exposure assessment.

- Frequency of consumption of raw fish and shellfish in various months - Currently there is only annual data on the frequency of consumption of raw fish and shellfish. Data on the frequency of consumption at different months of the year and in different countries would be beneficial.
- Data on the procedure of fish on the vessels - Information on any of the evisceration / processing procedures that are carried out on small fishing vessels.

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4 RISK ASSESSMENT OF *VIBRIO VULNIFICUS* IN RAW OYSTERS

This document outlines the objectives and approach for modelling the risk of *V. vulnificus* from the consumption of raw oysters. The feasibility of the proposed approach, adequacy of data and validity of assumptions need to be reviewed before the work is progressed.

There have been at least two previous risk assessments on *V. vulnificus*. McCoubrey (McCoubrey, 1996) reported on the risk of *V. vulnificus* infection following consumption of raw commercially harvested North Island oyster from New Zealand in 1996. The report concluded that environmental conditions, especially high salinities, were not suitable for *V. vulnificus* survival. Quantitative risk assessment procedures were not employed in this assessment. The European Commission's Scientific Committee on Veterinary Measures relating to Public Health has prepared a document on *V. vulnificus* and *Vibrio parahaemolyticus* in raw and undercooked seafood (European Commission, 2001). This work followed the general format of a risk assessment and noted variations in *V. vulnificus* prevalence on a global scale. However, for the European Union, it was concluded that it would be impossible to determine a numerical estimate of risk due to a scarcity of seafood consumption data, lack of quantitative estimates of *V. vulnificus* in seafoods and the absence of a consensus on dose response.

As neither of the above risk assessments were quantitative the general approach proposed and many of the parameters used in the following are the same as those used in the United Stares FDA Draft Risk Assessment on the Public Health Impacts of *V. parahaemolyticus* in Raw Molluscan Shellfish (FDA-VPRA) which is discussed in more detail in section 2.

4.1 Hazard identification

Since *V. vulnificus* was first reported in the 1970's it has been the subject of many research articles and these have been reviewed (Oliver, 1989; Strom and Paranjpye, 2000). Most of the studies have been conducted in the United States and outside of that country there is currently little epidemiological information as *V. vulnificus* is not a reportable disease in most countries and surveillance is limited. While foodborne *V. vulnificus* infections are relatively rare in the United States (approximately 40 reported cases of primary septicaemia per year), they have the highest case/fatality ratio among foodborne illnesses exceeding 50% (Mead *et al*., 1999; Glatzer, 2001). Individuals with pre-existing liver disease are at greatest risk for contracting primary septicaemia and death but other chronic illnesses and immune deficiency are also associated with increased risk. Healthy individuals may be at risk for relatively mild gastroenteritis, which is outside the scope of this assessment, but the risk for primary septicaemia in the absence of reported risk factors is considered negligible.

A number of factors have been reported as possible virulence determinants in *V. vulnificus*, including an extracellular cytolysin, protease, siderophores, a phospholipase, polysaccharide capsule, resistance to bactericidal effects of human serum, resistance to phagocytosis, and the ability to acquire iron from transferrin (Oliver, 1989; Strom and Paranjpye, 2000). The relevance of these factors has been examined in various *in vivo* or *in vitro* models. Production of disease by this bacterium appears to be multifaceted involving a variety of virulence attributes and host susceptibility factors. Most animal studies have not found major differences in virulence characteristics of clinical and environmental isolates of *V. vulnificus* (Table 4.1). However, this is inconsistent with the low attack rate in susceptible populations consuming seafood contaminated with *V. vulnificus*. Less than one illness occurs per 10,000 meals of raw Gulf oysters served to the highest risk population, people with liver diseases (Hlady, 1994), suggesting that environmental strains are not equally virulent or not all people with liver disease are equally susceptible.

Nearly all *V. vulnificus* primary septicaemia cases in the United States have been associated with consumption of raw oyster harvested from the Gulf Coast. These cases follow a seasonal distribution with approximately 90% of cases occurring from April through October (Glatzer, 2001). Only one confirmed

foodborne case has been associated with oysters harvested outside of the Gulf Coast and only one case has been linked to oysters harvested in January or February. The seasonal numbers of *V. vulnificus* in Gulf Coast oysters at harvest (Motes *et al*., 1998) and at retail (Cook *et al*., 2001) are distributed similarly to illnesses.

4.2 Exposure assessment

4.2.1 Microbial ecology

Vibrio vulnificus is a bacterium that occurs naturally in estuaries in many parts of the world (Oliver, 1989). Its distribution and abundance is affected by temperature and salinity of the seawater. *V. vulnificus* is present in waters, sediments, plankton, molluscs, crustacean and finfish of Gulf Coast estuaries (Tamplin, 1990; DePaola *et al*., 1994) in the United States. Attention is generally focused on oysters since most *V. vulnificus* related foodborne illnesses in the United States are linked to their raw consumption. *V. vulnificus* is found in various tissues of the oysters and may reside within oyster haemocytes (Tamplin and Capers, 1992; Harris-young *et al*., 1993). Each oyster may shed up to one million *V. vulnificus* cells per day into the water demonstrating its ability to multiply within the oyster (Tamplin and Capers, 1992). .

Typically United States Gulf Coast oysters harbour about 1000 *V. vulnificus* cells per gram during the warmer months of April through October and usually less than 10 per gram during other months although *V. vulnificus* may become undetectable in Gulf Coast oysters during unusually cold periods (DePaola *et al*., 1994; Motes *et al*., 1998). Some evidence suggest that this bacterium lives in oysters year round but may become dormant or VBNC during cold weather; a temporary condition reversible by increasing water temperature (Nilsson *et al*., 1991). *V. vulnificus* numbers in seawater are approximately 100-fold lower than in oysters but numbers frequently exceed 10^6 per gram in the intestines of bottom feeding fish that inhabit oyster reefs (DePaola *et al*., 1994).

Unlike many shellfish-borne human pathogens, *V. vulnificus* is not associated with human faeces and traditional indicators of faecal pollution (i.e. faecal coliforms) are not effective at predicting its abundance in oysters (Tamplin *et al*., 1982). A number of factors may interact with temperature and salinity to control *V. vulnificus* populations in oysters including nutrient availability, resuspension of sediments, plankton blooms, defecation's of vertebrates (i.e. finfish), phagocytosis by oyster haemocytes, competition, predation, phage infections and a variety of physical factors (pH, dissolved oxygen, water chemistry, and sunlight). The effect these factors on *V. vulnificus* ecology is unknown, making the prospect of developing a reliable indicator model in the near future extremely unlikely.

4.2.2 Growth and survival characteristics

The bacterium may grow at temperatures as low as 13^{o}C (Kaspar and Tamplin, 1993) but its numbers in the environment remain low at temperatures below 20^{o}C (Kelly, 1982; O'Neill *et al*., 1992). Highest concentrations occur when the water temperature is between 20^{o}C and 30^{o}C. Thus, *V. vulnificus* is more abundant along the Gulf Coast than in the cooler waters of the Atlantic and Pacific Coast of the United States (DePaola *et al*., 1994; Cook, 1994; Tamplin, 1990; O'Neill *et al*., 1992; Kaysner *et al*., 1987; Motes *et al*., 1998). *V. vulnificus* can be found at salinities ranging from 0.8 to 35 ppt (Tamplin, 1990; Kaysner *et al*., 1987). The salinity optimum for *V. vulnificus* appears to vary considerably from area to area but highest numbers are usually found at intermediate salinities of 5 to 25 ppt (Tamplin *et al*., 1982; Kelly, 1982; O'Neill *et al*., 1992; Tamplin, 1990; Motes *et al*., 1998).

Table 4.1. Summary of virulence testing from clinical and environmental *V. vulnificus* strains using various animal models.

			No. isolates tested/no. Virulent	
Model	**Dose/Route**	**Reference**	**Clinical strains**	**Environmental strains**
Normal adult mouse	10^6/i.p [c]. 10^6/s.c.[c]	Poole and Oliver, 1978	2/3	Not tested
	10^8/i.v. [c]		2/3	Not tested
	10^8/o.g. [c]		1/1	Not tested
			0/1	Not tested
Normal adult mouse	10^8/i.p.	Tison and Kelly, 1986	20/20	25/29
Normal adult mouse	10^8/i.p.	Kaysner *et al*., 1987	4/4	40/40
Normal adult mouse	10^6/i.p.	Stelma *et al*., 1992	7/11	9/13
Iron-overload mouse[a]	10^0/i.p.	Wright *et al.,* 1981	1/1	Not tested
Iron-overload mouse[a]	10^3/i.p.	Morris *et al*., 1987	4/8	2/8
Iron-overload mouse[b]	10^2/i.p.	Kaysner *et al*., 1987	3/4	4/7
Iron-overload mouse[b]	10^2/i.p.	Stelma *et al*., 1992	8/11	9/13
Iron-overload mouse[b]	10^3/i.p.	Jackson *et al*., 1997	1/1	1/8
Iron-overload and immunocompromised mouse	10^3/i.p.	Stelma *et al*., 1992	0/3	4/4
Suckling mouse	10^6/o.g.	Johnson *et al*., 1984	5/5	0/7
Suckling mouse	10^7/o.g.	Morris *et al*., 1987	4/8	2/8
Suckling mouse	10^5/o.g.	Reyes *et al*., 1987	5/6	2/5

[a]Ferric ammonium citrate (80 mg/kg) used to produce iron-overload

[b]Iron dextran (250 mg/kg) used to produce iron-overload

[c] Route of administration:
i.p.=intraperitoneal injection;
s.c.=subcutaneous injection;
i.v.=intraveneous injection;
o.g.=orogastric ingestion

V. vulnificus is more sensitive than other *Vibrio* spp. and most other foodborne pathogens to most inactivation techniques used in food processing. A mild heat treatment of 50°C for 5 minutes yielded a 6 $\log_{10}$ reduction of *V. vulnificus* in shucked oyster meats (Cook and Ruple, 1992). Freezing oysters at –40°C and storage for 3 weeks achieved a 4 to 5 $\log_{10}$ reduction of the natural *V. vulnificus* population

(Cook and Ruple, 1992). However, the effectiveness of freezing may be reduced in *V. vulnificus* cells subjected to a cold adaptation step of 15°C prior to freezing (Bryan *et al*., 1999). Similar reductions can be readily achieved by irradiation (Ama *et al*., 1994) and high hydrostatic pressure (Berlin *et al*., 1999). Low pH is quite lethal to *V. vulnificus* (Koo *et al*., 2001) but organisms within the oyster tissues would probably be protected from acidic hot sauces and other chemicals as these would probably not penetrate to the interior of an oyster (Sun and Oliver, 1994; Sun and Oliver, 1995). Depuration was shown not to be effective in elimination of *V. vulnificus* as it resides within various oyster tissues, but relaying oysters to high salinity (>32ppt) was shown to reduce *V. vulnificus* numbers by 3-4 logs (<10 per g) within 2 weeks (Motes and DePaola, 1996).

4.2.3 Consumption of oysters

The frequency of consumption and amount of raw oysters consumed would be the same as described in the FDA-VPRA (Anonymous, 2001).

4.2.4 *Modelling exposure to V. vulnificus*

A schematic diagram of the *V. vulnificus* risk assessment model is shown in Figure 4.1. Modelling exposure to *V. vulnificus* can follow the same approach and use many of the same assumptions as used for the FDA-VPRA (Anonymous, 2001). These are summarized in Table 4.2. While foodborne *V. vulnificus* infections have been reported in countries other than the United States such as Taiwan (Chuang *et al*., 1992) and Korea (Park *et al*., 1991), sufficient data is currently available only in the United States for most of the model inputs shown in Figure 4.1. This framework could be used by other countries to model the risk of *V. vulnificus* illness from raw oysters when they collect the appropriate data and may be modified to address other seafoods.

The production-process-retail-consumption continuum was modelled using a modular approach. Each of the modules – harvest, post harvest including storage and retail – and the data requirements for each are outlined in the following sections.

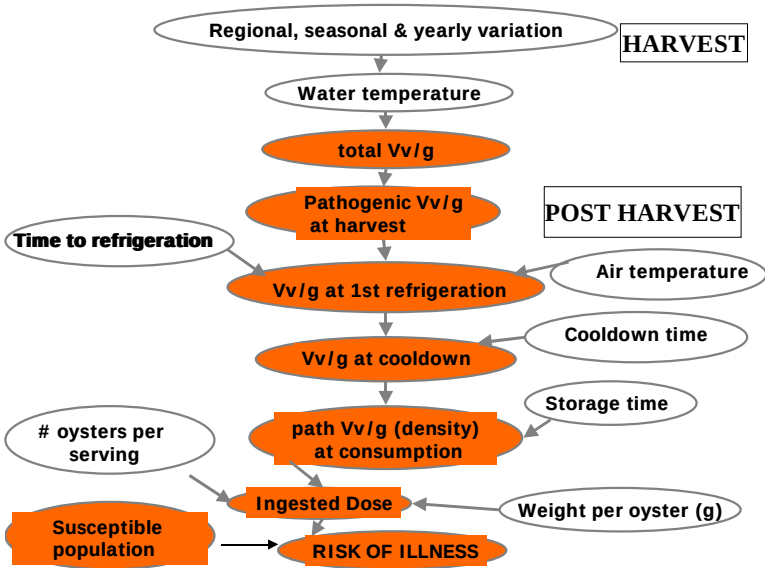

Figure 4.1. Schematic diagram of the *V. vulnificus* conceptual risk assessment model showing integration of all modules. The inputs that are not shaded can be transferred directly from the FDA-VPRA and inputs that are shaded require additional data.

Table 4.2. Model inputs, data sources and assumptions for the proposed *V. vulnificus* risk assessment.

Model Inputs	Data Source	Assumptions
Water temperature	FDA-VPRA; NOAA buoy (National Oceanic and Atmospheric Administration, 1999)	Buoy data representative of growing areas.
Total *V. vulnificus* numbers at harvest	Weekly oyster samples from 4 Gulf States 1994-1995 (Motes *et al*. 1998)	Data relevant for other years
Pathogenic *V. vulnificus* numbers at harvest	Weekly oyster samples from 4 Gulf States 1994-1995 (Motes *et al*. 1998)	All *V. vulnificus* strains equally virulent.
Air temperature	FDA-VPRA; NOAA buoy (National Oceanic and Atmospheric Administration, 1999)	Temperature of oyster meat equilibrates rapidly to that of air.
Time harvest vessel in water	FDA-VPRA; Dealer survey (Cook, 1997)	Harvest practices have not changed since 1996.
Time to first refrigeration	FDA-VPRA; Dealer survey (Cook, 1997)	Oysters harvested at a constant rate throughout the harvest period.
V. vulnificus growth rate	Natural populations in oysters at ambient temperature (Cook, 1997). Data lacking at lower temperature	*V. vulnificus* grows at similar rate in Gulf oysters from April through October.
V. vulnificus/g at first refrigeration	Dealer survey of Gulf oysters 1995-1996 (Cook, 1997)	Dealer practices in 1996 are typical of current practices.
Cool down time	FDA-VPRA; no data	Rectangular distribution between 1 and 10 h.
V. vulnificus/g at cool down	Dealer survey of Gulf oysters 1995-1996 (Cook, 1997)	Dealer practices in 1996 are typical of current practices.
V. vulnificus survival	Natural populations in oysters stored at 3°C for 14-17 d (Gooch, 2000; Cook *et al*., 2001)	*V. vulnificus* die off at 3°C similar to that at other temperatures between 0-13°C.
Pathogenic *V. vulnificus*/g at consumption	Retail study of U.S. oysters 1998-1999 (Cook *et al*. 2001)	Data relevant for other years.
Percentage population susceptible	Liver disease, immune disorder, etc. (Desenclos *et al*., 1991; Shapiro *et al*., 1998; Hlady, 1997; Klontz, 1997)	Infection rates in Florida among various susceptible populations applicable to other states.
Percentage of oysters consumed raw	50% oysters consumed raw FDA-VPRA	Susceptible individuals consume raw oysters at same rate as total population.
No. oysters per serving	FDA-VPRA	None
Weight per oyster	FDA-VPRA	None
Risk of illness	Relationship between monthly exposure and illness within defined (Gulf States) population (Cook *et al*., 2001; Glatzer, 2001; National Marine Fisheries Service, 1998)	Consumption of raw oysters among susceptible population does not vary from month to month Reporting of *V. vulnificus* septicaemia cases related to oyster consumption does not vary from month to month.

4.2.4.1 Harvest

Like *V. parahaemolyticus*, *V. vulnificus* numbers at harvest are determined primarily by water temperature and salinity. Other factors may also contribute to *V. vulnificus* numbers but only temperature and salinity have been quantified (Motes *et al*., 1998).

4.2.4.1.1 Water temperature and salinity distributions

Like *V. parahaemolyticus* the numbers of *V. vulnificus* at harvest are influenced predominantly by water temperatures and salinities. The FDA-VPRA did not quantify the distribution of salinities prevalent in the United States growing areas. It was noted that there was little systematic collection of salinity data outside of a few selected estuaries (e.g., sites within the National Estuarine Research Reserve System) and that what data was available was relatively recent and did not quantify extremes of salinities that might be expected over longer periods of time. Water temperature data was obtained from the National Buoy Data Center (NOAA) for sites considered representative of selected regions in the United States.

For the proposes of this exposure assessment, the FDA-VPRA water temperature distributions for the United States Gulf Coast based on the NOAA data were used. Exposure assessments were run for Gulf Coast summer and Gulf Coast winter (i.e., a cold versus a warm harvest season). The water temperature in the summer averages 29° C and varies from day-to-day with a standard deviation of 1.5° C. The corresponding average and standard deviation in the winter are 14.4 and 2.7° C, respectively. These parameters vary from year to year, as discussed in the FDA-VPRA (Anonymous, 2001), and this variation was incorporated into the present exposure assessment.

Although the effect of salinity has not been incorporated into this assessment, the effect of salinity on predicted *V. vulnificus* numbers merits consideration. Effects of salinities on *V. vulnificus* may be stronger than on *V. parahaemolyticus* and lack of salinity data presents a potential data gap.

4.2.4.1.2 Prediction of the distribution of V. vulnificus numbers at harvest

Numbers of *V. vulnificus* in United States Gulf Coast oysters at harvest have been investigated in a number of studies (Tamplin *et al*., 1982; Motes *et al*., 1998; Vanoy *et al*., 1992; Jackson *et al*., 1997). The study by Motes *et al*. 1998 examined *V. vulnificus* numbers in 3 major Gulf Coast estuaries at weekly intervals for 15 months and was selected for the exposure assessment as samples were collected more intensively and extensively than in previous studies (Motes *et al*., 1998). Additionally, their enumeration procedures are the same as used for other model inputs in this exposure assessment (i.e. to examine market oysters and determine *V. vulnificus* growth and survival).

Like *V. parahaemolyticus*, ambient water temperatures and salinities influence *V. vulnificus* numbers at harvest (Oliver *et al*., 1982; Motes *et al*., 1998). On the basis of correlation statistics of harvest studies (Motes *et al*., 1998), temperature and salinity appear to account for about 60% to 70% of the total variation of observed *V. vulnificus* numbers. Other factors may influence the regional/seasonal variation but there is no data available at the present time for the purpose of quantitative modelling.

Motes *et al*. found the effect of salinity on *V. vulnificus* harvest numbers to be significant (Motes *et al*., 1998). The R^2 of a temperature only regression model was 0.60 in comparison to an R^2 of 0.70 when the effects of salinity were added to the regression model. This suggests that effects of salinity explain an additional 10% of the total variation of *V. vulnificus* numbers observed in the United States Gulf Coast.

A temperature only-regression analysis of the Motes *et al*. data is shown in Figure 4.2. The data shown are averages of duplicate samples of composites of 12 oysters. Unlike *V. parahaemolyticus*, numbers of *V. vulnificus* are not linear versus temperature and noticeably plateau at higher temperatures in the Gulf Coast. Thus the regression fitted to the *V. vulnificus* data is a quadratic regression. Predictions of mean log *V. vulnificus* numbers based on the regression are appropriate only in the range of temperature

from 10 to about 32°C (i.e., near the maximum of the quadratic). In the regression *V. vulnificus* numbers are log-transformed because untransformed numbers are positively skewed (i.e., the distribution of MPN/g at any given temperature is not symmetric about the mode). Very few samples in the study had nondetectable counts and for the purpose of estimating the regression half the limit of detection (<0.3 or 3.0 per g) was substituted for nondetectable outcomes obtained by the MPN method used. Due to the small number of nondetectable samples, very little bias is introduced by this substitution.

The best fitting temperature-only (quadratic) regression of the data was (Motes *et al*., 1998):

$$\log(Vv/g) = \alpha + \beta_1 * TEMP + \beta_2 * TEMP^2 + \varepsilon$$
$$\varepsilon \sim Normal(0, \sigma^2)$$

The parameter estimates obtained by regression were:

$$\alpha = -5.503$$
$$\beta_1 = 0.539$$
$$\beta_2 = -0.008$$
$$\sigma^2 = 0.60$$

The estimate of variance about the regression mean (σ^2) includes the effect of method error (3-tube MPN) which has been estimated to be 0.12 (Motes *et al*., 1998). Consequently, "true" population variation of numbers in averages of 2 composites is estimated as 0.48 (i.e., 0.60 – 0.12). Very little variance was found between duplicate samples, which were taken from the same dredge (Motes *et al*., 1998). Therefore, variance of numbers in 12 oyster composites (more typical of serving size) is not anticipated to be substantially different from 0.48 and this value has been used in the present simulations.

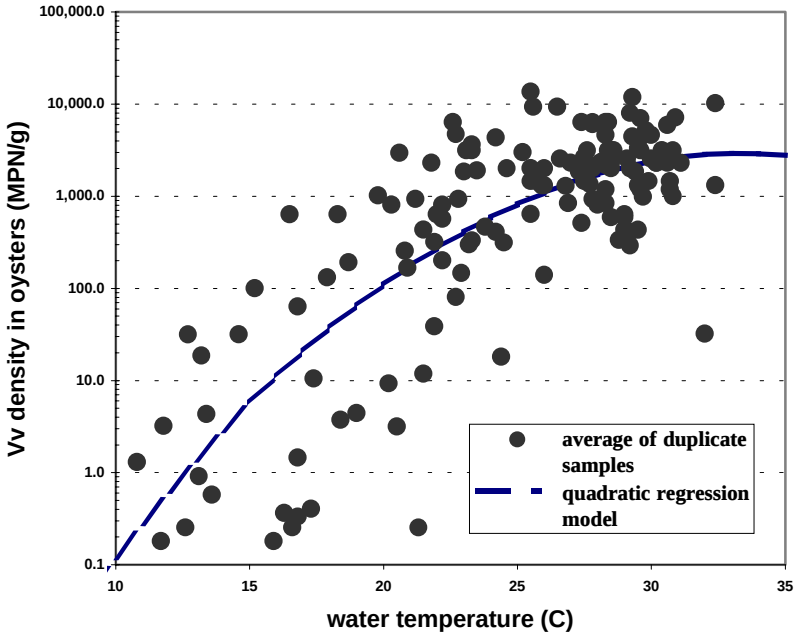

Figure 4.2. *Vibrio vulnificus* numbers in United States Gulf Coast Oysters (Motes *et al*., 1998)

Although not incorporated in the present assessment, Motes *et al*. found that salinity had an appreciable effect on *V. vulnificus* numbers in the Gulf of Mexico. Based on a combined temperature and salinity regression model that is quadratic for both of these factors, Figure 4.3 illustrates the effect of ignoring salinity and predicting *V. vulnificus* numbers based on the temperature only. The temperature and salinity regression indicates that optimal salinity for *V. vulnificus* was approximately 17 ppt. For salinities in the range of 12 to 20 ppt, ignoring the effect of salinity will at most overpredict log *V. vulnificus*/g by 5%. However, extremes of salinities in the range of <10 or >25 ppt can be detrimental to *V. vulnificus* survival, and predictions of *V. vulnificus* numbers at these extremes based on temperature only may overpredict by >20% depending upon the temperature. This may be an important consideration in countries that have a major portion of their oysters production in high salinity waters, for example New Zealand.

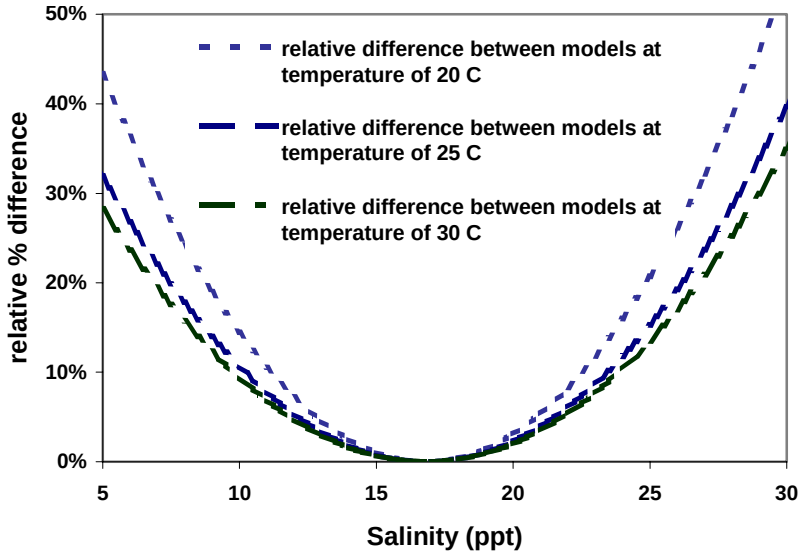

Figure 4.3. Temperature only versus temperature + salinity model for $\log^{10}$ *V. vulnificus* at harvest

4.2.4.2 Post harvest

This section follows closely the corresponding section in the FDA-VPRA (Anonymous, 2001). Like *V. parahaemolyticus* the numbers of *V. vulnificus* at consumption would be influenced by ambient air temperatures at harvest, time from harvest until oysters are placed under refrigeration, time it takes oysters to cool once under refrigeration and length of refrigeration time until consumption.

4.2.4.2.1 Growth of V. vulnificus from harvest to first refrigeration

The model of growth used in the present assessment is the 3-phase linear growth model advocated for microbial risk assessment by Buchanan *et al*. (Buchanan *et al*., 1997). This is the same growth model used in the FDA-VPRA (FDA, 2001). The growth prediction equation of this model is:

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

where N(0) denotes the initial number of organisms per g (i.e., at time of harvest), N(t) denotes the predicted number at t intervals of time (hours) post-harvest. The parameters of the equation are the maximum growth rate (μ_m) and the maximum density (A).

As in the FDA-VPRA, a secondary model of microbial growth relating the growth rate to ambient holding temperature is assumed. This secondary model is:

$$\mu_m(T) = \max\{0, \alpha * (T - T_0)\}$$

where T denotes hold temperature and the parameters of the equation are the temperature below which growth does not occur (T_0) and the slope of a growth rate versus temperature (α) relationship.

Comparative studies of numbers in oysters received at processing plants versus oysters at harvest (Ruple and Cook, 1992) and experimental studies with shellstock oysters stored under different temperature regimes clearly indicate that postharvest multiplication is substantial at ambient air temperatures of ~25°C which are typical in the United States Gulf Coast in late spring through early fall. The best available data to estimate this parameter are provided by two studies (Cook, 1994; Cook, 1997). It is also apparent that the minimum temperature required for growth of *V. vulnificus* is approximately 13°C (Kaspar and Tamplin, 1993). Below this temperature, *V. vulnificus* numbers decrease over time and/or *V. vulnificus* can enter a viable but nonculturable (VBNC) state (Oliver, 1995).

Figure 4.4 shows the data where oysters were held for up to 14 hours at ambient air temperatures ranging from 24 to 32°C (Cook, 1997). Table 4.3 lists *V. vulnificus* growth rates at various temperatures. A 0.75 log increase in numbers was observed over a period of 30 hours when oysters were held at 18°C (Cook, 1994). For oysters harvested during the summer and stored at ambient air temperatures ranging from 24°C to 33°C (with average 28°C), a 1.3 $\log_{10}$ increase in *V. vulnificus* numbers was observed over 7.5 hours with a plateau of approximately 2 $\log_{10}$ increase after a period of 14 hours (Cook, 1997). In an earlier study, oysters stored under refrigeration at 18°C were found to have average increase of approximately 0.75 $\log_{10}$ over a period of 30 hours(Cook, 1994). Thus, the maximal growth rate is approximately 0.025 hr^{-1} at 18°C and 0.19 hr^{-1} at 28°C (at times below 14 hours). Assuming no growth at 13°C, regression of maximal growth rate against temperature gives an estimate of 0.011 $hr^{-1}{}^{o}C^{-1}$ for the slope factor α above the threshold temperature of 13°C (i.e., a linear regression of growth rate versus temperature above 13°C where growth rate is assumed to equal zero at 13°C)

Based on the parameter estimates for the primary and secondary growth models, predictions of *V. vulnificus* growth from an initial level of 3 $\log_{10}$ per g are illustrated in Figure 4.5 for ambient air temperatures of 18, 20, 26 and 32°C.

4.2.4.2.2 Distribution of ambient air temperature

The distribution of ambient air temperatures before first refrigeration of oysters would be the same as in the FDA-VPRA (Anonymous, 2001). The air temperature in the summer averages 1.7°C cooler than the water temperature. The day to day standard deviation of difference between air and water temperature is 1.3°C. The corresponding average and standard deviation in the winter are 1.1 and 3.3°C, respectively, with air still generally cooler than water. These parameters vary from year to year, as discussed in the FDA-VPRA (Anonymous, 2001) and this variation has been incorporated into the present exposure assessment.

4.2.4.2.3 Distribution of time oysters are left unrefrigerated

The distribution of times oysters are left unrefrigerated would be the same as in the FDA-VPRA (Anonymous, 2001).

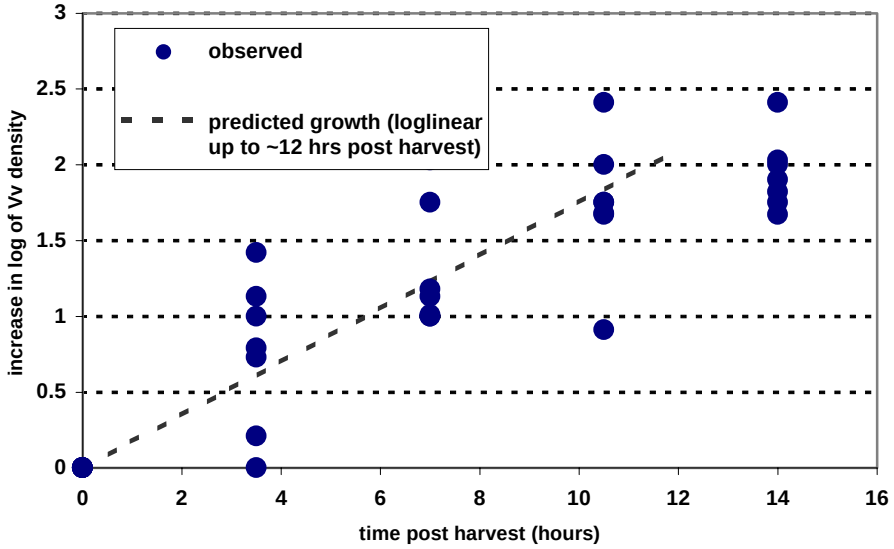

Figure 4.4. Postharvest growth of *V. vulnificus*

Table 4.3. *V. vulnificus* growth rate versus temperature**.**

Study	Holding temperature (Celsius)	Growth rate (log10 per hr)	Assumptions/Limitations
(Cook, 1997)	28	0.175	Ambient air temperature varied from 24 -33, assumed average of 28 ^{o}C
(Cook, 1994)	18	0.025	Rate per hour assumed constant with observed average 0.75 log increase (n=5) over period of 30 hours
(Kaspar and Tamplin, 1993)	13		Presumed no growth temperature

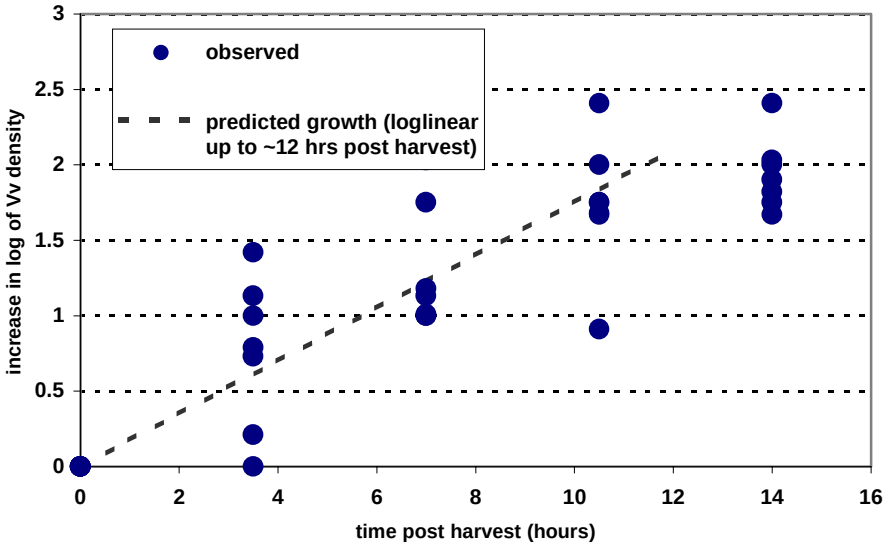

Figure 4.5. Predicted Loglinear growth of *V. vulnificus* as a function of ambient air temperature

4.2.4.2.4 Growth of V. vulnificus during cooling

Model assumptions of growth during the process of cooling are as described in the FDA-VPRA (Anonymous, 2001) (see also section2.2.5.4.4). Briefly, oysters are assumed to take anywhere from 1 hour to 10 hours to reach no growth temperature, with all values in this range being equally likely regardless of initial air temperature (i.e., temperature difference). The growth rate (which varies in proportion to the air temperature) is assumed to fall linearly to zero during the period of time required to reach no growth temperature.

4.2.4.2.5 Die-off of V. vulnificus during cold storage

V. vulnificus is more susceptible to cold than *V. parahaemolyticus*. Based on ISSC/FDA retail data (Cook *et al*., 2001), it has been estimated that *V. vulnificus* numbers decline by 0.041 logs per day under normal conditions of cold storage in the United States marketplace. Minimum, maximum and mean duration of storage of oyster lots sampled in this same study were used in the FDA-VPRA to define a distribution of storage times for the United States marketplace.

4.2.5 Simulation Results

Monte Carlo simulations of the distribution of *V. vulnificus* at harvest and at selected points in the production-consumption process continuum were obtained using the simulation program Analytica 2.0.5 (Anonymous 1999). As outlined in the discussion above, the output distributions were based primarily on water temperature, the derived regression relationship for $\log_{10}$ *V. vulnificus* numbers versus water temperature, *V. vulnificus* growth rate versus temperature and various distribution parameters (from the FDA-VPRA), which effect the extent of microbial growth and survival post-harvest.

V. vulnificus exposure associated with United States Gulf Coast oysters was simulated for both the summer (July-September) and winter (January-March) harvest seasons (i.e., a warm versus a cool harvest season). Parameter distributions were obtained by the Monte Carlo method using a sample size of 1,000. The effect of year-to-year variation in mean and variance of water temperature distributions was evaluated based on 20 Monte Carlo samples of water temperature parameters (i.e., mean and variance). However, the effect of these year-to-year temperature variations on the distributions of *V. vulnificus* at harvest and post-harvest were minimal. Statistical summaries of the parameter distributions obtained by Monte Carlo sampling, averaged over the year-to-year variations in water temperature, are shown in Table 4.4.

Overall, the simulation results suggest that *V. vulnificus* numbers increase post-harvest an average of 0.80 $\log_{10}$ MPN per gram during the summer harvest season and decrease an average of 0.2 $\log_{10}$ MPN per gram during the winter harvest season. Variation in water/air temperatures and the characteristics of harvesting duration and storage time have the effect of increasing the variation of *V. vulnificus* numbers at each point along the harvest to consumption continuum. For the United States Gulf Coast summer, the standard deviation of *V. vulnificus* numbers (in 12 oyster composites) is 0.79 $\log_{10}$ at consumption compared to 0.70 $\log_{10}$ at harvest. Due to the positive skew of the distributions, the mean density of *V. vulnificus* per gram is greater than the antilog of mean $\log_{10}$ MPN per gram. Mean density of 54,000 and 43 per gram were obtained for the summer and winter harvest seasons, respectively. Given an average serving size of 330 grams of oyster meat weight, these average numbers correspond to average ingested doses of 1.8×10^8 and 1.4×10^4 respectively.

Representative output of the analytical simulation is shown in Figures 4.6 through 4.8. These graphs illustrate the effect of post-harvest parameters on the location and shape of the distribution of *V. vulnificus* per gram. Generally, each stage of the harvest to consumption continuum shifts the mean numbers per gram with a concomitant increase in the variability about the mean from one sample of oysters to the next.

Figure 4.6 shows typical distributions of water an air temperature obtained for the Gulf summer. These distributions are normal by assumption. As evident in the figure, the distribution of air temperature has a mean that is slightly less than that of water and exhibits more variation (i.e., spread). The variation in temperatures drives numbers at harvest and determines variability in extent of growth occurring after harvest. Together with parameters effecting harvest duration (e.g., distribution of time prior to 1st refrigeration shown in Figure 4.7), the growth rate of *V. vulnificus* post-harvest and survival during storage is used to derive the distribution of numbers at the time of consumption. The difference in *V. vulnificus* numbers at harvest versus at consumption is shown is Figure 4.8. The *V. vulnificus* numbers at consumption are slightly less than 1 $\log_{10}$ higher than that at harvest.

Table 4.4. Summary output of the simulation of environmental parameters, oyster handling conditions, *V. vulnificus* growth, survival and numbers from harvest to consumption in United States Gulf Coast oysters in the summer and winter.

	GULF SUMMER	**GULF WINTER**
Distribution Parameter	**Mean (std dev)** [1]	**Mean (std dev)** [1]
Water temperature, °C	29 (1.52)	13.7 (2.68)
Log *V. vulnificus*/g at harvest	3.29 (0.70)	0.27 (1.13)
V. vulnificus/g at harvest	7050	46
Air – water temperature difference, °C	-1.66 (1.33)	-1.07 (3.3)
Air temperature, °C	27.3 (2.04)	12.6 (4.3)
Time on the water, hours	6.8 hrs	7.5 hrs
Time oysters unrefrigerated, hours	3.9 hrs	4.25 hrs
$\log_{10}$ growth prior to refrigeration	0.62 (0.31)	0.075 (0.13)
$\log_{10}$ growth during cooldown	0.50 (0.22)	0.06 (0.093)
Die-off during storage (in logs)	0.31	0.31
$\log_{10}$ *V. vulnificus*/g at consumption	4.10 (0.79)	0.087 (1.13)
V. vulnificus/g at consumption	54,000	43
Oysters per serving	15.75	15.75
Grams per serving	333	333
Total *V. vulnificus* ingested per serving	1.8×10^8	1.4×10^4

[1] For distributions that are approximately normally distributed the standard deviation is given in parentheses; no standard deviation is tabulated for those distributions that are highly skewed.

4.2.6 Model Validation

Results of simulations were compared to available data on the density of *V. vulnificus* in United States market oysters. Summary statistics for the density of *V. vulnificus* in market oysters obtained by the ISSC/FDA collaborative retail study (Cook *et al*, 2001) are presented in Table 4.5. The model simulation output is consistent with these measurements. In particular, the means compare favourably for both summer and winter with a mean of 54,000 per gram predicted versus 62,100 observed for the United States Gulf Coast summer harvest. For the winter the model predicted mean is 43 per gram versus an observed mean of 60 per gram.

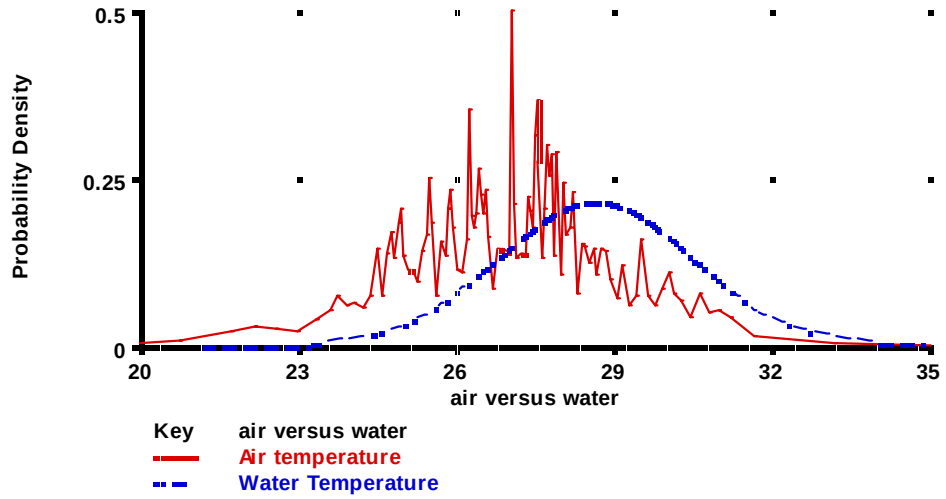

Figure 4.6. Typical day-to-day "noontime" water/air temperatures for the United States Gulf Coast summer (units of the x-axis are °C).

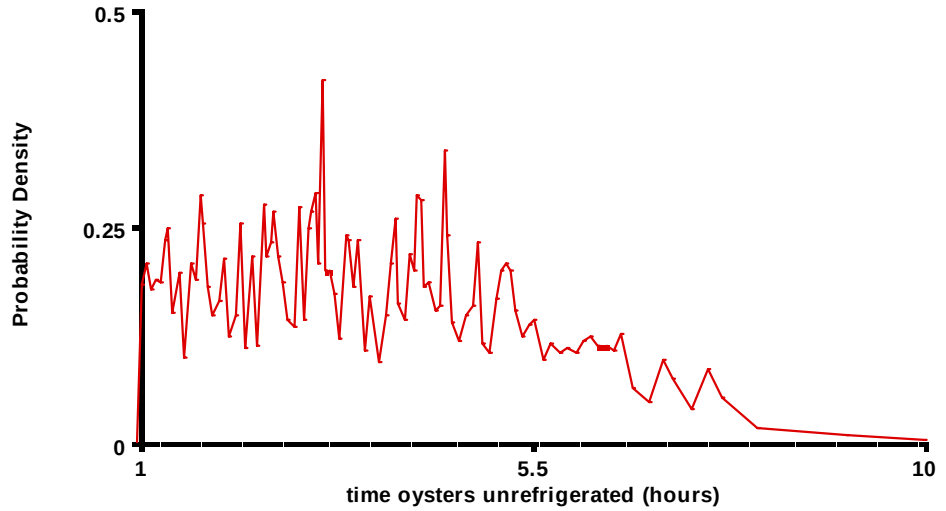

Figure 4.7. Distribution of the time that oysters are left unrefrigerated post-harvest.

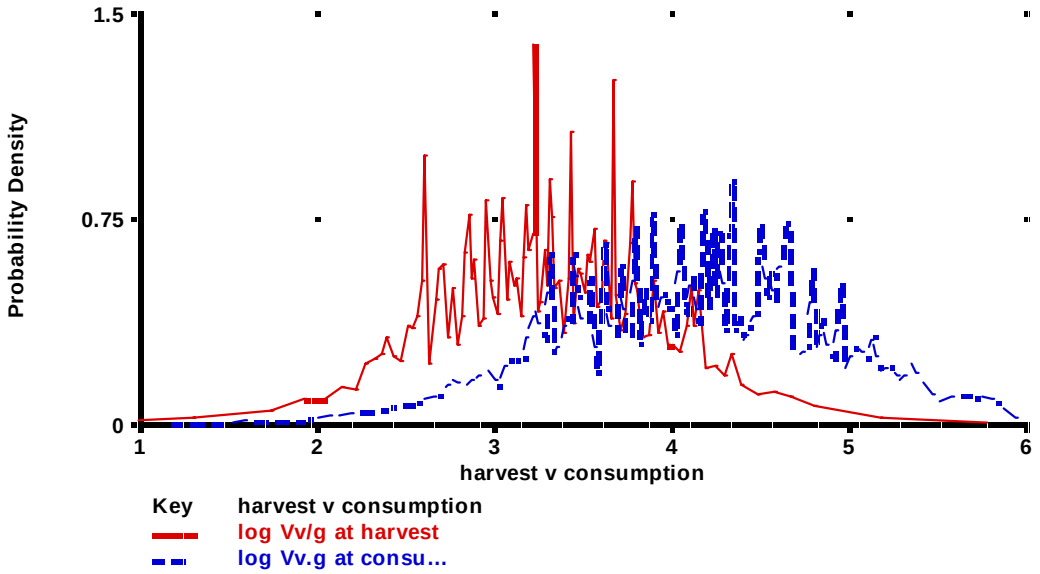

Figure 4.8. Distribution of *V. vulnificus* numbers at harvest versus consumption (units of x-axis are $\log_{10}$ MPN per g).

Table 4.5. ISSC/FDA retail data on the numbers of *V. vulnificus* in United States Gulf Coast oysters during the summer and winter.

Region / Season in the United States	**# samples**	**% samples with detectable *V. vulnificus***	***V. vulnificus*/g** [1]	**Log10 *V. vulnificus*/g** [1]
Gulf/Summer	41	97.5%	62,100	4.0 (1.16)
Gulf/Winter	37	84%	60	0.60 (1.12)

[1] mean or mean and standard deviation

The difference in predicted versus observed MPN per gram for the Gulf of Mexico summer may be partially due to effect of method error on the estimate for the ISSC/FDA data. The difference between the mean and variance of predicted versus observed $\log_{10}$ numbers suggest that the higher estimate of mean *V. vulnificus*/g based on retail data is due to the higher variance of $\log_{10}$ numbers for the ISSC/FDA data. In the model simulation, error associated with measurements of *V. vulnificus* taken by Motes *et al.*(1998) has been subtracted out. The ISSC/FDA retail study also employed an MPN procedure for enumeration and a percentage of the variance of $\log_{10}$ numbers is attributable to method error over and above true variation in numbers from one sample to the next. This method error is symmetric and unbiased on the $\log_{10}$ scale but not on the untransformed scale (i.e., MPN or count per gram). However, the magnitude of method error associated with the retail data is not known since a modified MPN procedure was employed to compensate for the effect of interference observed in the MPN series for these data.

With respect to the winter harvest season, the mean V. *vulnificus*/g was slightly higher than that predicted by the model simulation (60/g versus 43/g). Although the model predicted variance of $\log_{10}$ *V. vulnificus*/g at harvest is comparable to the variance of $\log_{10}$ *V. vulnificus*/g observed in the ISSC/FDA study, the variance in the ISSC/FDA data is both inflated due to method error and biased downwards by the effect of substitution of half the limit of detection for all samples with unquantifiable numbers. The effect of this bias on statistical calculations of mean and variance is more pronounced for the samples obtained during the winter (16% with unquantifiable numbers).

4.3 Hazard characterization

4.3.1 Description of the pathogen, host, and food matrix factors and how these impact the disease outcome.

4.3.1.1 Characteristics of the pathogen

4.3.1.1.1 Infectivity, virulence/pathogenicity

V. vulnificus potentially causes mild to severe gastroenteritis in most people who consume contaminated food; however, for a specific subpopulation of susceptible people, *V. vulnificus* can cause a serious septicaemia that frequently leads to death.

4.3.1.1.2 Genetic factors (e.g. antimicrobial resistance and virulence factors).

The virulence factors associated with *V. vulnificus* are less well characterized. Factors like cytolysin, protease/elastase, and phospholipase all may play a role, but none of them appear to be essential for virulence as some mutant strains with these factors deleted do not appear to exhibit decreased virulence. The presence of a capsule appears to be well correlated with virulence, however most freshly isolated environmental strains appear to have a capsule irrespective of their virulence (Morris, 1994 and Strom *et al*., 2000).

4.3.1.2 Characteristics of the host

4.3.1.2.1 Immune and physiological status of the host

Foodborne *V. vulnificus* infection is clearly associated with underlying medical conditions (ICMSF, 1996). Liver disease is a prominent risk factor for *V. vulnificus* infection; including cirrhosis due to alcohol consumption. Additional risk factors include diabetes, gastrointestinal disorders (surgery, ulcer), haematological conditions, and immunodeficiency due to underlying conditions such as cancer and treatment of chronic conditions with immunosuppressive agents (arthritis, etc.). As with many other microorganisms the pathogenicity of *V. vulnificus* appears to be associated with the availability of free iron in the host (Wright *et al*. 1981). Many of the known predisposing conditions for infection, particularly chronic liver diseases, are associated with impaired iron metabolism. *V. vulnificus* may pose a small risk to otherwise "healthy" individuals since a small fraction of cases (<5%) occur in individuals without any identifiable risk factor.

Normal population

The normal population may be susceptible to a relatively mild gastroenteritis from consumption of seafoods harbouring *V. vulnificus* but this rarely leads to primary septicaemia. Thus healthy individuals will be excluded from this assessment as the focus is the more serious primary septicaemia cases.

Susceptible population

The prevalence of predisposing conditions among the adult population (>18 years of age) in the United States has been estimated in a 1997 memorandum from to the FDA Office of Seafood Director (Klontz, 1997). These estimates are expressed in Table 4.6.

Table 4.6. Prevalence rates of *V. vulnificus* risk factors per 100,000 individuals assuming a total US adult population of 185,000,000 individuals.

Risk factor	Prevalence per 100,000 individuals
Diabetes (insulin-dependent)	540.5
Liver disease (cirrhosis)	2000.0 (range: 1600 - 9900)
Gastric acidity	38.9
Cancer	1420.0
Hepatitis (B and C)	(range: 400 - 1600)
Kidney disease	108.0
Haemochromatosis	1081.1
AIDS	540.5
Immune-compromised due to treatment/surgery	
Asthma	25.7
Rheumatoid arthritis	51.4
Psoriatic arthritis	37.9
Lupus	(range: 4 - 250)
Polymylagia rheumatica	53.0
Giant cell arthritis	12.0
Transplant recipients	59.5

Using a median value for the prevalence of hepatitis and lupus, these numbers suggest that approximately 7% of the United States adult population are susceptible to infection. Given the uncertainty in prevalence of liver disease (including hepatitis), this number could be as high as ~16%. However, the median estimate of prevalence of liver disease is consistent with results of the 1988 Florida behavioural survey in which 2.4% of raw oyster consumers surveyed reported that they were aware that they had a liver disease (Hlady *et al*., 1993). Consequently, although it can not be ruled out that up to ~8% of the population have undiagnosed chronic liver conditions, a figure of 7% appears to be a more reasonable estimate of the susceptible population. This represents a population of 13 million individuals in the United States at "high risk" of infection. However, it must be noted that this could be different for other countries and regions in particular where large numbers of the population suffer from hepatitis.

The overall estimate of size of the susceptible population is somewhat imprecise due to varying case definitions of the disease conditions. For most disease conditions, the estimates presented in Table 4.x (Klontz, 1997) estimate the prevalence based on cases defined by relatively severe progression (e.g., long term corticosteroid treatment, end-stage renal disease, etc.). It is not know which of these disease conditions are truly associated with increased risk. There may be a distribution of susceptibility related to progression of the various predisposing conditions.

4.3.1.2.2 Age, sex and ethnic group

The vehicle of infection under consideration in this risk assessment for *V. vulnificus* is raw seafood. The consumption patterns for raw oysters the United States have been estimated for age, sex, and ethnic group (Desenclos *et al*., 1991 and Timbo *et al*., 1995).

4.3.1.2.3 Health behaviours

All *Vibrio* spp. are relatively susceptible to inactivation by cooking. Most of the risk associated with the relevant strains of *Vibrio* spp. in food comes from the consumption of raw seafood or from cross contamination of other foods by raw seafood or contaminated water. Health behaviours leading to impaired liver function as the result of long-term heavy alcohol consumption are a major risk factor for septicaemia from *V. vulnificus* infection (Klontz, 1997).

4.3.1.2.4 Genetic factors

Host genetic factors related to susceptibility to *V. vulnificus* gastrointestinal infections are unknown, however, there are many genetic factors associated with the likelihood of the infection to proceed to septicaemia. The presence of a human genetic mutation leading to reduced levels of transferrin, such as hereditary haemochromatosis, results in increased likelihood of septicaemia for the infected individual.

4.3.1.3 Characteristics of the food matrix

4.3.1.3.1 Fat and salt content

Fat and salt content are probably not relevant in the determination of risk with respect to *Vibrio* spp. While the fat content of a matrix may be relevant with respect to the increase of effective dose of pathogens through protection of *Vibrio* spp. in micelles during gastric passage, there is insufficient evidence to model the degree of increased survival.

4.3.1.3.2 pH and water activity

Vibrio spp. appear to be relatively sensitive to both low pH and dehydration. Because of the nature of most foods associated with the unintended consumption of *Vibrio* spp., pH and water activity are probably not relevant in modelling survival of *Vibrio* spp. in raw seafood, however these parameters may be relevant in modelling the growth of *Vibrio* spp. in other foods as the result of cross contamination.

4.3.2 Public Health Outcomes

There are numerous reports of sporadic foodborne cases of *V. vulnificus* but outbreaks of *V. vulnificus* have not been associated with consumption of food (Shapiro *et al*., 1998). An outbreak of wound infections caused by a single clone designated biogroup 3 was reported among fish handlers in Israel but there have not been subsequent reports of outbreaks (Bisharat and Raz, 1997).

4.3.2.1 Manifestations of disease

V. vulnificus causes mild to severe gastrointestinal illness, potentially progressing to septicaemia with a significant mortality in a susceptible population.

4.3.2.2 Rational for the biological end points modelled

Septicaemia is the endpoint modelled as patients typically present to health systems with this symptom. Because of the severity of the septicaemia, underreporting is not a substantial consideration.

4.3.3 Dose-response relationship

4.3.3.1 Summary of available data

4.3.3.1.1 Probability of illness given exposure.

There are no human volunteer studies with *V. vulnificus* on which a dose-response relationship may be estimated. Data are available to estimate the relationship by comparing monthly exposure estimates to sensitive populations with monthly-observed epidemiological data in the United States using a method proposed by Hitchins (1996) and Buchanan *et al*. (1997).

4.3.3.1.2 Probability of sequellae given illness.

Culture-confirmed *V. vulnificus* infections are usually characterised by septicaemia (43%), the remaining infections being gastrointestinal (5%), wound (45%), and uncharacterized (7%). If septicaemia and gastrointestinal illnesses are pooled, approximately 90% of culture-confirmed illnesses associated with the consumption of food result in septicaemia (Hlady and Klontz, 1996).

4.3.3.1.3 Probability of secondary and tertiary transmission.

There are few, if any, reports of secondary or tertiary transmissions of illnesses caused by *V. vulnificus*.

4.3.3.1.4 Probability of death given illness.

For *V. vulnificus* in the United States, mortality rates are between 50% and 60% for patient with septicaemia (Hlady and Klontz, 1996).

4.3.3.2 Sources of data used

Modelling of the dose-response for *V. vulnificus* utilized estimates of exposure per eating occasion developed by DePaola (2001) and average monthly number of oyster associated cases reported to the United States CDC from 1988 through 1996. Both the Beta-Poisson and the exponential model were fit to the data and uncertainty of the Beta-Poisson dose-response fit was characterized by considering uncertainty or variability of the number cases likely to occur in a given month and the uncertainty of the mean $\log_{10}$ density of *V. vulnificus* at harvest associated with average monthly water temperatures in the Gulf of Mexico.

Calculations of mean *V. vulnificus* dose per serving and United States CDC statistics for average number of cases per month are shown in Table 2. The mean $\log_{10}$ *V. vulnificus* numbers in oyster tissue at harvest were obtained by combining data for the United States Gulf coast water temperatures with a *V. vulnificus* density versus water temperature regression relationship presented in the exposure assessment. The mean $\log_{10}$ *V. vulnificus* levels at retail were developed in the exposure assessment based on post-harvest handling assumptions as reported in the. FDA-VPRA (Anonymous, 2001) together with estimates of *V. vulnificus* specific growth rate post-harvest and survival during cold storage. The same methods outlined in the exposure assessment were used here to estimate monthly mean $\log_{10}$ *V. vulnificus* numbers at retail.

The number of servings per month was estimated assuming that 50% of the average landings for that month are consumed raw and then converting to the corresponding number of meals based on average oyster weight and typical number of oysters per serving. The number of meals consumed by the susceptible population was estimated as being 7% of the total meals. For the purpose of dose-response

modelling, the average ingested *V. vulnificus* dose per serving is more pertinent than the median. Thus the mean *V. vulnificus* dose per serving was matched with the average number of cases for that month. Average numbers of cases of septicaemia are from United States Gulf coast oyster harvest (Shapiro, *et al*., 1998). Based upon these dose and epidemiological data, Beta Poisson and Exponential curve fits were obtained by maximum likelihood.

An uncertainty analysis of the Beta-Poisson fit was obtained by generating 1,000 alternative data sets representing uncertainty in the number of cases occurring in a given month and uncertainty of mean $\log_{10}$ *V. vulnificus* numbers at harvest. The exposure assessment utilized a quadratic regression to predict *V. vulnificus* numbers versus temperature based on a 1994/1995 harvest study (Motes *et al*, 1998). The asymptotic standard errors and correlations between the parameter estimates in the regression fit were used to define a multinormal uncertainty distribution for the parameters. Monte Carlo samples from this distribution were used to generate the 1,000 alternative sets of mean ingested dose (associated with each month) by applying the same harvest to consumption calculations discussed above. The effect of uncertainty in growth and survival rates post-harvest was not considered. The number of illnesses occurring in a given month was varied as a Poisson random variable with mean given by the average number of cases shown in Table 4.7.

Figure 4.9. shows the maximum likelihood Beta Poisson dose-response curve fit for *V. vulnificus*. The dots represent the best estimates of the average monthly risk of illness based upon an analysis of exposure and observed epidemiology. The solid line is the most likely Beta Poisson model and the parallel dashed lines are 95% upper and lower uncertainty limits on the predicted risk based on the uncertainty factors identified and considered here. The dotted line represents maximum likelihood fit of the exponential model to the data. The best estimates of the parameters for the Beta Poisson model are $\alpha=9.0\cdot10^{-6}$ and $\beta=1.2\cdot10^{4}$. The best estimates of the parameter for the Exponential was $\beta=8.0\cdot10^{-12}$. Figure 4.9b illustrates the confidence region for alpha and beta parameters of the Beta-Poisson model based on the 1,000 Monte Carlo uncertainty sample.

Table 4.7. Environmental and epidemiological data for *V. vulnificus*

Month	WTEMP	Mean log10 Vv/g at harvest	Mean log10 Vv/g at consumption	Landings (pounds),Average 1990-1998	All meals	Servings for at risk individuals	Median Vv per serving dose	Mean Vv per serving dose	Log10 of the Mean Vv per serving dose	Average # cases in month
Jan	12.5	-0.03	-0.34	1,586,000	883,000	62,000	1.5E+02	2.8E+02	2.45	0
Feb	15	0.76	0.61	1,626,000	906,000	63,000	1.4E+03	2.5E+03	3.40	0
Mar	17.5	1.45	1.51	1,868,000	1,040,000	73,000	1.1E+04	2.0E+04	4.30	0.2
Apr	22.5	2.52	2.96	1,617,000	900,000	63,000	3.1E+05	5.6E+05	5.75	1
May	26	3.04	3.75	1,359,000	757,000	53,000	1.9E+06	3.5E+06	6.54	3
Jun	28.5	3.28	4.19	1,297,000	722,000	51,000	5.2E+06	9.6E+06	6.98	2.5
Jul	30	3.38	4.41	1,195,000	665,000	47,000	8.6E+06	1.6E+07	7.20	2.5
Aug	30	3.38	4.41	1,085,000	604,000	42,000	8.6E+06	1.6E+07	7.20	3.5
Sep	28	3.24	4.11	1,224,000	682,000	48,000	4.3E+06	8.0E+06	6.90	3
Oct	23	2.61	3.12	1,567,000	872,000	61,000	4.4E+05	8.2E+05	5.91	3
Nov	18	1.57	1.68	1,803,000	1,004,000	70,000	1.6E+04	2.9E+04	4.46	1.5

Dec	15	0.76	0.61	1,841,000	1,025,000	72,000	1.4E+03	2.5E+03	3.40	2

4.3.3.3 Assumptions

In developing the dose-response model for *V. vulnificus* the following assumptions were made:

- Meal size is a constant 333 g per serving
- 7% of meals are consumed by at risk individuals (Klontz, 1997)
- The estimated mean number of. *V. vulnificus* cells per gram at consumption is based on a regression equation for $\log_{10}$ *V. vulnificus* per gram versus temperature at harvest, average time unrefrigerated, estimated growth rate versus temperature, and survival during cold storage (DePaola, 2001)
- The estimates of monthly mean *V. vulnificus* per serving, based on the exposure analysis conducted, are assumed accurate
- The use of mean *V. vulnificus* /g rather than median *V. vulnificus* /g (i.e. mean $\log_{10}$ *V. vulnificus* /g) as a summary measure of exposure for a group is considered appropriate for dose-response analysis (i.e., for grouped data with varying individual doses within each group it is considered more appropriate to relate the average response to average (mean) dose).

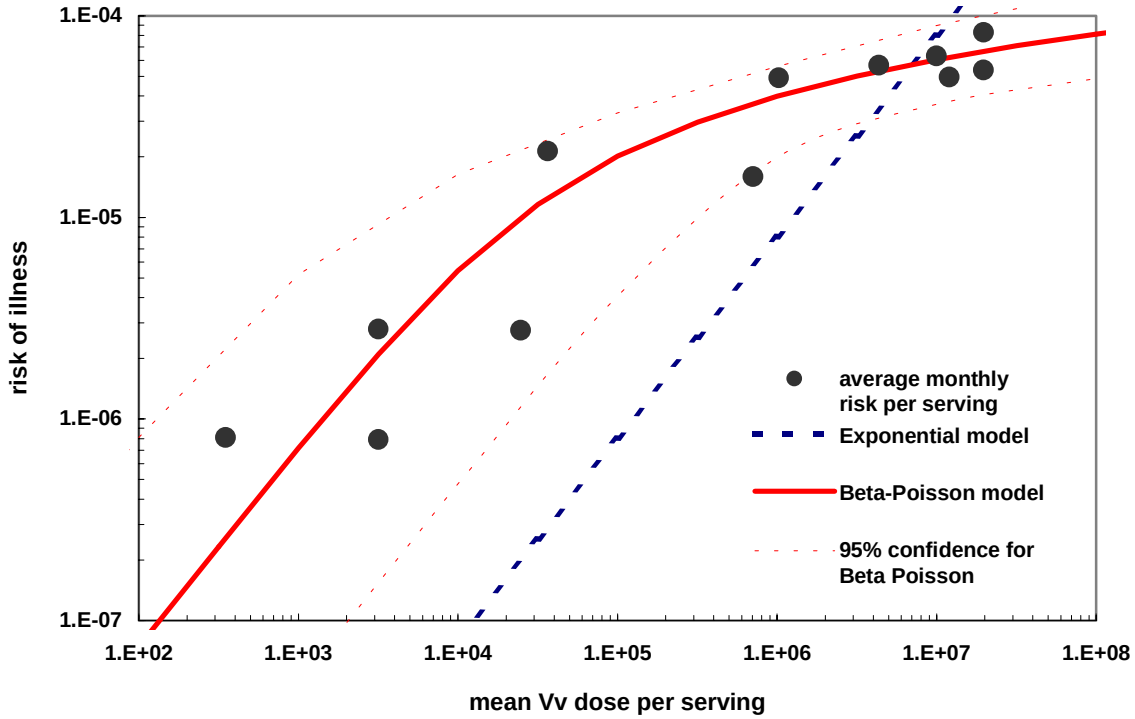

Figure 4.9a. Beta Poisson dose-response curve for *V. vulnificus* (monthly average risk per serving versus monthly average dose per serving)

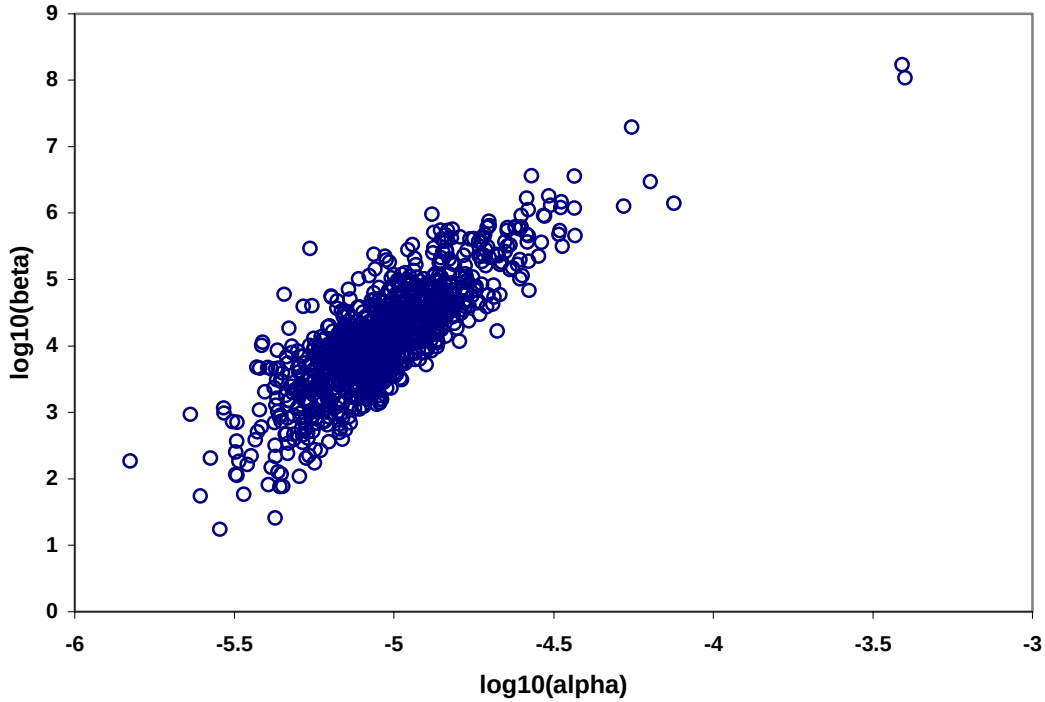

Figure 4.9b. Parameter uncertainty of Beta Poisson dose-response fit to *V. vulnificus* epidemiological data

4.3.3.4 Goodness of fit of the distribution

The goodness of fit of the distribution is uncharacterised as a family of dose-response parameters are used to represent the parameter uncertainty.

4.3.3.5 Uncertainty and variability in the estimates

This analysis incorporates both uncertainty and variability in the estimates.

4.4 Risk characterization

If feasible this will be completed in 2002. If not the work will be continued at a later date

4.5 Gaps in Data

In the United States, especially for the Gulf Coast, there is available data for each of the model inputs using the framework outlined in the FDA-VPRA (Anonymous, 2001). A key assumption is that all *V. vulnificus* strains are virulent. Most studies indicate that a large majority of food and environmental strains are virulent. Outside of the United States, there is a scarcity of useful data for quantitative risk assessment for *V. vulnificus* including numbers of *V. vulnificus* in foods, consumption patterns and epidemiology.

In the course of the work to date a number of questions arose which need to be addressed in order to complete this risk assessment. These are as follows;

- Are all *V. vulnificus* strains equally virulent?
- If *V. vulnificus* strains vary in virulence, how can degrees of virulence be assigned to various subpopulations within the population in oysters?

- Does virulence vary by season, region, or source (shellfish, finfish, water, etc.)?
- Are all members of the at risk population equally susceptible?
- If susceptibility varies among individuals and/or susceptibility is not constant within an individual, how can the at-risk population be defined and properly weighted for degree of susceptibility?
- Is it appropriate to weight susceptibility by a factor of increased incidence of *V. vulnificus* infections in a particular at-risk population (i.e. *V. vulnificus* infections were 4.5 times higher in diabetics and among nondiabetics in a Florida study)?
- Is there month to month bias in reporting of *V. vulnificus* infections within a defined epidemiological region?
- Is risk of *V. vulnificus* infection dependent on exposure and independent of vehicle of infection?
- What are the weaknesses of developing a dose response relationship based on monthly differences in exposure and reported illnesses within a defined epidemiological unit?
- What are the obstacles of applying this dose response relationship to other foods, regions or countries?

4.6 Conclusion and recommendations

The FDA-VPRA framework (Anonymous, 2001) and many of the model inputs are applicable for modelling *V. vulnificus* risk in United States oysters and sufficient data are available to conduct a useful risk assessment.

Countries outside the United States that wish to pursue *V. vulnificus* risk assessment should initiate data collection efforts on *V. vulnificus* numbers in relevant seafoods at harvest and the point of consumption and characterize the susceptible population. The dose response data from the United States should transfer to other countries.

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Appendix.

Analytica 2.0.5 Model Simulation Structure for Exposure Assessment of V. vulnificus in Raw Oysters

Calculations in this exposure assessment were performed using the Monte Carlo simulation software "Analytica". The structure of the simulation was organized into two parts: (a) simulation of the distribution of *V. vulnificus* numbers in oysters at the time of harvest and (b) the simulation of the effect of post-harvest processing on the distribution of *V. vulnificus* numbers up to the point of consumption. Specification of a model is accomplished by specifying "chance" nodes and interrelationships between these nodes in an influence diagram for the simulation. Additional nodes of the influence diagram are used to contain supporting information such as function and index definitions that are referenced in the specification of the chance nodes representing the physical parameters of the model to be simulated per se.

Figure 1 shows the Analytica influence diagram of the model for harvest density calculations. Water temperature influences log *V. vulnificus*/g at harvest, from which the distribution of *V. vulnificus* /g is obtained by taking the antilog. The definition of the water temperature node is:

Normal(Temps[parm='mu'], Temps[parm='sigma'])

The temperature parameter array "Temps" (the "temperature distributions" node) contains a sample of 20 year-to-year variations of mean and standard deviation of the day-to-day variability of water temperature. Each element in an array defines a separate simulation so that a sequence of 20 simulations is obtained once values of parameters are loading into the array.

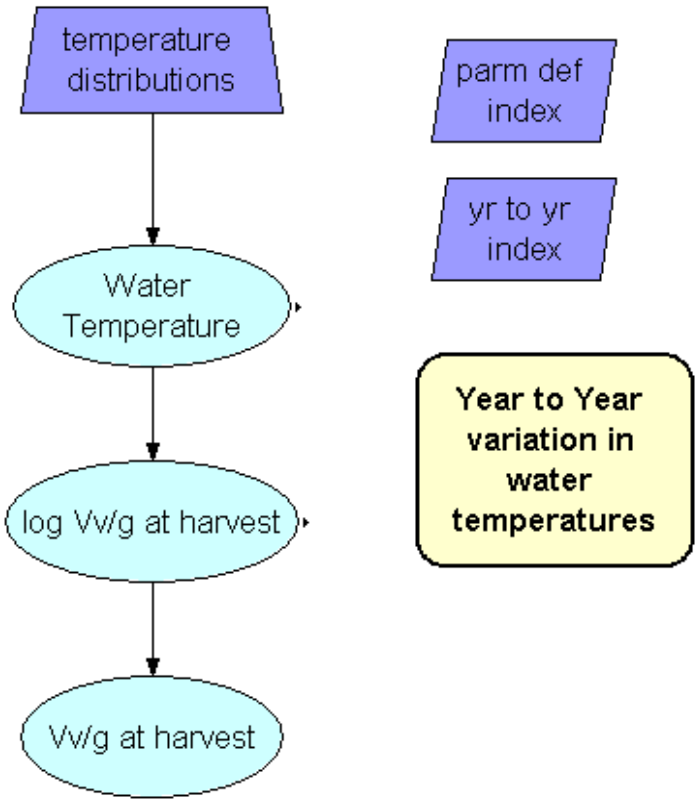

Figure 1. Structure of Model of Harvest Numbers

The definition of the log *V. vulnificus* /g at harvest node is:

Normal(-5.503+0.539*Wtemp-0.0081*Wtemp*Wtemp, 0.69)

This is simply the Motes *et al.* temperature relationship for the mean and the method error adjusted population standard deviation of 0.69 (=square root of 0.48). The relationship to *V. vulnificus* /g is simply the antilog.

The Analytica influence diagram for the effect of post-harvest processing is shown in Figure 2.

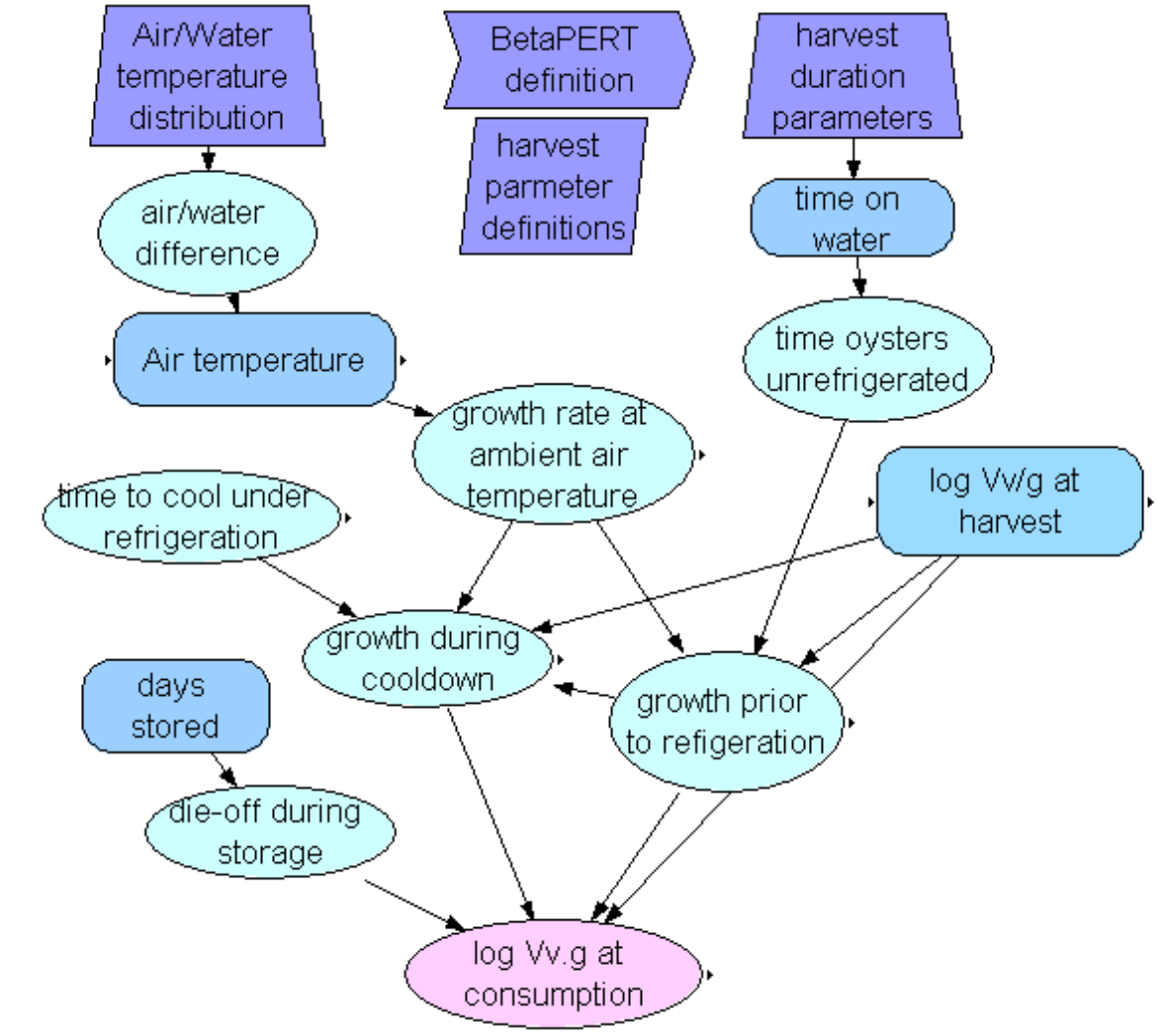

Figure 2. Structure of Model of Effect of Post-Harvest Handling

Water temperature and Air/Water temperature differences define the node representing the variation of ambient temperature of oyster prior to refrigeration. For each Monte Carlo random sample of ambient air temperature the corresponding growth rate is defined as

Max([0,0.011*(Air_temperature-13)])

The chance nodes representing variation of time on the water (length of harvesting operations) and time required to cooldown oysters to no growth conditions (13°C) influence the chance nodes for the amount of growth that occurs prior to time of 1st refrigeration and then subsequently as the oysters cool. These relationships are defined as:

Min([L_har_vv_g + Growth_rate*Time_oysters_unrefr, 6]) - L_har_vv_g

and

Min([L_har_vv_g+Outgrowth_1 + Growth_rate*(Time_to_cool+1)/2, 6]) - L_har_vv_g+Outgrowth_1)

The variable L_har_vv_g is the density per g at harvest (from previous influence diagram). Outgrowth_1 is the number of logs of growth occurring prior to 1st refrigeration if oysters are unrefrigerated for a duration of time defined by Time_oysters_unrefr. However, no growth is permitted which would raise numbers above 6 $\log_{10}$. Similarly the 2nd equation defines the extent of growth during cooldown duration "Time_to_cool" subject to a restriction that no growth is allowed which would raise numbers above 6 $\log_{10}$.

The final output parameter in the influence diagram is the density at consumption:

L_har_vv_g + Outgrowth_1 + Outgrowth_2 - Die_off_during_stor

Simulated samples of density at harvest are adjusted upward by the distributions of extent of growth prior to and during refrigeration. Then the distribution is adjusted down by the effect of random extent of die-off during storage of 1 to 14 days.

5 RISK ASSESSMENT OF *VIBRIO CHOLERAE* IN SHRIMP FOR DOMESTIC AND EXPORT MARKETS

5.1 Hazard Identification

Toxigenic *V. cholerae* O1 and O139 are the causative agents of cholera, a water- and food-borne disease with epidemic and pandemic potential. Non-O1/non-O139 strains may also be pathogenic but are not associated with epidemic disease. Non-O1 strains are generally nontoxigenic, usually cause a milder form of gastroenteritis than O1 and O139 strains,-and are usually associated with sporadic cases and small outbreaks rather than epidemics (Desmarchelier, 1997).

Outbreaks of cholera have been associated with consumption of seafood including oysters, crab and shrimp (Oliver and Kaper, 1997). The largest outbreak was a pandemic in South America in the early 1990s when *V. cholerae* O1 caused more than 400,000 cases and 4,000 deaths, in Peru (Wolfe, 1992). Contaminated water used to prepare food, including the popular, lightly marinated fish *ceviche*, was the cause of the outbreak.

The most important virulence factor associated with *V. cholerae* O1 and O139 causing epidemic cholera is the cholera toxin. The *ctx* gene encoding the production of cholera toxin has been sequenced and this has enabled development of DNA probes and Polymerase chain reaction (PCR) based methods for detection of this gene in environmental isolates of *Vibrio cholerae* O1 and O139. Isolation of non-toxigenic *V. cholerae* O1 from the environment has been reported by several workers (Kaper *et al.,* 1979, Colwell *et al*., 1981, Sakazaki and Donovan, 1984; Minami *et al*., 1991). Therefore, if *V. cholerae* O1 is isolated from shrimp, it would be important to determine whether they are toxigenic or not.

5.2 Exposure assessment

5.2.1 Microbial ecology

Though over 130 serovars of *V. cholerae* are recognised, only *V. cholerae* O1 and O139 are associated with epidemic and pandemic cholera. Cholera is exclusively a human disease and no animal species has been found to be consistently infected. The primary source of *V. cholerae* O1 and O139 is the faeces of persons acutely infected with the organism. The organism reaches water through sewage. The causative organism can survive in waters for long periods of time and there are several instances where water has been implicated by epidemiological studies as vehicles of *V. cholerae* O1 (Table 5.1).

*5.2.2 **Growth and survival characteristics***

The physicochemical factors limiting the growth of *V. cholerae* O1 have been summarised by ICMSF (1996). The optimum temperature for the growth in 37°C and the temperature range at which growth can occur in 10-43°C. The pH optimum for growth is 7.6 and *V. cholerae* can grow in the pH range of 5.0 to 9.6. The water activity optimum for growth is 0.984 and growth can occur between 0.970-0.998. *V. cholerae* can grow in the salt range of 0.1-4.0% NaCl, while optimum is 0.5% NaCl.

5.2.2.1 Growth rate

Kolvin and Roberts (1982) estimated the growth of *V. cholerae* O1 in raw and cooked seafood. No growth was observed in raw prawns, mussels and oysters but in cooked shellfish, rapid growth occurred. Levels of 10^{10} cells/g was attained in cooked prawns and mussels at 37°C. At 22°C, there was a lag phase of 8 h for classical biotype and 4 h for eltor biotype.

5.2.2.2 Death or inactivation

V. cholerae O1 is highly sensitive to acidic environments and is killed within minutes in gastric juice with pH < 2.4. Therefore, normochlorohydric individuals are resistant to attack by cholera. *V. cholerae* O1 is also highly sensitive to desiccation. Therefore well dried vessels may not be involved in transmission of cholera. This organism is also heat sensitive with a D-value of 2.65 min at 60°C (ICMSF, 1996). The pathogen survives refrigeration. In shrimp homogenates spiked with *V. cholerae* O1 at a level of 7.8 log/g, survival up to 21 days at 7°C was observed (Reilly and Hackney, 1985). In raw shrimp with an initial count of 5 log /g, survival of 4-9 days was observed at 5-10°C (Pesigan *et al.,* 1967). Survival in frozen seafood has been estimated to be more than 6 months (ICMSF, 1996)

Table 5.1. Water implicated by epidemiological studies as vehicles of *V .cholerae*

Year	Isolation site	Implicated water	Reference
1974	Portugal	Commercially bottled uncarbonated spring water, well water	Blake *et al*., 1977a, 1977b
1980	Thailand	Ice	Morris *et al*., 1982
1981	South Africa	River water	Sinclair *et al*., 1982
1984	Mali	Well water	Tauxe *et al*., 1988
1990	Malawi	Water stored at home, well water	Swerdlow *et al*., 1991
1991	Bolivia	River water	Gonzalus *et al*., 1992
1991	Equador	Street Vendor drinks	Weber *et al*., 1992
1991	Peru	Street Vendor drinks	Swerdlow *et al*., 1991
1991	Peru	Municipal water stored at home	Ries *et al*., 1992
1991	Peru	Ice	Ries *et al*., 1992

5.2.3 Prevalence in shrimp and water.

The survival time of *V. cholerae* in water has been estimated (Feacham *et al*., 1981). The average time required to achieve a 1 log decline in cell number (t_{90}) is a function of organism as well as biotype (Feacham *et al*., 1981) as shown in Table 5.2. The work of Colwell and co-workers has shown that *V. cholerae* O1 can survive in water almost indefinitely and the organism can be said to be an autochthonous aquatic organism (Colwell and Spira, 1992). The conclusion that *V. cholerae* O1 can persist for long periods of time in water is supported by the observation that *V. cholerae* O1 of the same biotype, serotype, phage type and toxin profile have been isolated over a 10 year period in locations such as Gulf of Mexico (Blake *et al*., 1980; Shandera *et al*., 1983). In Australia, *V. cholerae* O1 could be isolated intermittently over 22 month period from river water, which was used as an auxiliary town water supply that was implicated in a case of cholera in 1977 (Rogers *et al*., 1977). However, *V. cholerae* O1 and O139 is confined to fresh water and estuarine environments. There are no reports of the presence of these organisms in off shore environments.

In the aquatic environment, a strong association between levels of zooplankton and incidence of *V. cholerae* has been observed. Adherence to chitin has been shown to strongly influence the ecology of *V. cholerae*. Toxigenic *V. cholerae* has also been reported to attach to hindgut of crabs (Huq *et al*., 1996). The hindgut of crustacea is an extension of exoskeleton and it is lined with chitin. The seasonality of cholera in Bengal may be explained by the model in that primary transmission would be controlled by

environmental factors such as temperature, salinity, nutrient concentration, zooplankton blooms as well as seasonal variation in seafood harvesting and consumption and in direct water contact (Colwell and Spira, 1992).

Table 5.2. Survival of *V. cholerae* in water (Feacham *et al*., 1981)

Biotype	Water type	t_{90}
Classical	Fresh water(non sterile)	184 (0.16-36 h)
	Seawater (non sterile)	95 h (0.36-161 h)
Eltor	Fresh water (non sterile)	53h (1-230 h)
	Seawater (non sterile)	56h (8-235 h)

However, shrimp body surface, gut or gills is not the primary habitat for *V. cholerae* O1 and O139, which are involved in epidemics of cholera. In fact, there are very few records of isolation of *V. cholerae* O1 and O139 from shrimp. Most studies from south-east Asia indicate the absence of *V. cholerae* O1 from raw shrimp (Karunasagar *et al.,* 1990; Fonseka, 1990, Rattagool *et al.,* 1990; Karunasagar *et al.,* 1992), but Dalsgaard *et al*. (1995) noted that the *V. cholerae* O1 was present in 2% of brackish water cultured shrimp in south-east Asia. Data from India indicate the presence of *V. cholerae* O1 in 0.2% of raw shrimp (Ministry of Agriculture, India). However, it is not known whether these shrimp associated strains are toxigenic or not.

5.2.4 Concentration in shrimp and water

Most studies on the incidence of *V. cholerae* in the environment have been conducted using enrichment techniques and therefore, there is little data on the levels of this organisms in natural waters. Some studies indicate that numbers could be low e.g. 46/L (Colwell and Spira, 1992). In Bangladesh, the level of *V. cholerae* O1 in household water was determined and in most cases, *V. cholerae* O1 could be found only after enrichment and the probable level in water was estimated to be 0-500cfu/100ml (Spira *et al*., 1980). With respect of shrimp, there is no data on the numbers of *V. cholerae* O1 and most reported isolations have been done following enrichment.

There are several reports of the involvement of fish and shellfish in outbreaks of cholera (Table 5.3). The problem is acute where fish and shellfish are consumed raw. There is only one record of an outbreak of cholera linked the consumption of raw shrimp in the Philippines in 1961, however, the source of the shrimp is not known (Joseph *et al*., 1965). Therefore, it is not possible to assess whether *V. cholerae* O1 was naturally present in the shrimp, or whether the shrimp was contaminated after harvest. Shellfish most often involved are molluscan shellfish (oysters) and crabs. While oysters are consumed raw in many places, crabs are generally cooked, but crabs boiled for less than 10 minutes or steamed for less than 30 minutes may still harbour viable *V. cholerae* O1 (Blake *et al*., 1980).

V. cholerae O1 can persist in both free living and refrigerated molluscs for many weeks (DePaola, 1981). Kolvin and Roberts (1982) noted that *V. cholerae* O1 did not multiply in any raw shellfish. But in cooked shellfish rapid growth occurred. In cooked prawns and mussels, at 37°C numbers of 10^{10} were reached within 12 h. At 22°C there was a lag phase of 8 h for the classical biotype and 4 h for the eltor biotypes. It has been shown that *V. cholerae* O1 adheres to chitin, a component of crustacean shells and survival of chitin–adsorbed *V. cholerae* in dilute hydrochloric acid of approximately the same pH as gastric acid is prolonged (Nalin *et al*., 1979). Thus, chitin adsorbed *V. cholerae* O1 may be protected from the lethal effect of gastric acid.

Table 5.3. Instances of fish implicated as vehicles for transmission of *V. cholerae*

Site	Year	Fish involved	Reference
Philippines	1962	Raw shrimp	Felsenfeld, 1972
Italy	1973	Raw mussels	Baine *et al*., 1974
Portugal	1974	Raw and undercooked shellfish	Blake *et al*., 1977b
Guam	1974	Salted raw fish	Merson *et al*., 1977
Gilbert Islands	1977	Raw and salted fish And clams	McIntyre *et al*., 1979
Louisiana	1978	Cooked crabs	Blake *et al*., 1980
Singapore	1982	Cooked squid	Goh *et al*., 1984
Louisiana	1986	Cooked crab, cooked or raw shrimp	Lowry *et al*., 1989
Guinea-Bissare	1987	Cooked crabs	Shaffer *et al*., 1988
Colarado	1988	Raw oyster	CDC, 1989
Chuck	1990	Raw fish	Swerdlow *et al*., 1991
Ecuador	1991	Seafood	CDC, 1991
Japan	1991	Imported clams	Anon, 1991
Hong Kong	1994	Seafood	Kam *et al*., 1995

5.2.5 Consumption of shrimp

Shrimp is widely consumed both in developing countries and developed countries. In most Asian shrimp producing countries, large sized high quality shrimp goes to the export market and only small sized varieties are consumed locally. Estimates of the volume of shrimp consumed are available for very few countries. In Australia, 18,791 tonnes of locally produced and 10,106 tonnes of imported prawns are consumed annually. Assuming that the consumption in one meal is 150g, this equates with 192,647,000 servings. It is also estimated that 25% of Australians consume prawns.

5.2.6 Modelling exposure

5.2.6.1 Approaches

Exposure assessment involves estimation of the likelihood of ingesting toxigenic *V. cholerae* O1 and O139 by eating shrimp contaminated with these organisms and the amount of the organisms consumed. Since most of the world's shrimp production and processing occurs in developing countries in Asia and Latin America, where cholera is endemic, there can be multiple modes of contamination. Therefore, exposure assessment should consider possibilities of contamination during harvesting, post-harvest handling, processing, retail and at the consumer end during preparation for consumption. Two models are being proposed, one for shrimp harvested from tropical coastal environment, handled and marketed in domestic markets of developing countries (Figure 5.1) and another for shrimp harvested/cultured and processed in tropical developing countries, and consumed in developed countries (Figure 5.2).

5.2.6.2 Production to consumption chain

5.2.6.2.1 Preharvest

There is no evidence to show that shrimp caught by trawling in offshore waters harbour toxigenic *V. cholerae* O1 and O139.

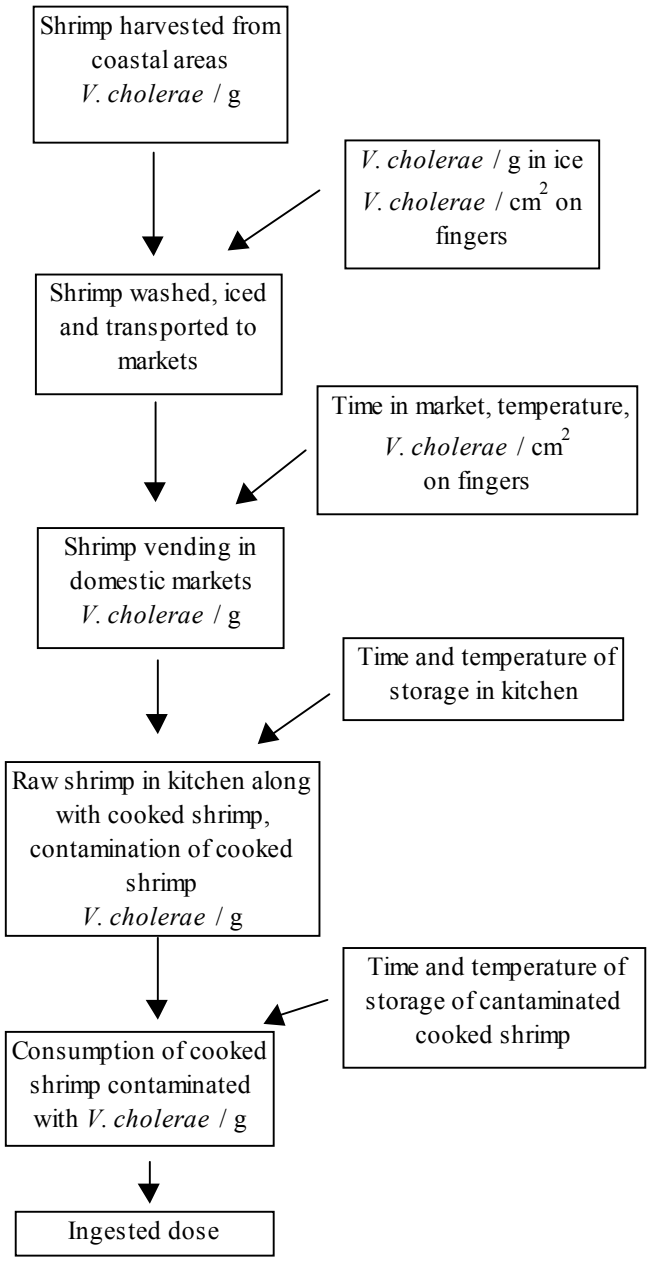

Figure 5.1. Model for exposure assessment for *V. cholerae* in shrimp for domestic consumption in developing countries.

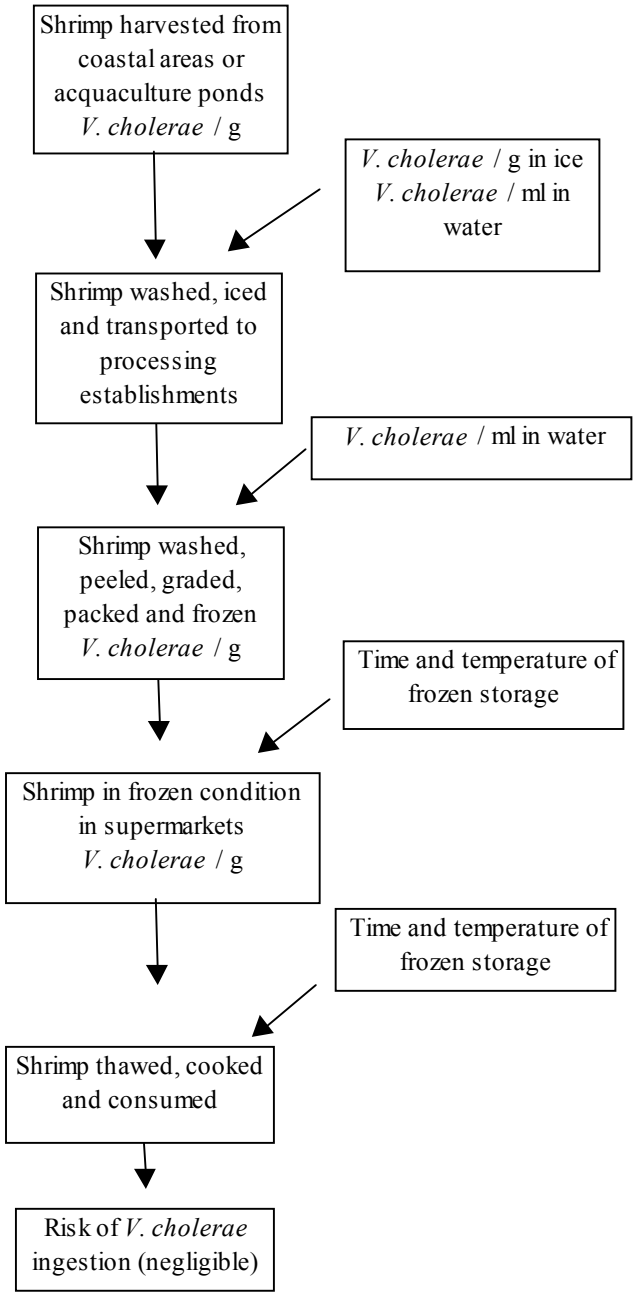

Figure 5.2. Model for exposure assessment for *V. cholerae* in shrimp for international markets.

5.2.6.2.2 *Production*

The contribution of aquaculture to shrimp production is increasing in most countries. Most shrimp aquaculture activity is in coastal areas and the water source is generally estuaries or bays. In this situation, introduction of *V. cholerae* O1 or O139 is possible as evidenced by the data of Dalsgaard *et al*. (1995). There is no data on the level of *V. cholerae* O1 and O139 likely to be present. However, considering the data on the low level (46/L) of these organisms in natural waters (Colwell and Spira, 1992), it can be expected that the level of these organisms in cultured shrimp is rather low. *V. cholerae* O1 has been shown to adhere to chitin, but association has been demonstrated only with zooplankton and crabs. The observation that shrimp is rarely associated with outbreaks of cholera suggest that shrimp body surface and gut are not the preferred habitat for *V. cholerae* in natural waters. Ravi Kiran (1992) analysed shrimp gut for the presence of potential human pathogens and noted the absence of *V. cholerae* O1. Since shrimp is

generally consumed after cooking, the risk of cholera transmission through cultured shrimp is very low. This is supported by epidemiological data, which shows that though developed countries such as the United States and Japan import and consume large quantities of cultured shrimp from Asia and Latin America, there are no outbreaks of cholera associated with these products.

5.2.6.2.3 Transport and distribution

After harvest of marine shrimp by trawling, the shrimp are separated from the by-catch by manual sorting and iced on board the trawlers. Ice is generally produced using potable water in coastal ice plants and carried on board in insulated containers. During this process, there is a limited possibility of contamination with *V. cholerae* O1 if the person handling the shrimp and ice is a carrier of *V. cholerae* O1. In cholera endemic areas, asymptomatic carriers play an important role in transmission of the pathogen. For example, the contamination of water stored in households from the fingers of carriers has been observed to be a major route of transmission of cholera. This is borne out by studies conducted in Calcutta, India. A series of studies were conducted in which sanitary wells were installed in communities and the effect on incidence of cholera and other enteric diseases measured. The results have generally indicated no reduction in the incidence of cholera or diarrhoea in users of sanitary wells. The major reason for this appears to be that water is rarely ingested directly from source, but is stored in the household in a variety of ways. These studies suggested that asymptomatic carriers contaminated waters by putting their fingers or utensils in the water that is stored in wide mouthed containers. This suggestion is supported by the observation that introduction of narrow necked earthenware vessels for storing water reduced the *V. cholerae* O1 infection rate by 75% (Deb *et al*., 1986).

In the case of cultured shrimp, following harvest, the shrimp are washed in tap water, iced and packed in plastic crates for transport by trucks to the processing centre. The marine shrimp caught by trawlers is re-iced at the landing centre. At this point, contamination with *V. cholerae* O1 is a possibility, since the level of hygiene in the landing centres can be quite low. Often, ice crushing is done on the floor, which is washed with tap water that may not be of potable quality. Contamination with *V. cholerae* O1 by water or asymptomatic carriers is therefore also possible at this point. However, the level of the pathogen would be very low.

The experimental study of Kolvin and Roberts (1982) suggests the *V. cholerae* O1 does not multiply in raw prawns. Further, the temperature of iced shrimp during transport would be less than 10°C and at this temperature, *V. cholerae* O1 does not multiply. The temperature range at which growth of *Vibrio cholerae* O1 could occur is 10-43°C (ICMSF, 1996). Once the shrimp arrives in a processing unit, they are taken to peeling sheds where they are peeled manually, washed, graded and packed for freezing. In this situation again the possibility for contamination via water or asymptomatic carriers exists.

Often, the shrimp processors pay great attention to the quality of shrimp intended for export market. Thus, in spite the many potential routes for contamination with *V. cholerae* O1 during handling and processing of shrimp, the incidence of this pathogen in exported shrimp is rather low and this explains the lack of involvement of shrimp in outbreaks of cholera in shrimp importing countries. The care taken by shrimp exporters is evident from data from Peru that was collected during the Peruvian cholera epidemic (De Paola *et al*., 1993). During this epidemic, *V. cholerae* O1 was present in all the 5 samples of raw seafood sampled from street vendors in Lima and Callao, but only one sample out of 1011 samples of seafood destined for export was positive for *V. cholerae* O1. (De Paola *et al*., 1993). This shows that even during large epidemic situations, contamination of seafood with *V. cholerae* O1 can be prevented through adoption of strict hygienic measures during handling and processing.

On the other hand, shrimp intended for domestic consumption is not so carefully handled. Since these are low value shrimp compared to that intended for export, they are poorly iced, and transported to domestic fish markets where sanitary conditions are poor. In this situation, contamination with *V. cholerae*

O1 through poor quality water, and thus ice, exists. Another factor could be frequent handling and touching of shrimp by vendors and prospective buyers. This could substantially increase chances of contamination and the situation could be similar to contamination of water stored in households described earlier (Deb *et al*., 1986). Since shrimp intended for domestic consumption is poorly iced, the temperature would be higher than 10°C. Though slow multiplication in this situation can be expected, the data from Kolvin and Roberts (1982) shows that *V. cholerae* O1 does not multiply in raw prawns. Poorly handled shrimp would also carry a heavy load (10^5-10^6/g) of spoilage flora with which a low number (perhaps <100/g) of *V. cholerae* O1 has to complete. Thus unless the initial level of contamination is high, the risk of getting cholera through seafood is rather low.

There is no data on the extent of contamination that can occur through asymptomatic carriers. De Paola (1981) cited data that indicates that during the carrier state of 5-19 days, *Vibrio cholerae* O1 concentration in stools could be 10^2 to 10^5/g. Therefore, contamination via fingers can be estimated to be less than 100 *V. cholerae* O1 cells. Further, carrier states of a short duration and chronic carriers are rare and are not known to play any role in cholera transmission or persistence (Tauxe *et al*., 1994).

5.2.6.2.4 Storage and retail

As discussed in the previous section, the chances for *V. cholerae* O1 contamination are high during retailing of shrimp in domestic markets in developing countries. The data of DePaola *et al*. (1993) from the Peruvian epidemic shows high level of contamination (100%) in raw seafood from street vendors. As shown in Table 5.3, there is only one recorded outbreak of cholera linked to raw shrimp. This episode also could be due to unhygienic retailing conditions. However, there are no data on the numbers of *V. cholerae* O1 found in raw shrimp from street vendors, therefore, it is difficult to understand whether *V. cholerae* O1 would multiply to infectious levels in shrimp under retailing conditions.

The retail market in developed countries does not provide any chance for contamination or multiplication of *V. cholerae* O1 in shrimp. This is supported by epidemiological data from countries such as the United States and Japan, where shrimp consumption is high and cholera is a reportable disease. There are no reports of either outbreaks or sporadic cases of cholera associated with imported shrimp.

5.2.6.2.5 Home and food services

In developing countries, particularly in rural areas, hygienic standards in the kitchen can be low. Though shrimp is generally consumed after cooling, the cooked food may not be consumed several hours after cooking and even sometimes will not be consumed until the next day and then without reheating. Contamination of cooked shrimp from raw shrimp might occur via vessels and containers that are not sufficiently washed between use. The experimental study of Kolvin and Roberts (1982) shows that *V. cholerae* O1 can multiply rapidly in cooked shrimp. In places like Calcutta, ambient temperature could be between 30-40°C. At this temperature, *V. cholerae* O1 can multiply rapidly and reach numbers of 10^{10} in 12 h. Cooked food contaminated with *V. cholerae* O1 and eaten without heating would be expected to have a infective dose of the pathogen.

Even in food catering facilities such as rural restaurants, contamination of cooked shrimp with *V. cholerae* O1 from raw seafood could occur. Mild heating of such stored contaminated food would not totally eliminate *V. cholerae* O1, if multiplication has occurred. The decimal reduction time (D) for *V. cholerae* O1 has been reported to be 2.65 min at 60°C (ICMSF, 1996). If *V. cholerae* O1 has multiplied to a level of 10^{10} cells, the shrimp needs to be boiled for 30 minutes to eliminate the organism.

5.3 Hazard Characterization

5.3.1 Description of the pathogen, host, and food matrix factors and how these impact the disease outcome.

5.3.1.1 Characteristics of the pathogen

5.3.1.1.1 Infectivity, virulence/pathogenicity

V. cholerae is well known for causing the gastrointestinal illness known as cholera, which in its severe form is an illness characterized by the passage of voluminous stools leading to dehydration. If untreated, the resulting dehydration can lead to hypovolemic shock and the death of the patient. Only the toxigenic strains of *V. cholerae* O1 and O139 are considered in this analysis.

5.3.1.1.2 Genetic factors (e.g. antimicrobial resistance and virulence factors).

V. cholerae is known to have several genetic factors related to virulence, but the *ctx* operon, is the primary factor associated with toxigenicity. The *ctx* operon codes for cholera toxin (CT), which is made up of an A and B subunit, and is responsible for the symptoms of cholera. This is the toxin secreted by the toxigenic *V. cholerae* O1 and O139 strains. The O1 serogroup can be subdivided into two main sub-groups, the Ogawa and the Inaba. The strains may be further subdivided into two biotypes: classical and El Tor. The *V. cholerae* O139 toxigenic strain is an El Tor biotype (Kaper, *et al*., 1995 and Faruque *et al*., 1998).

V. cholerae O1 are extremely sensitive to acidic environment. In gastric juice with pH less than 2.4, *V. cholerae* O1 are killed rapidly (Levine *et al.,* 1984; Nalin *et al.,* 1978). Since *V. cholerae* O1 are contacted via oral ingestion, the organisms must pass through the gastric acid of the stomach to colonise the intestine. In normochlorohydric adult volunteers, doses of up to 10^{11} pathogenic *V. cholerae* O1 given without buffer or food did not reliably cause illness, whereas dose of 10^4-10^8 organisms given with 2g of $NaHCO_3$ resulted in diarrhoea in 90% of individuals (Cash *et al.,* 1974). The spectrum of illness with 10^6 organisms was similar to that of cholera. In another volunteer study, doses of 10^5, 10^4 and 10^3 resulted in a 60% attack rate, although the diarrhoeal illness at the two lower doses were milder and appeared to have long incubation period (Levine *et al.,* 1981). The dose response study of Levine *et al.* (1981) is indicated in Table 5.z.

Table 5.4. Clinical response of healthy North American volunteers to various doses of *V. cholerae* E1 Tor Inaba Strain N 16961

Dose[a]	Clinical Mean Attack rate(h)	Mean incubation ill volunteer (range)	Mean diarrhoeal stool vol. per ill volunteer(range)	Mean no. of loose stools per ill volunteer(range)
10^6	9/10[c]	25.5	3.2 litres (0.4-13.1)	12.9 (2-39)
10^5	3/5	18	3.1 (0.4-3.7)	15 (9-21)
10^4	4/5	36.5	1.1 (0.6-1.5)	6.5(4-10)
10^3	4/6	33.3	0.9 (0.4-1.9)	5.8

[a] Volunteers ingested 2 g sodium bicarbonate prior to ingesting inoculum

[b] Number ill/number of volunteers challenged.

5.3.1.2 Characteristics of the host

5.3.1.2.1 Immune-status

The host immune system is the critical host defence mechanism against *Vibrio* spp. infection. The immunocompromised are at special risk for both infection and for more severe sequelae associated with that infection.

5.3.1.2.2 Age, sex and ethnic group

In cholera endemic areas, children in the age group 2-15 are considered most susceptible to cholera. This is due to their experiencing initial infection (Glass *et al*., 1982). These first infections are severe, but rarely are people hospitalised twice for the disease suggesting that immunity is long lasting and protects against severe illness. Where endemic, the second highest number of cases in women of childbearing years 15-35. In developed countries where hygienic standards are high, all age groups are equally susceptible (Kaper, *et al*., 1995).

5.3.1.2.3 Health behaviours

All *Vibrio* spp. are relatively susceptible to inactivation by cooking. Most of the risk associated with the relevant strains of *Vibrio* spp. in food comes from the consumption of raw seafood or from cross contamination of other foods by raw seafood or contaminated water.

5.3.1.2.4 Physiologic status

V. cholerae infection is known to be more severe in individuals suffering from malnutrition. Hypochlorohydria associated with malnutrition, B_{12} deficiency and gastritis predispose to the development of cholera. In endemic areas, only a minority(20-40%) of infections with *V. cholerae* O1 El Tor results in any illness (Sahid e*t al.,* 1984; Bart *et al.,* 1970). Studies from rural Bangladesh indicate that only 11% of persons exposed to *V. cholerae* O1 in household water developed infection and only half of the infected individuals developed illness (Spira *et al.,* 1980). The level of contamination in water ranged from 1-500 cfu/100ml. The minimum infective dose of *V. cholerae* has been estimated to be 10^6-10^8 and this level is reached only in food subjected to time-temperature abuse (WHO, 2000).

Among host susceptibility factors, notable is the association with blood group. Barua and Paguio (1977) and Chaudhuri and De (1977) noted the incidence of cholera in patients with blood group A was lower than that in general population and incidence with blood type O was significantly higher.

5.3.1.2.5 Genetic factors

The likelihood of *V. cholerae* infection progressing to the severe form, cholera gravis appears to depend upon the individual's ABO blood group (Levine *et al*., 1979). Individuals with blood group O appear more likely to exhibit severe diarrhoea. Individuals who are heterozygous for the cystic fibrosis allele are apparently less susceptible to severe cases of cholera. This resistance to cholera of the heterozygote is presented as justification for the prevalence of an allele that causes a fatal genetic disease in the homozygous form, cystic fibrosis. (Rodman and Zamudio, 1991).

5.3.1.3 Characteristics of the food matrix

V. cholerae O1 ingested with food is likely to be protected from gastric acid. In an experimental study, human volunteers ingested 10^6 *V. cholerae* O1 Eltor with 2g $NaHCO_3$ in 300 ml water or in a meal of fish, rice, milk and custard (Levine *et al.,* 1981). Volunteers who ingested *V. cholerae* with water

alone did not become infected, but those who ingested organism in a meal had cholera of similar severity to those who had buffered gastric acidity with $NaHCO_3$ (Levine *et al.,* 1981).

5.3.1.3.1 Fat and salt content

Fat and salt content are probably not relevant in the determination of risk with respect to *Vibrio* spp. However, while the fat content of a matrix may be relevant with respect to the increase of effective dose of pathogens through protection of *Vibrio* spp. in micelles during gastric passage, there is insufficient evidence to model the degree of increased survival.

5.3.1.3.2 pH and water activity

Vibrio spp. appear to be relatively sensitive to both low pH and dehydration. Because of the nature of most foods associated with the unintended consumption of *Vibrio* spp., pH and water activity are probably not relevant in modelling survival of *Vibrio* spp. in raw seafood, however these parameters may be relevant in modelling the growth of *Vibrio* spp. in other foods as the result of cross contamination.

5.3.2 Public Health Outcomes

5.3.2.1 Manifestations of disease

V. cholerae - mild to severe gastrointestinal illness, may cause patient dehydration leading to death.

5.3.2.2 Rational for the biological end points modelled

V. cholerae - gastrointestinal illness is modelled as the endpoint as this is the more common outcome of infection and there are human volunteer data to correlate dose with likelihood of diarrhoea.

5.3.3 Dose-response relationship

5.3.3.1 Summary of available data

5.3.3.1.1 Probability of illness given exposure.

Human volunteer data is available for both sub-type serogroups and both biotypes of *V. cholerae*. Cash *et al.* (1974) have studied classic Inaba and Ogawa strains; Levine *et al*. (1988) and Black *et al*. (1987) have studied El Tor Inaba and Ogawa strains.

5.3.3.1.2 Probability of sequellae given illness.

Many *V. cholerae* infections result in a serious condition called cholera gravis which can be life threatening. There are no specific sequellae associated with the severe form of illness other than the risk of death.

5.3.3.1.3 Probability of secondary and tertiary transmission.

V. cholerae is observed to occur in families; however, it is thought that this is the result of a common primary infection rather than by secondary transmission (Glass and Black, 1992).

5.3.3.1.4 Probability of death given illness.

The probability of death as the result of *V. cholerae* is dependent on the public health infrastructure of the locality where the case of cholera is acquired. In areas where cholera is poorly treated, mortalities range between 20% and 50%. In localities where rehydration therapies are readily available, the mortality rate is less than 1% (Glass and Black, 1992).

5.3.3.2 Dose-response model

Figure 5.3 shows results of the analysis of *V. cholerae* data using the same methods applied to the *V. parahaemolyticus* data (section 2). Other analyses of the data were done by Teunis, *et al*. (1996) and calculated the following parameters in Table 5.5. This issue is also discussed in section 2.2.3.2.

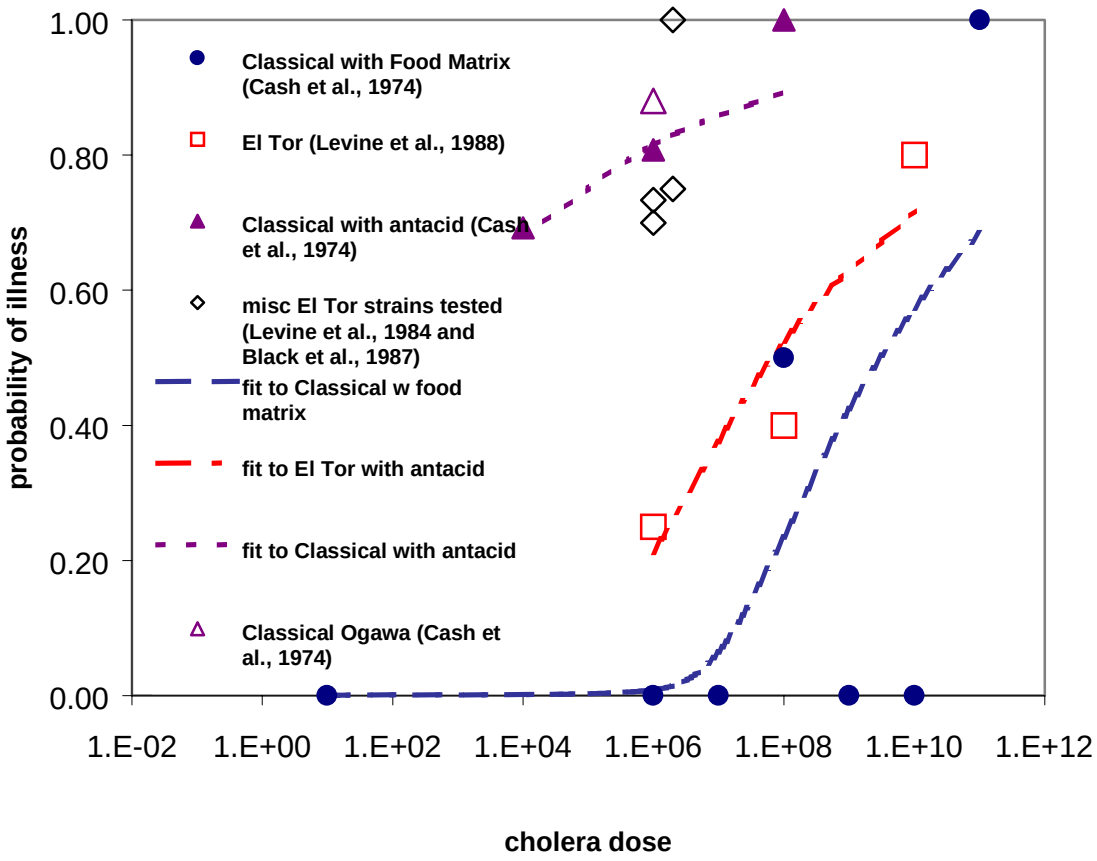

Figure 5.3. Beta-Poisson dose response curves for different strains of *V. cholera*

Table .5.5. Beta-Poisson parameters for *Vibrio cholerae* strains

Strain	Buffer	Alpha	Beta	Deviance	Df	By
classic inaba 569B	no	0.508	7.52×10^7	1.75	5	Teunis *et al.*(1996)
classic inaba 569B	no	0.1312	1.49×10^7	7.36	5	Bowers (2001)
classic inaba 569B	yes	0.164	0.149	0.149	1	Teunis *et al.*(1996)
classic inaba 569B	yes	0.119	0.717	0.48	1	Bowers (2001)
El Tor inaba JBK	yes	0.113	1.38×10^5	0.526	1	Bowers (2001)

The human volunteer trials conducted by Cash, *et al*., 1974 specifically looked at the effect of *V. cholerae* administered with a buffer and with a meat broth. The column in Table 5.5 labelled "buffer" indicates whether the dose was administered with a stomach pH neutralising buffer, "yes", or not "no". Note the shift in the dose-response curve in Figure 5.3 for Classical with food matrix compared to Classical with antacid (Cash, 1974). The values derived by Bowers, 2001 are within the confidence intervals of Teunis *et al*. (1996) and differ slightly because of differences in pooling of severity of illness categories to define cases of illness.

Table 5.6. Uncertainty of Beta Poisson dose-response for *V. cholerae* (Cash *et al*., 1974, classical biotype with food matrix): MLEs of parameters and non-parametric probability of bootstrap resamples.

	Possible resample			**MLEs of parameters**		**Likelihood of resample**		**MLE of Log ID50**		**Deviance of fit to resample**		**p-value of fit to resample**	
boot	x1	x2	x3	x4	x5	x6	x6	alpha	beta				
1	0	0	0	0	0	1	2	1.75E+08	1.07E+18	0.0156	9.63	1.2329	0.9417
2*	0	0	0	0	1	1	2	53.09	9.77E+10	0.0313	9.11	0.5487	0.9902
3*	0	0	0	0	2	1	2	14.66	8.43E+09	0.0156	8.61	2.3954	0.7922
4	0	0	0	1	0	1	2	1.73	2.94E+09	0.0625	9.16	3.8519	0.5709
5	0	0	0	1	1	1	2	0.91	4.46E+08	0.1250	8.71	0.7033	0.9828
6*	0	0	0	1	2	1	2	10.75	3.18E+09	0.0625	8.33	0.5368	0.9907
7	0	0	0	2	0	1	2	0.28	4.66E+07	0.0937	8.69	5.8755	0.3185
8	0	0	0	2	1	1	2	0.51	7.52E+07	0.1875	8.34	1.7501	0.8825
9*	0	0	0	2	2	1	2	8.68	1.36E+09	0.0937	8.05	0.6195	0.9871
10	0	0	0	3	0	1	2	0.28	2.02E+07	0.0625	8.35	8.2791	0.1415
11	0	0	0	3	1	1	2	0.49	3.45E+07	0.1250	8.04	3.4549	0.6302
12*	0	0	0	3	2	1	2	7.34	6.14E+08	0.0625	7.78	1.1706	0.9477
13	0	0	0	4	0	1	2	0.31	1.29E+07	0.0156	8.04	13.2628	0.0210
14	0	0	0	4	1	1	2	0.55	2.28E+07	0.0313	7.77	7.6107	0.1790
15*	0	0	0	4	2	1	2	6.24	2.49E+08	0.0156	7.47	3.2040	0.6686

* unconverged estimates

Resampled Beta-Poisson dose-response parameters were obtained for each of the *V. cholerae* trials in a manner similar to that done for *V. parahaemolyticus*. Only data for *V. cholerae* O1 biotype Classical Inaba is shown.

5.3.3.2.1 Assumptions

The primary assumptions are:

- healthy volunteers responses to oral challenge is representative of the general population.
- the virulence of the pathogens or susceptibility of the host does not vary
- the Beta-Poisson dose-response model is reasonable for use in characterizing risk of illness when consuming *Vibrio* spp.

While these assumptions are not representative of what we believe is the nature of the human dose-response relationship, they form the basis of the first iteration of the hazard characterization. As more information becomes available, these assumptions will be revised to better reflect our understanding of dose-response relationships.

5.3.3.2.2 Goodness of fit of the distribution

The goodness of fit of the distributions for all of the *Vibrio* spp. are uncharacterised as a family of dose-response parameters are used to represent the parameter uncertainty. The deviance for the fitting of the most likely parameters obtained for the *V. cholerae* dose-response data reported by Teunis *et al*. (1996) is 1.75 for *V. cholerae* 569B classical Inaba administered with without a pH buffer and 0.149 for *V. cholerae* administered with a pH buffer.

5.3.3.2.3 Uncertainty and variability in the estimates

This analysis incorporates both uncertainty and variability in the estimates. This issue is discussed in more detail in section 2.3.3.2.4.

5.4 Risk characterization

To be completed in 2002

5.5 Gaps in data

For exposure assessment, the most important data would be numbers of the pathogen in raw foods, seasonal variations in numbers and numbers at retail level in domestic markets. The risk assessment for *V cholerae* O1 is not straight forward due to multiple routes of entry of the organism in the farm to fork model. For example through water, through food handlers etc.. Quantitative data is not available for water, ice, and contamination via carriers or post cooking. Some of the questions that need to be answered before completing this risk assessment are;

(i) What is the level of *V. cholerae* O1 in shrimp cultured in coastal environment? Are the shrimp associated strains toxigenic?

(ii) What is the level of *V. cholerae* O1 in domestic water supply in rural areas in developing countries? Are the water associated strains toxigenic?

(iii) What is the incidence carrier state for *V. cholerae* O1 in shrimp handlers in coastal areas?

(iv) Can *V. cholerae* O1 survive and multiply in the floors of fish landing centres, shrimp peeling sheds and fish markets?

(v) What is the situation with *V. cholerae* O139?, its natural ecology, association with seafood, survival and multiplication abilities?

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