Food and Agriculture Organization of the United Nations

World Health Organization

JOINT FAO/WHO ACTIVITIES ON RISK ASSESSMENT OF MICROBIOLOGICAL HAZARDS IN FOODS

BACKGROUND PAPER

for the

JOINT FAO/WHO EXPERT CONSULTATION ON

Development of Practical Risk Management Strategies based on Microbiological Risk Assessment Outputs

Kiel, Germany, 3-7 April 2006

Case study: *Vibrio vulnificus* in oysters

Prepared by Working Group members:
Angelo DePaola[1], Ron Lee[2], Deon Mahoney[3], Irma Rivera[4], Mark Tamplin[5]

Working draft, February 2006

[1] FDA Gulf Coast Seafood Laboratory, Dauphin Island, AL, USA
[2] CEFAS Weymouth Laboratory, Weymouth, Dorset, UK
[3] Food Standards Australia New Zealand, Canberra, Australia
[4] University of S. Paulo, S. Paulo, Brazil
[5] Coordinator WG5, USDA-ARS-ERRC, Wyndmoor, PA, USA

Disclaimer

This case study was prepared by a group of independent experts as a background paper for an FAO/WHO expert meeting. The views expressed herein are those of the authors and do not necessarily reflect the views of the Food and Agriculture Organization of the United Nations or the World Health Organization. The World Health Organization and the Food and Agriculture Organization of the United Nations do not warrant that the information contained in this publication is complete and shall not be liable for any damage incurred as a result of its use. The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization nor of the Food and Agriculture Organization of the United Nations concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

This material is provided to facilitate transparency and international discussion and further development of microbiological risk management and food safety metrics. It should not in any situation be referenced as the opinion of FAO, WHO or the Codex Alimentarius.

For further information on the joint FAO/WHO
activities on microbiological risk assessment, please contact:

Food Quality and Standards Service
Nutrition and Consumer Protection Division
Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla
I-00100 Rome, Italy

Fax: +39 06 57054593
E.mail: jemra@fao.org

Web site: http://www.fao.org/ag/agn/agns/

or

Department of Food Safety, Zoonoses and Foodborne Diseases
World Health Organization
20, Avenue Appia
CH-1211 Geneva 27
Switzerland

Fax: +41 22 7914807
E.mail: foodsafety@who.int

Web site: http://www.who.int/foodsafety

Acknowledgements

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) would like to express their appreciation to all those who contributed to the preparation of this report through the provision of their time, expertise, data and other relevant information.

In addition the Working Group would like to acknowledge the generous contributions of John Bowers and Marc Glatzer of the US Food and Drug Administration.

Contents

1. BACKGROUND

1.1 Task and Approach of the Working Group

The Codex Committee on Food Hygiene (CCFH) has requested that the Food and Agriculture Organization of the united Nations and the World Health Organization (FAO/WHO) provide scientific advice on how Performance Objectives (PO) and Performance Criteria (PC) can be utilized to achieve Food Safety Objectives (FSO). The ultimate goal is to produce a reference document that will assist CCFH and countries in using risk assessment to develop risk management measures.

This Working Group was assigned the task of interpreting data and information on *Vibrio vulnificus* illness associated with the consumption of oysters, including the FAO/WHO Risk Assessment of *Vibrio vulnificus* in Raw Oysters (FAO/WHO, 2005), and transforming it into practical guidance that can be used by risk managers to elaborate potential risk management options. Such guidance involves knowledge of the dynamics of pathogen growth, survival and inactivation in order to establish scientifically defensible POs and PCs at specific points within the food chain, as well as to identify potential microbiological, process and product criteria.

The form of this guidance may include advice on public health goals and targets and how they can be translated into FSOs that articulate tolerable levels of pathogens in oysters at the time of consumption. It is also important to consider the levels of these pathogens at key points along the supply chain and to describe POs and PC at these steps. Such information will assist risk managers in seafood industries and in regulatory agencies. The output should also consider the issue of uncertainty surrounding the results of the risk assessment and how risk managers may address such uncertainty.

The working group has reviewed the key papers (see Bibliography) and drawn upon the expertise of the members in drafting this report. The need for specific additional expertise was identified and was addressed by personal contact with selected experts from the shellfish industry and Government during the preparation of this document.

The approach taken was to demonstrate how risk assessment can be applied to developing hypothetical risk management practices for generic oyster production and processing operations consistent with operations in the Gulf Coast of the United States (USA).

1.2 Background on Hazard-Commodity Pair

1.2.1 Disease

The bacterium *Vibrio vulnificus* is a natural inhabitant of warm estuarine waters. Presently, three biotypes have been identified associated with infections of human and marine animals. Biotype 1 is typically isolated in human cases resulting from seafood consumption; Biotype 2 is associated with infections of cultured eels; whereas Biotype 3 is limited to human wound infections associated with handling aquacultured fish in Israel (Bisharat et al., 1999; Bisharat and Raz, 1997). The risk of infection is linked to high levels of *V. vulnificus* in the environment and in shellfish that are eaten raw or mildly cooked.

V. vulnificus can infect humans through wound exposure or seafood consumption. Infections are rare, and generally limited to the immunocompromised or to individuals with pre-existing chronic illnesses *e.g.* liver disease, chronic alcohol use, cancer, diabetes mellitus (Hlady and Klontz, 1996; Strom and Paranjpye, 2000). In such individuals, the organism causes a mild to severe gastrointestinal illness, potentially passing through the intestinal wall into the bloodstream and causing septicaemia.

The foodborne route of infection is associated with a high mortality rate exceeding 50% (Mead et al., 1999). In the USA, 30-40 cases of septicaemia are reported annually (Mead et al., 1999), and almost all cases are associated with the consumption of raw oysters harvested from waters of the Gulf Coast states (Texas, Louisiana, Mississippi, Alabama and Florida) during the warmer weather months. The risk of infection has been linked with high environmental levels and rapid growth of the pathogen in oyster shellstock held without adequate refrigeration (Hlady and Klontz, 1996).

Vibrio vulnificus is an autochthonous bacterium of estuarine environments and is often present in waters that meet sanitary standards for the harvest of oysters (Tamplin et al., 1982). Two large environmental surveys of *V. vulnificus* levels in oysters and seawater have shown that temperature and salinity are the primary environmental factors that influence *V. vulnificus* growth and survival, thereby affecting the likelihood of illness for consumers (Motes et al., 1998; Tamplin, 1994).

As *V. vulnificus* illness is usually severe, and is relatively easy to diagnose as the organism grows readily on ordinary laboratory media, it is unlikely to go undetected in countries with good medical and laboratory facilities. While *V. vulnificus* illness has been frequently reported in the USA, it is apparent that infections occur in other parts of the world. In particular, it is becoming clear that the occurrence of *V. vulnificus* infection, including that associated with the consumption of shellfish, is more common in some Asian countries than previously thought (Chuang et al., 1992; Park et al., 1991). The infection appears to be relatively rare in Europe and Australasia. Within the Nordic and Low Countries of Europe, occasional wound infections have been reported but not primary septicaemia associated with shellfish consumption (Dalsgaard et al., 1996; Mertens et al., 1979; Veenstra et al., 1993).

1.2.2 Ecology

Vibrio vulnificus can grow at temperatures of 13°C and above, but maximum concentrations in the environment occur when water temperature is in the range of 20 and 30°C (Kaspar and Tamplin, 1993; Motes et al., 1998; Tamplin, 1994). Increased levels of nutrients, suspended solids and plankton have also been identified as having a positive correlation with levels of *V. vulnificus* (Montanari et al., 1999). While the organism will tolerate salinities of up to 60 parts per thousand (ppt), more rapid growth occurs between 5 and 25 ppt.

The organism has been reported in relatively high concentrations in the marine environment in the USA and Asia (Amaro and Biosca, 1996; Arias, Garay and Aznar, 1995, 1998, 1999; Chuang et al., 1992; Park et al., 1991). Isolations at a lower frequency and concentration have been reported from parts of Europe. The organism has also been found in cultured finfish in Israel (Bisharat and Raz, 1997).

1.2.3 Effect of interventions

The application of various microbial inactivation techniques to oyster shellstock has shown that *V. vulnificus* is more sensitive than other *Vibrio* spp. and most foodborne pathogens. For example, treatment of shellstock at 50°C for 5 minutes has been shown to result in a 6 log decrease in *V. vulnificus* levels in oyster meats (Cook and Ruple, 1992). Similar reductions have been achieved by application of irradiation (Ama, Hamdy and Toledo, 1994), high pressure processing (Berlin et al., 1999), and freezing oysters at -40°C followed by storage for 3 weeks (Cook and Ruple, 1992).

Depuration at ambient temperatures has been shown to be ineffective in reducing *V. vulnificus* levels since this organism resides deep within oyster tissues (Harris-Young et al., 1993; Tamplin and Capers, 1992), however relaying oysters to high salinity waters greater than 32 ppt has been shown to reduce *V. vulnificus* numbers by 3 to 4 logs within 2 weeks (Motes and DePaola, 1996).

1.2.4 Current risk management strategies

Worldwide, few countries have implemented risk management practices to control *V. vulnificus* levels in oysters. In New Zealand, a qualitative risk assessment concluded that the local high salinities were not conducive for *V. vulnificus* survival, plus there was insufficient epidemiological evidence to indicate that *V. vulnificus* was a significant foodborne disease (McCoubrey 1996). The European Commission's Scientific Committee on Veterinary Measures relating to Public Health prepared a qualitative risk assessment on *V. vulnificus* and *V. parahaemolyticus* in raw and undercooked seafood, and concluded that current available data do not support setting specific standards or microbiological criteria for pathogenic *V. vulnificus* (European Commission, 2001). However, in the latter report it was recognized that some management strategies might prove effective at reducing *V. vulnificus* levels, including proper refrigeration temperatures during harvest, processing, transport and storage.

In the USA, the National Shellfish Sanitation Program (NSSP) has implemented control measures to reduce post-harvest levels of *V. vulnificus* (USDHHS, 1999). Specifically, states that have previously reported two or more human *V. vulnificus* infections must limit the time between oyster harvest and the first refrigeration step. For example, during summer months when ambient air temperature is greater than 28°C, oysters must be placed under refrigeration within 10 hours of harvest.

In other countries where *V. vulnificus* disease occurs, its full impact is not realized due to other infrastructure priorities. For example, epidemiological resources may be targeting well defined and more significant illnesses, and not opportunistic organisms such as *V. vulnificus* that affect many fewer persons. In such cases, it is not unexpected that these countries do not have risk management practices for shellfish-borne *V. vulnificus* disease.

2. PROCESS

2.1 Oyster Production, Harvesting and Marketing

A generic oyster production and processing flow chart applicable to different countries is shown in Figure 1. It identifies the major steps from oyster production to placing product in the marketplace, and indicates factors (Key Inputs) that influence *V. vulnificus* incidence, growth, survival and inactivation at different process steps.

Conditions in the harvesting area that may influence *V. vulnificus* numbers depend on the location (coastal/estuarine), seasonal effects and pre- and at-harvest meteorological conditions. Harvesting practices and post-harvest transport, storage, processing and marketing conditions will depend on both local and destination market practices and requirements, and differences will occur depending on the species of oyster, whether shucking is practiced (and if so, whether this takes place at sea), and where additional steps such as relaying or depuration are undertaken. From a risk assessment perspective, actual practices and conditions may include those that are without local (if applicable) or destination market hygiene control requirements.

In Australia there are two main species of cultured oysters: the Sydney rock oyster (*Saccostrea glomerata*) and the Pacific oyster (*Crassostrea gigas*). Sydney rock oysters are grown in estuarine and riverine habitats, with the most popular method of culture involving sticks and trays. Pacific oysters are grown on plastic baskets on intertidal racks, using deep-water raft culture, or in mesh bags on longlines. Oysters are not in contact with the marine substrate.

Management of the sanitary status of shellfish in Australia involves the classification of growing waters on the basis of a sanitary survey and an on-going water sampling program. Oyster harvest is also managed according to rainfall events - typically oysters are not harvested when the salinity falls below about 23 ppt. Depuration and relaying is practiced in some States before oysters are marketed for human consumption. The Sydney rock oysters are transported from the farm live in the shell, and may survive out of water at ambient temperatures for 2-3 weeks at 8-15°C while Pacific oysters are refrigerated around 4°C and have a shelf-life of 7-10 days (Nell, 2001).

In other countries, there can be different processing schemes based on market infrastructure. For example, in Brazil there are two processes: small scale with process nodes for harvest, transportation, and distribution; and large scale processes that also include post-harvest depuration and ozone treatments.

Figure 1 seeks to represent the generic steps in the production of oysters for human consumption. Differences occur depending on the region, the species of oyster, the nature of production operations, if oysters are shucked at sea, and where additional steps such as relaying or depuration are imposed.

Figure 2 shows an oyster production and processing flow diagram for sub-littoral Gulf Coast oysters (*Crassostrea virginica)* in the USA. The production of Gulf Coast oysters has been significantly associated with the incidence of *V. vulnificus* infection in susceptible consumers and is considered in more detail in later sections of this report. The flow chart includes the consideration that post-harvest processing control measures (Key Inputs) may be applied to certain steps in production in order to reduce the risk of *V. vulnificus* infection.

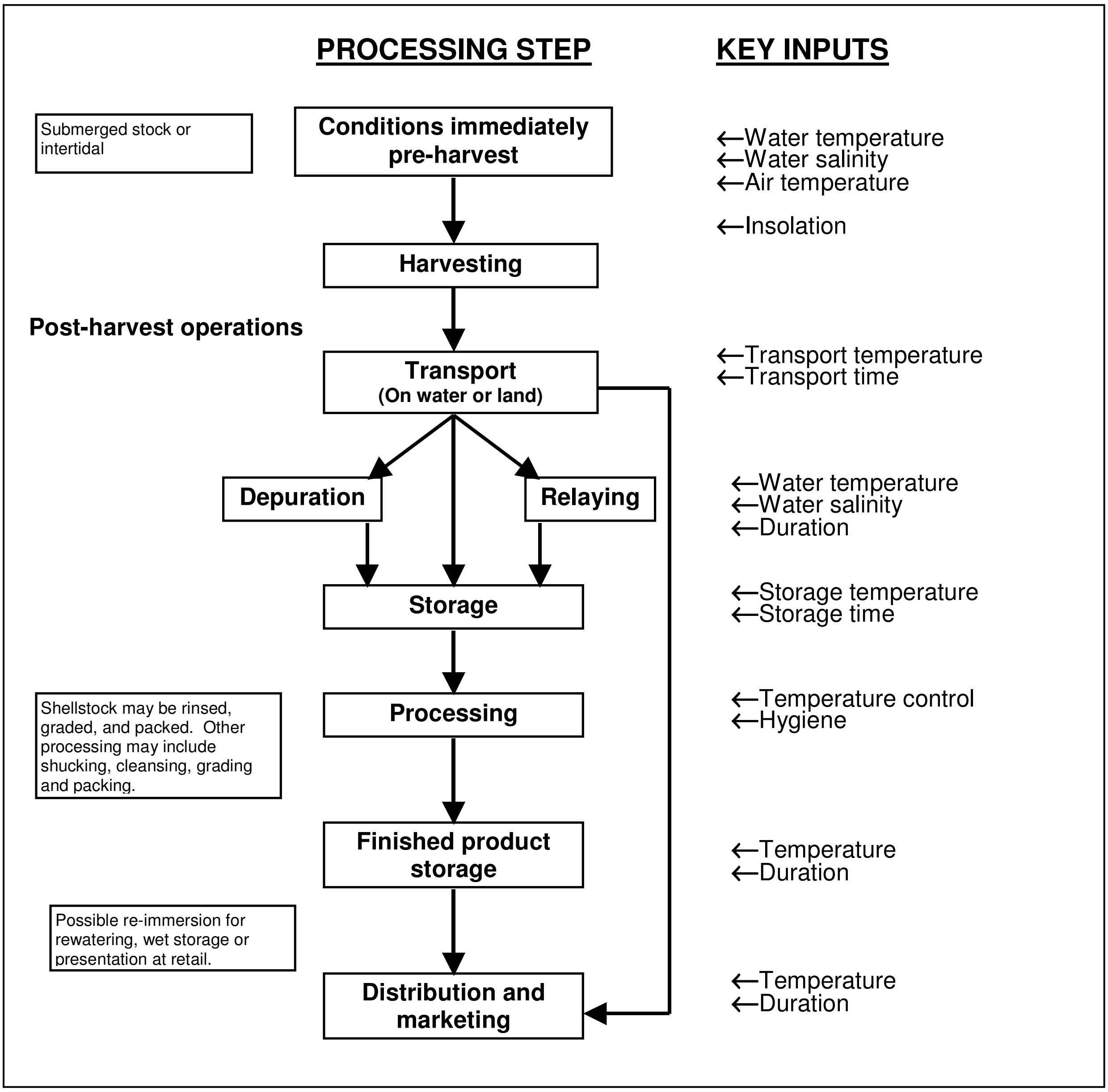


Figure 1. Oyster Production, Harvesting and Marketing Flow Chart

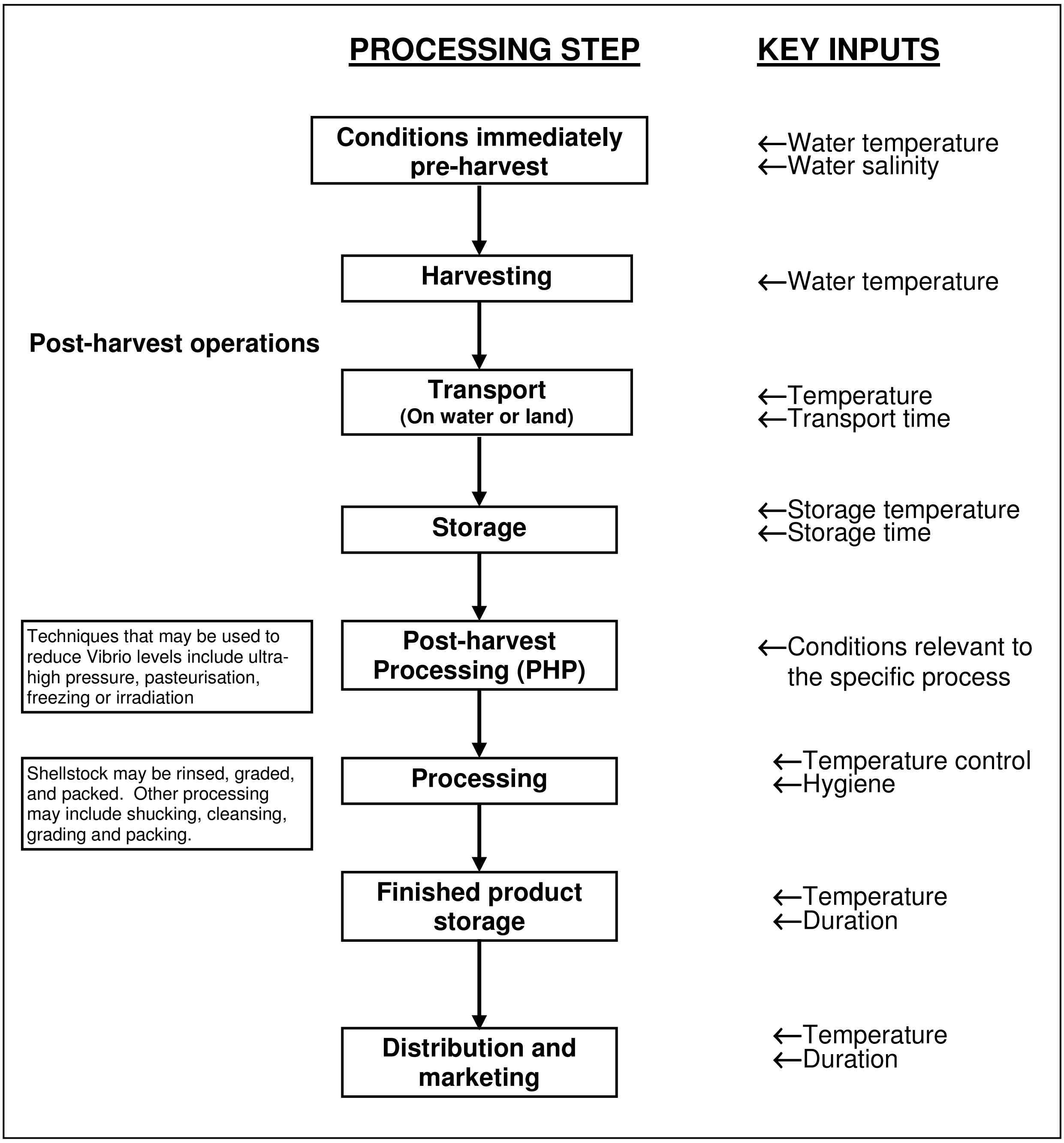


Figure 2. Gulf Coast Oyster Production, Harvesting and Marketing Flow Chart

3. AVAILABLE RISK ASSESSMENT MODELS AND OTHER RELEVANT DATA

Prior to the work of the FAO/WHO risk assessment team, there have been previous qualitative risk assessments on *V. vulnificus*. McCoubrey (1996) reported on the risk of *V. vulnificus* infection following consumption of raw commercially harvested oysters from the North Island of New Zealand. The report concluded that local environmental conditions, especially high salinities, were not suitable for *V. vulnificus* survival and that the risk would be extremely low. This conclusion was corroborated by a lack of epidemiological evidence of foodborne related cases of *V. vulnificus* illness in New Zealand.

Similarly, the European Commission's Scientific Committee on Veterinary Measures relating to Public Health prepared a document on *V. vulnificus* and *V. 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. In this assessment they concluded that "Currently available data do not support setting specific standards or microbiological criteria for pathogenic *V. vulnificus* and *V. parahaemolyticus* in seafood." However, the report recognized potential interventions including 1) prohibiting harvest of shellfish from areas with environments conducive to high levels of *V. vulnificus*, 2) rapidly cooling of shellstock after harvest and subsequent temperature control under 10°C, 3) heat treatment and 4) irradiation.

As neither of the above risk assessments was quantitative, it was decided to extend the *V. parahaemolyticus* models described in the USA Food and Drug Administration "Quantitative Risk Assessment on the Public Health Impact of Pathogenic *V. parahaemolyticus* in Raw Oysters" (US FDA, 2005) and the Joint FAO/WHO "Risk Assessment of *V. parahaemolyticus* in Raw Oysters" (FAO/WHO, 2002) to consider the risk associated with *V. vulnificus* in oysters. The general approach and many of the parameters and assumptions in the "Risk Assessment of *Vibrio vulnificus* in Raw Oysters" (FAO/WHO, 2005) are the same as those in the draft Joint FAO/WHO "Risk Assessment of *V. parahaemolyticus* in Raw Oysters" (FAO/WHO, 2002).

The FAO/WHO "Risk Assessment of *Vibrio vulnificus* in Raw Oysters" (FAO/WHO, 2005) establishes a relationship for predicting *V. vulnificus* levels at harvest based on seasonal and yearly variations of water temperature. Industry data representative of storage times and temperatures were used to predict growth and survival of *V. vulnificus* post-harvest. These data along with surveyed oyster consumption patterns were used to determine exposure to *V. vulnificus*. Predicted exposure levels were validated using data from a national market survey of *V. vulnificus* in raw oysters. Hazard characterization relied primarily on a dose-response relationship derived from the seasonal relationship of predicted exposure and reported illness frequencies.

Most of the available data on levels of *V. vulnificus* in shellfish are from USA Gulf Coast environments and shellfish, and this was used in the modelling. In the USA, *V. vulnificus* levels in oysters and oyster-associated cases of illness follow a seasonal trend, with approximately 90% of illnesses occurring during the months of April to October. A schematic diagram of the *V. vulnificus* risk assessment model is shown in Figure 3.

The FAO/WHO *V. vulnificus* risk assessment focuses on modelling risk based on current production and management practices, and the output of the assessment includes a dose-response curve, describes the impact of typical seasonal conditions on numbers of *V. vulnificus* per gram of oysters and calculates the predicted number of cases based on seasonal estimates of mean risk per serving. Table 1 provides an example of the outputs.

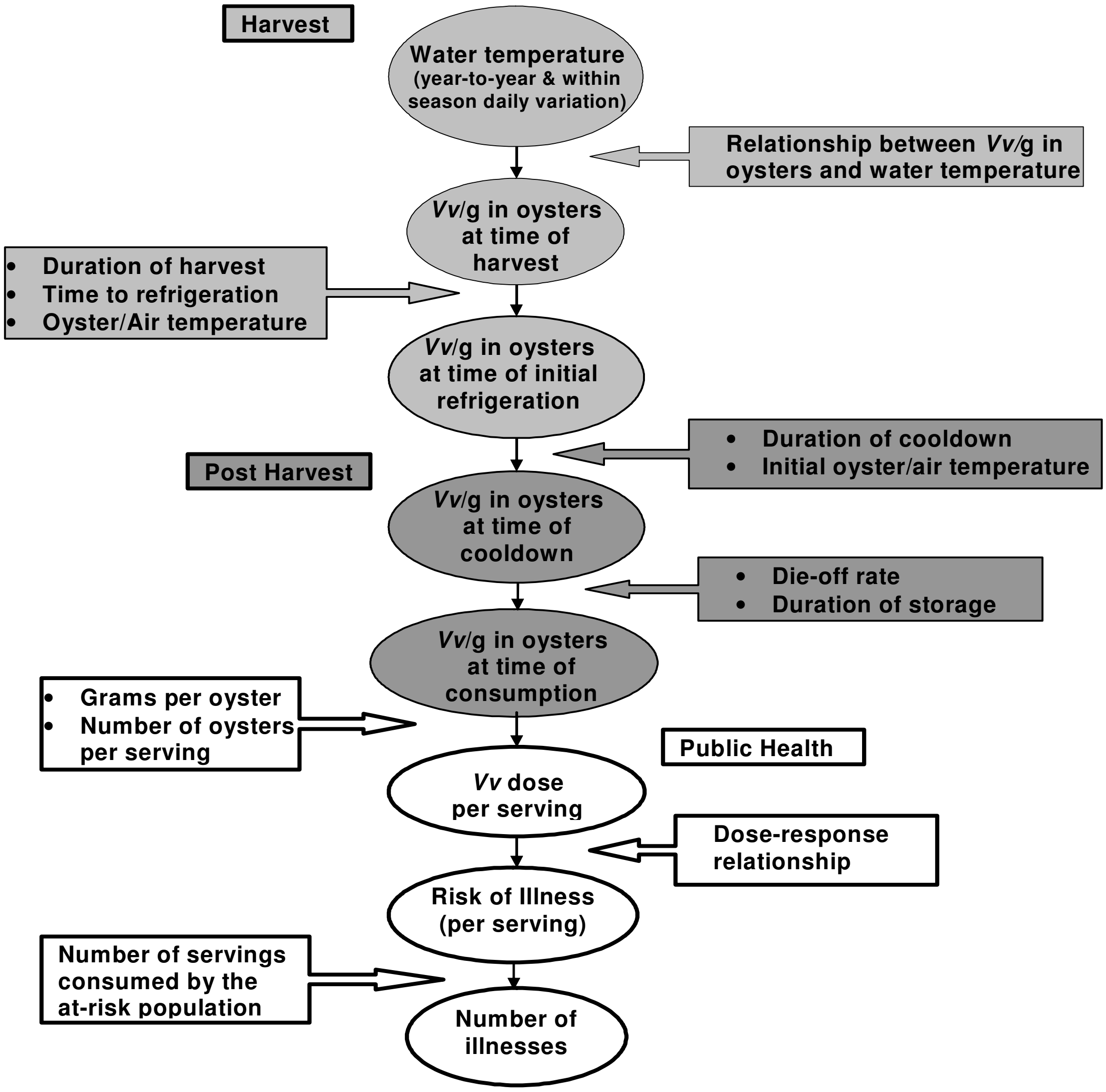


Figure 3. Risk Assessment Model for USA Gulf Coast Oysters

Table 1. Summary of seasonal predictions of mean number of cases based on the FAO/WHO *V. vulnificus* risk assessment model (FAO/WHO, 2005).

Season	*V. vulnificus per* gram at harvest	*V. vulnificus* per gram at consumption	Predicted mean number of cases
Winter (Jan-Mar)	40	80	0.5
Spring (Apr-June)	2,600	21,400	11.7
Summer (July-Sept)	5,600	57,000	12.2
Autumn (Oct-Dec)	500	3,700	8.0
Total			**32.4 (25.9-42.8)**[1]

[1] 90% uncertainty intervals

Modelling assumptions included: 50 percent of the oysters landed are consumed raw; seven percent of oyster meals are consumed by at-risk consumers, and the mean meal size is 196 grams per serving.

These data demonstrate the seasonal variation in the predicted number of cases of illness, and an approximate 1 log increase in MPN/gram increase in *V. vulnificus* numbers at post-harvest during the summer season.

In the FAO/WHO *V. vulnificus* risk assessment, the effects of three arbitrary FSOs on predicted annual number of cases were considered. For a scenario with an endpoint criterion of <3 Most Probable Number (MPN)/gram, candidate technologies included mild heat (50°C) treatment, freezing with extended storage, and high hydrostatic pressure. These interventions would reduce illness to a mean of 0.16 predicted annual cases. If the target was increased to 30 and 300 MPN/gram, the mean annual number of cases would be 1.2 and 7.7, respectively.

The risk assessment also examined the impact of varying the time from harvest to first refrigeration (0 to 20 hours) and found that the range of expected annual cases ranged from 17.7 to 59.3. This illustrated that this intervention alone would not be effective for achieving very low annual frequencies of illness. It was pointed out that in 1996, the NSSP implemented a new practice, specifying that oysters be refrigerated within 10 hours of harvest. A survey of industry refrigeration practices before and after the 1996 intervention program showed there was only a small improvement (0.14 hour) in the mean time to refrigeration, reflected in an estimated 1-2 fewer *V. vulnificus* cases per year.

Finally, the FAO/WHO *V. vulnificus* risk assessment addressed the effects of harvesting oysters from only high salinity (>30 ppt) waters. However, there were significant data gaps about the growth of *V. vulnificus* in high salinity oysters that could influence the estimated exposure levels at consumption. Nevertheless, assuming that all oysters were harvested from such high salinity waters, the estimated incidence would be greatly reduced to <1 case per year.

4. APPLICATION OF MICROBIOLOGICAL RISK ASSESSMENT IN DEVELOPING RISK MANAGEMENT OPTIONS

The output of the risk assessment demonstrates that the risk of illness following consumption of USA Gulf Coast oysters can be reduced by the application of various control measures. For the risk manager, the challenge is to use risk mitigation measures that are realistic, achievable and have a benefit that exceeds the cost.

An important early step is to establish the appropriate level of protection (ALOP). This could lie anywhere between the status quo (approximately 30-40 USA cases/annum) and zero cases per annum. These represent the two extremes. With this in mind, the working group examined the current USA situation and made judgements about what would need to be done if the ALOP was:

A 75 percent reduction in the number of cases of Vibrio vulnificus illness following the consumption of raw oysters

There are a number of points in the supply chain where interventions to reduce *V. vulnificus* numbers and the potential risk to consumers could be applied effectively. The data generated during the FAO/WHO *V. vulnificus* risk assessment assists in identifying the most effective and appropriate of these risk management measures. These measures will be addressed sequentially along the oyster harvest and processing chain and the impact of interventions will be considered in terms of the reduction in risk.

4.1 Oyster Harvest

The first point of control is at harvest. In general, the risk of oyster-associated *V. vulnificus* illness is extremely low when water temperature is below 20°C or if salinity is greater than 30 ppt. These conditions, separately and together, are known to occur in certain geographical areas where molluscan shellfish are produced and if these areas and times of the year were identified, risk managers could minimize restrictions for these oysters. For example, if *V. vulnificus* is shown not to grow in high salinity oysters, then post-harvest temperature controls could be eased and this would reduce industry operating costs. However, this potential intervention must also consider effects on other pathogens able to grow at high salinities, such as *V. parahaemolyticus.*

Restricting oyster harvest during high-risk months is an obvious means of preventing illness, but this has a major impact on trade and the livelihood of oyster producers and related sectors. In the mid 1990’s, the FDA proposed a total ban on harvest of Gulf Coast oysters from May through October but this was rejected by the Interstate Shellfish Sanitation Conference (ISSC). This was a clear example of circumstances where economic matters took precedence over public health considerations. Nevertheless, restricting harvest to the cooler months of the year would reduce illnesses. For example, restricting harvest from October to March would decrease annual cases from 32.4 to 8.5, a 74 percent reduction in risk (Table 1).

4.2 Post-harvest Handling

Risk managers in the oyster industry may be more receptive to risk mitigation measures rather than a total ban on harvest during high-risk months. Such measures include controls over post-harvest handling, post-harvest processing (PHP), and labelling which recommends cooking and/or education programs for at-risk consumers.

If temperature and salinity are favourable for *V. vulnificus* growth, the first point in the process chain for intervention would be post-harvest storage. In this regard, levels of *V. vulnificus* depend on the ambient temperature and the time to reduce oyster temperatures to levels that prevent growth of

V. vulnificus (<13°C). While ambient temperature cannot be controlled, other options would be to harvest at cooler times of the day (*i.e.* early morning and at night) and to rapidly cool shellstock immediately after harvest.

Currently a requirement in the USA is to place oysters under refrigeration within 10 hours of harvest during summer months for states that have reported two or more cases of *V. vulnificus*. Figure 4 illustrates the relative reduction in risk based on the time from harvest to initial refrigeration of shellstock. Using conventional refrigeration, the time to reach a shellstock temperature of <13°C will vary between 1-10 hours. In contrast, placing shellstock in an ice slurry can reduce the time to less than 1 hour. For example, the latter process intervention reduces the risk per serving by approximately 50 percent, while icing within 4 hours of harvest achieves an approximate 30 percent reduction in risk. Approximately 12 cases could be prevented if icing occurred within 1 hour of harvest in the spring and summer, or about 33% of the annual cases. While this does not achieve the ISSC stated ALOP of a 60% reduction, it only leaves 27% of the reduction to PHP and education in order to reach the target level.

While there is a requirement to place oysters under refrigeration (7°C) within 10 hours, there is no guidance on cooling rates and the risk assessment assumes that it takes 1-10 hours for oysters to reach at least 13°C. Increasing the cooling rate to achieve an oyster temperature of less than 13°C more rapidly would substantially reduce *V. vulnificus* growth and subsequent risk. Various cooling regimes and scenarios could be modelled, and enhanced industry education would be most valuable.

Because of extreme year-to-year variations in climate in recent years, it may be appropriate to consider water temperature instead of month as the trigger for interventions. Most of the earth's surface is scanned daily by satellite imagery and water temperatures can be accurately determined in 1 kilometre grids. Because the data are in digital form, it is relatively straightforward to integrate sea surface temperature data into the risk assessment model and predict *V. vulnificus* densities in oysters and the risk of illnesses from their consumption in real time.

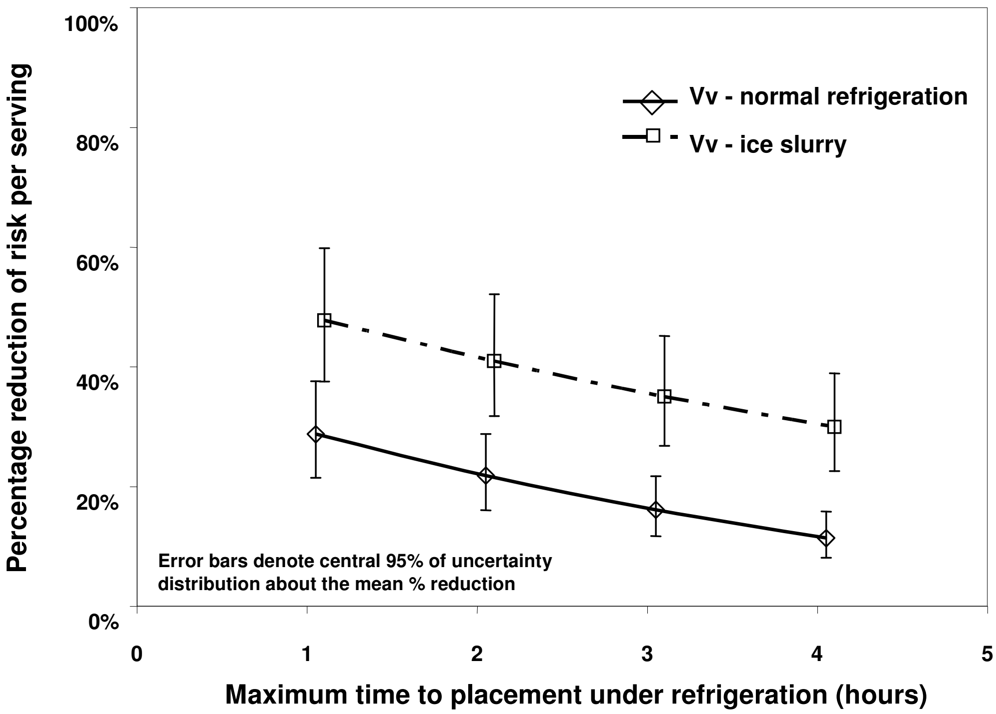


Figure 4. Predicted effectiveness of rapid cooling on risk per serving for summer harvested Gulf Coast Oysters

Examples of how water temperature imagery can be utilised is provided by the Naval Research Laboratory (Stennis Space Center, Bay St. Louis, Mississippi). The following graphics illustrate conditions and *V. vulnificus* levels on 18 September 2004 and the risk to consumers under current handling practices. Figure 5 illustrates the standard sea surface water temperature for the Mississippi and Alabama coasts. Figure 6 provides an approximation of *V. vulnificus* density after water temperature has been integrated to predict *V. vulnificus* levels using the FAO/WHO *V. vulnificus* risk assessment model. Figure 7 shows *V. vulnificus* levels at the time of consumption under the 10 hour refrigeration requirement, and Figure 8 indicates the log mean risk when oysters are iced within 1 hour.

Figure 5: Water temperature

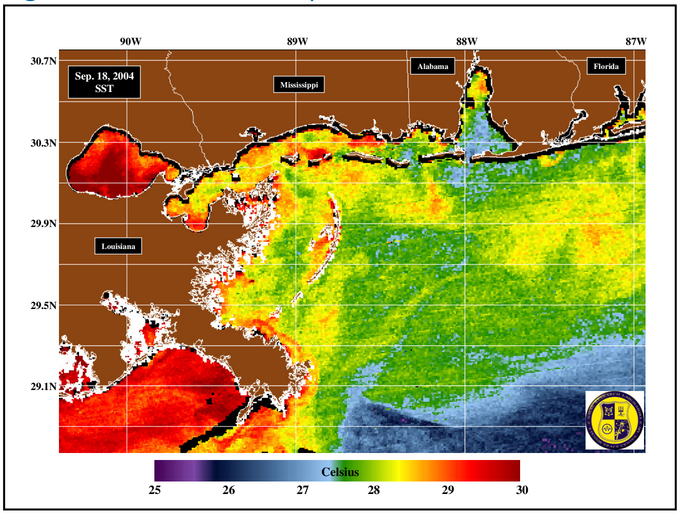


Figure 6: *V. vulnificus* baseline levels

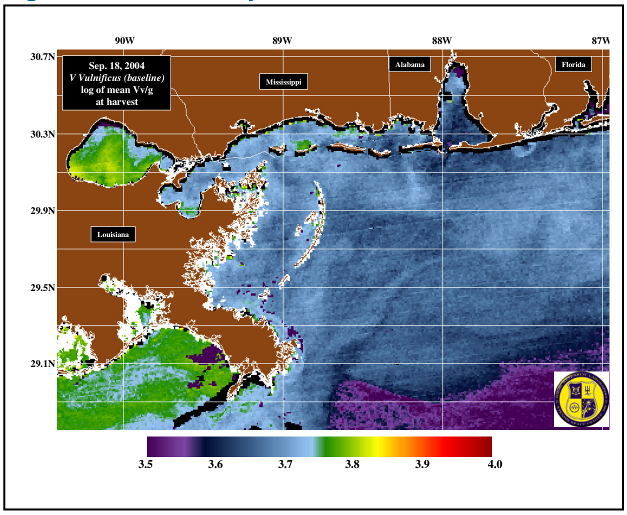


Figure 7: *V. vulnificus* levels at time of consumption

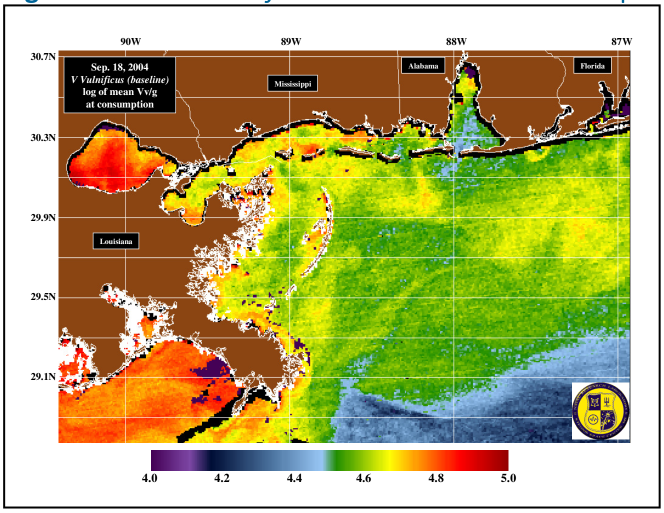
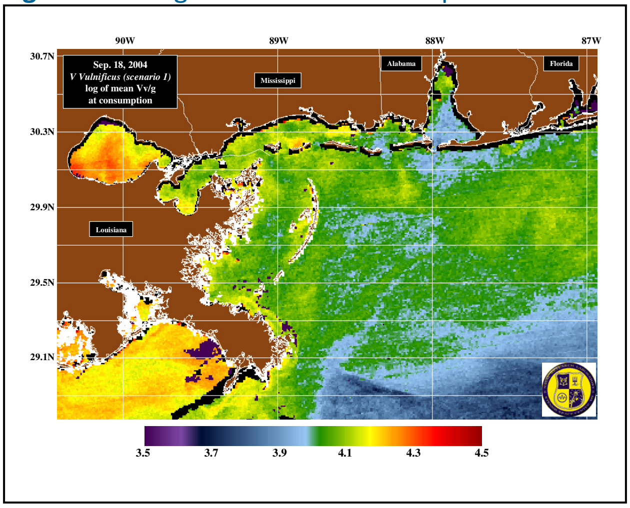


Figure 8: Log mean risk at consumption



4.3 Post-harvest Processing (PHP)

V. vulnificus is sensitive to most inactivation techniques used in food processing, hence there are several validated post-harvest processing (PHP) methods for reducing the *V. vulnificus* load in oysters to non-detectable levels. They include heating (pasteurization) at 50°C, freezing with extended frozen storage and high hydrostatic pressure.

Depuration for short periods, *e.g.* 24 hours, may reduce faecal coliform levels, but is ineffective for reducing *V. vulnificus* and may actually result in large increases if the system is not functioning properly or refrigerated (Tamplin and Capers, 1992). Relaying to high salinity waters for 2 weeks has been shown to be effective in reducing *V. vulnificus* levels by several logs and may be a suitable option in some geographical areas (Motes and DePaola, 1996). More work would be required to determine its efficacy.

A range of other PHP measures have been proposed. For example, a mild heat treatment of 50°C for 5 minutes will yield a 6-log reduction in *V. vulnificus* levels in shucked oyster meats (Cook and Ruple, 1992). Freezing oysters at -40°C and storage for 3 weeks achieves a 4- to 5-log reduction in the *V. vulnificus* population (Cook and Ruple, 1992). However, the effectiveness of freezing may be reduced in cases where cells are subjected to a cold adaptation step. Similar reductions can be readily achieved by irradiation (Ama, Hamdy and Toledo, 1994) and using high hydrostatic pressure (Berlin et al.,1999). While these methods are effective in reducing *V. vulnificus* levels, only a very small proportion of the harvest is currently being processed by these methods. Nevertheless, as technologies become more widespread and cost-effective, such PHP methods may be valuable interventions to reduce *V. vulnificus* risk to less than one case per year.

Effective post-harvest temperature controls and the establishment of a performance criteria that PHP must achieve at least a 4-log reduction of *V. vulnificus* would enable most oysters to meet a microbiological limit of less than <3 MPN *V. vulnificus*/gram, and therefore a reduction in illness to a mean of 0.16 annual cases. Cooking would also achieve at least a 4-log reduction in *V. vulnificus* levels and would essentially eliminate illnesses if it was universally applied during restricted periods.

4.4 Oyster Consumption

The final point for intervention is at the point of consumption. Consumer education has been emphasized by the ISSC but is difficult to model without knowing how it has affected behaviour. It is reasonable to assume that illnesses will be reduced proportionally by each meal of raw oysters avoided by high-risk consumers.

In 1999, the ISSC adopted an illness reduction plan to reduce the level of illnesses by 60% in seven years. The focus has been on education of high-risk consumers and increasing the capacity for PHP to reduce *V. vulnificus* to non-detectable levels. The target level was an end point criterion of <3 MPN/gram. Unfortunately, reducing *V. vulnificus* to <3 MPN/gram was impractical for much of the industry.

Table 2. Reported cases of seafood-related *V. vulnificus* septicaemia in the USA

Year	Cases	Year	Cases	Year	Cases
1995	27* (32)	1999	36 (37)	2003	33 (34)
1996	33 (35)	2000	30 (30)	2004	32 (37)
1997	20 (23)	2001	40 (41)	2005	11 (13)**
1998	40 (42)	2002	35 (35)		

* M. Glatzer (personal communication) – number of cases reported by the ISSC. Excludes cases involving consumption of only clams, cases where type of shellfish consumed is unknown, and cases involving self-harvest. Numbers in parentheses are the total number of cases in the FDA database.

** Partial results for 2005

4.5 Approaches to achieve an ALOP

While USA Gulf Coast oysters can be harvested and consumed safely for a limited period each year, for much of the year post-harvest interventions are needed to reduce the risk to consumers.

In setting FSOs, the working group considered a series of ALOPs. These ranged from the status quo (approximately 26-43 cases) to less than one case per annum (Table 3).

In the scenarios that only involved a seasonal harvest control, a FSO/PO/PC was not proposed because *V. vulnificus* levels are based on historical meteorological trends in the USA that may substantially vary with year. However, it is still possible to develop FSOs from these environmental data, *e.g.* setting maximum seawater temperature criteria for specific harvesting months.

Eliminating summer harvest (July-September) would reduce the number of cases from a mean of 32.4 to 20.2 (an approximate 38 percent reduction), while banning harvest for the period April through September results in 23.9 fewer cases (an approximate 74 percent reduction). Harvest restrictions can be extended to other combinations of months to achieve a desired risk reduction. Requiring all oysters harvested outside of a 'safer' period to be labelled as requiring cooking could increase the impact of such measures.

A related approach would be to restrict harvest to periods with specific maximum seawater temperatures, rather than months. Figure 9 shows the percent reduction in the number of illness (from baseline) and Figure 10 displays the resulting percent of annual harvest that would not be available for raw consumption. For example, by applying a limit of 20°C to all four seasons and then aggregating the results on an annual basis, restrictions at 20°C would prevent 96.3 % of illnesses and affect 50.9% of the harvest.

Table 3. Impact of control measures on the Appropriate Level of Protection (ALOP)

Oyster production	**Post harvest handling**	**Post harvest processing** (PHP)	**Oyster consumption**	**FSO**	**ALOP** (mean no. cases/annum)
Harvest all year	No change[1]	No change	No change	Not established[2]	32.4[3]
Harvest only Oct-Jun	No change	No change	No change	Not established	20.2 (37.8)[5]
Harvest all year	Rapid chilling (<1 hour on ice)	No change	No change	Not established	16.2 (50.0)
Harvest only Oct-Mar	No change	No change	No change	Not established	8.5 (73.8)
Harvest all year	No change	<300/gram	No change	<300/gram[4]	7.7 (76.2)
Harvest only Oct-Mar	Rapid chilling (<1 hour on ice)	No change	No change	Not established	4.3 (86.7)
Harvest limited to seawater <20°C	No change	No change	No change	Not established	1.2 (96.3)
Harvest all year	No change	<30/gram	No change	<30/gram[4]	1.2 (96.3)
Harvest all year	No change	<3/gram	No change	<3/gram[4]	0.16 (99.5)

[1] No change indicates that practices and *V. vulnificus* levels would be the same as those described in the FAO/WHO *V. vulnificus* risk assessment (Table 9) and vary by season.
[2] No FSO was established because it assumes that *V. vulnificus* levels would be based on the exposure levels described in Table 9 of the FAO/WHO *V. vulnificus* risk assessment.
[3] Mean number of cases is based on data in Table 1.
[4] Assumed no growth after PHP.
[5] Values in parentheses are percent reduction compared to a mean of 32.4 annual cases.

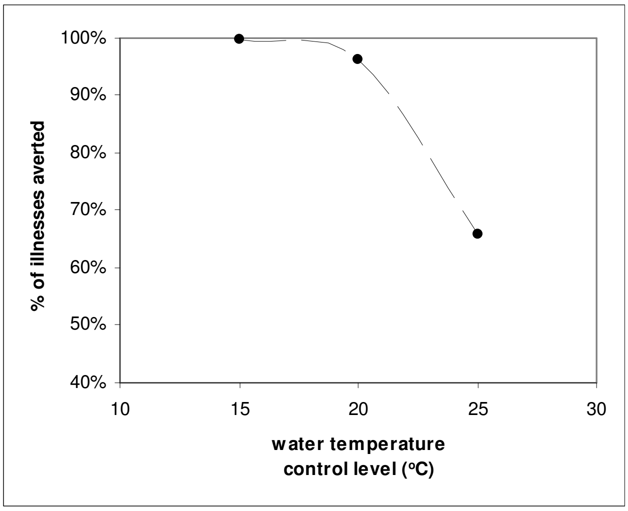


Figure 9. Percent reduction in the number of illness (from baseline).

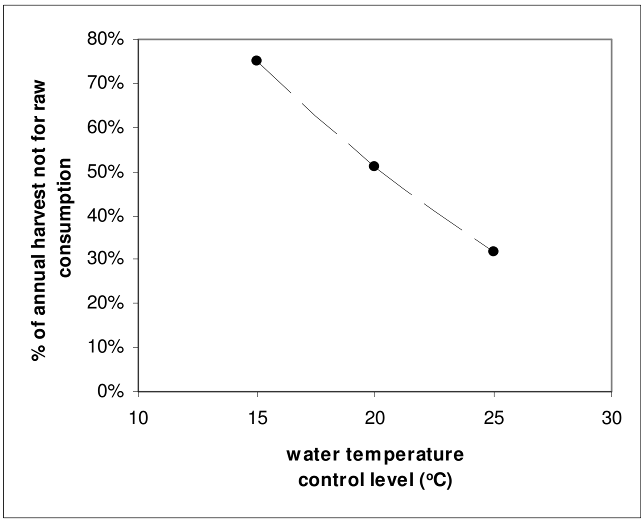


Figure 10: Percent of annual harvest not available for raw consumption

A requirement for summer shellstock to be placed in an ice slurry within one hour of harvest is predicted to result in a 50 percent reduction of *V. vulnificus* cases. This practice coupled with seasonal harvest restrictions, or a PHP, would result in substantial reductions in risk. Another approach is to require iced-chilling of harvest shellstock at various maximum seawater temperatures. At a limit of 20°C, there would be a reduction of approximately 55% annual cases with an associated effect on 50% of harvested oysters.

The risk assessment modelling demonstrated that a *V. vulnificus* end-point criterion of <3 MPN/g would reduce the risk of *V. vulnificus* in the USA to less than 1 case per year (specifically 0.16 cases). Measures that would need to be implemented would include efficient handling and post-harvest temperature controls, and the implementation of PHP for at least 9 months of oyster production when *V. vulnificus* levels are elevated. Such processing would need to achieve a 4-log reduction in *V. vulnificus* levels.

While the implementation of PHPs could achieve a relatively large reduction, considerable time may be necessary for the industry and consumers to transition totally to these interventions. Alternatively, PHP could be applied to a smaller segment of the total harvest while the technologies are developing and becoming more cost-effective. As PHP implementation increases, risk managers could tailor customized risk reduction plans to fit their specific circumstances.

The modelling also showed that the implementation of a *V. vulnificus* end-point criterion of <300 MPN/g would reduce the risk of *V. vulnificus* to the stated hypothetical reduction of at least 75 percent from current levels. Using Motes et al. (1998) data as a basis for prediction, such an end-point criterion would reduce the current mean level of 32.4 reported cases in the USA per year to approximately 7.7 cases per year.

Considering the overall flow process for oysters intended for raw consumption, the following series of measures may be considered to reduce *V. vulnificus* risk:

(a) **Control over harvest** - Oysters harvested at temperatures below 20°C and/or above 30 ppt salinity can go straight to market, provided storage temperature is controlled from the point of harvest.

(b) **Post-harvest handling** - If oysters are placed in an ice slurry within 1 hour of harvest this would dramatically reduce the risk per serving. Estimates indicate that around 50 percent of annual cases could be prevented by this low technology post-harvest control. This may be a suitable option for countries with fewer available resources.

(c) **Post-harvest processing** - Measures designed to kill *V. vulnificus* in oysters prior to their marketing will reduce the risk of illness. A 4-log reduction will result in oysters meeting an FSO of <3 MPN/gram. Less severe processing is necessary to meet the FSO of <300 MPN/gram, and will result in fewer physico-chemical changes to oyster structure and flavour. End product monitoring is an important means of verifying that oysters meet a microbiological criterion of <300 MPN *V. vulnificus*/gram at the time of shipping to retailers and restaurateurs.

(d) **Education** – Educating at-risk consumers of the dangers of consuming raw oysters and the need to change behaviour is an important risk management strategy. Unfortunately this measure cannot be as confidently relied upon as consumers are typically reluctant to change behaviour and attitude, even in instances of behaviour known to be linked to high risk. It should be also noted that education changes population exposure rather than individual exposure for those who do not respond to the message.

Figure 11 outlines examples of performance objectives and performance criteria that can be applied along the oyster harvest and supply chain in order to meet a FSO of <300 MPN *V. vulnificus*/gram and an ALOP of 75 percent reduction in cases of *V. vulnificus* illness.

Figure 11. Examples of performance objectives and performance criteria along the oyster harvest and supply chain to meet a FSO of <300 MPN *V. vulnificus*/gram and an ALOP of 75 percent reduction from current USA practices.

***Vibrio vulnificus* in Gulf Coast oysters (*Crassostrea virginica*)**

Through chain requirements to manage oyster safety

KEY:	
ALOP	Appropriate level of protection
FSO	Food safety objective
PO	Performance objective
PC	Performance criteria

Harvest

Important parameters are temperature and salinity of seawater – seasonal and diurnal influences

PC: Harvest when water temperature and salinity are unfavourable for *V. vulnificus* growth

Process criteria: Temperature <20°C and/or salinity >30 ppt [1]

1 Outside this range, PHP is necessary in order to achieve the ALOP

Chilling

Chill oysters to <13°C to prevent *V. vulnificus* growth

PC: Minimize opportunity for *V. vulnificus* growth

Process criteria: Rapid icing of oysters, less than 1 hour after harvest [2]

2 When temperature and salinity favour V. vulnificus growth this step achieves a 50% reduction in risk, and is consistent with good oyster handling practice. Further PHP is required in order to achieve the ALOP

Transport

Temperature control

PC: Prevent growth of *V. vulnificus*

Process criteria: Maintain oysters at <13°C

Post harvest processing (PHP)

Where PHP is applied, the goal is to reduce levels of *V. vulnificus*

PO: <300 MPN *V. vulnificus/gram*

PC: 4-log reduction in levels of *V. vulnificus*

Process criteria: Heat at 50°C for 5 minutes, etc [3]

3 *For Gulf Coast oysters, PHP is usually necessary during the months April to January* when water temperature and salinity favour V. vulnificus growth

Marketing

Maintenance of temperature control.

Education of at-risk consumers

PO: <300 MPN *V. vulnificus/gram*

Process criteria: Maintain oysters at <13°C

Micro limit: <300 MPN/gram [4]

4 Micro limit same as FSO as temperature control prevents outgrowth of V. vulnificus during marketing. Failure to maintain temperatures would result in rapid deterioration of oyster rendering them inedible.

During correct cold storage, V. vulnificus will decline by approximately 0.041 log/day. Mean storage time 7.7 days.

Consumption

Number of *V. vulnificus* at consumption

FSO: <300 MPN *V. vulnificus/gram* at time of consumption

Appropriate Level of Protection (ALOP) – 75 *percent reduction in the number of cases of Vibrio vulnificus illness, following consumption of raw oysters.*

5. LESSONS LEARNED

There are substantial barriers to overcome in order to better understand the outputs and limitations of microbiological risk assessment and to utilize the information to develop effective approaches for food safety risk management. This exercise demonstrated the difficulties of interpreting a microbiological risk assessment and identifying appropriate risk management strategies. Such difficulties are even more acute in developing countries where microbiological risk assessment is still an emerging science and country priorities are to elaborate and strengthen existing food safety programmes.

Developing countries are often constrained in terms of the technical infrastructure and scientific and financial resources that are necessary to carry out these assessments, including a source of trained assessors. They will need to invest more resources in food monitoring and public health surveillance and establish robust links between risk assessors and risk management at both national and international levels.

In the context of tasks performed by WG5, gathering local information and data on oysters would enable assessors to determine the risks to consumers and to identify region-specific control measures. Local data would also facilitate risk assessment for other pathogens.

The FAO/*WHO* *V. vulnificus* risk assessment (FAO/WHO, 2005) was facilitated by the availability of a US FDA *Vibrio parahaemolyticus* risk assessment (US FDA, 2005). Data produced for the latter risk assessment, such as monthly water/air temperature, salinity, monthly or seasonal harvest levels for various regions, harvest practices (submerged or intertidal), consumption patterns (percent consumed raw per month in each region), will also facilitate risk assessments for other pathogens, such as norovirus and hepatitis A, associated with molluscan shellfish consumption.

While the focus of this study has been on USA Gulf Coast oysters, the approach utilized can be applied to oysters from other regions of the world, with appropriate assumptions and data on water and air temperature, salinity, harvest and handling practices, seasonal consumption patterns and estimates of the size of the high-risk population. The approach is applicable in developing country situations and has substantial advantages as a model pathogen:commodity pair risk assessment because:

- There is a high reporting rate for primary septicaemia (>50%) compared with low and highly variable reporting for mild gastrointestinal infections;
- The dominance of a single vehicle of transmission (>90% of *V. vulnificus* cases of illness are associated with the consumption of raw oysters);
- Oysters are usually stored in the shell until consumption which, in a practical sense, eliminates potential for cross-contamination, thereby reducing uncertainty; and
- Raw oyster consumption eliminates variability and uncertainty on pathogen survival during preparation, in contrast to modelling the diverse cooking procedures used with other commodities.

5.1 Limitations

Worldwide, there is very little available and reliable information on oyster production and handling practices and their impact on the risk to consumers for *V. vulnificus* or *V. parahaemolyticus* disease. Information such as monthly or seasonal oyster production data, handling practices (*e.g.* time to refrigeration, product temperature profiles), and consumption patterns (*e.g.* percent consumed raw) is frequently incomplete. The default is a relatively large amount of USA Gulf Coast specific information, although still much is based on limited surveys and assumptions. These data are not resource-intensive to gather but would need to be collected by governments to assure reliability.

Extrapolating the risk assessment from a USA scenario to other countries requires an in-depth knowledge of variation in oyster harvesting, handling, processing and consumer practices. In the course of the working groups deliberations, efforts were made to obtain input from government and industry risk managers in both developed and developing countries, including Australia, Brazil, Chile, India, Korea, Japan, and the United Kingdom. In general, it was difficult to identify active risk management practices directed at *V. vulnificus*. This was attributed to a lack of epidemiological data for *V. vulnificus* foodborne shellfish-borne disease, as well as assumptions that this pathogen did not present a significant health threat.

These findings underline the need to gather specific types of information before risk assessment can be translated into risk management practices. Most notable is the need for epidemiological data before an ALOP could be established. If *V. vulnificus* foodborne illness is not reported, this could reflect a lack of surveillance and/or a low level of susceptibility within the shellfish-consuming population. High-risk groups have been identified within the USA and it seems likely that there are discrete subpopulations with these groups that have the greatest risk for *V. vulnificus* illness. Consequently, this situation may complicate utilization of the present risk assessment to other countries where susceptibility groups have not been identified.

V .vulnificus has been isolated from Australian waters (Myatt and Davis, 1989) and cases of both wound sepsis and foodborne illness have been reported. McAnulty (1990) found 40 percent of oysters in the state of New South Wales were contaminated with *V .vulnificus*. Available data would indicate that *V. vulnificus* levels are low and water temperatures are sufficiently low in most oyster-growing areas to limit pathogen growth. During the period 1988-1992, four cases of *V. .vulnificus* foodborne illness were reported (Kraa, 1995). All of these cases had chronic liver disease, and two died. There have been no further reported cases of *V. .vulnificus* illness in Australia despite close surveillance of oyster-borne illnesses.

Beyond epidemiological considerations, additional data will need to be collected in order to develop risk management practices based on a defined ALOP. Figure 1 illustrates a number of key inputs that influence the growth, survival and inactivation of *V. vulnificus*. The post-harvest handling practices, species of oysters, and/or consumption patterns differ between countries. Fortunately, time and temperature are the predominant effectors of *V. vulnificus* growth and simplify monitoring and predictions of exposure levels. In addition, there are also limited data on the ecology of *V. vulnificus* (especially numbers of *V. vulnificus* in food) in tropical countries and countries with different environmental conditions.

In countries where the temperature of shellstock oysters is not, or minimally, controlled following harvest, cost-effective, low capital interventions may be considered. Examples include the use of ice slurries to chill harvested oysters, treatment of shellstock at 50°C to obtain undetectable levels of *V. vulnificus*, and freezing oysters at -40°C followed by extended frozen storage.

Public health considerations are also worthy of additional attention. The proportion of susceptible individuals may differ between countries due to high prevalence of HIV/AIDS, hepatitis C or alcoholism. There are complexities associated with the dose-response relationships and it is influenced by human and environmental conditions. Furthermore, the dose-response assessment and risk assessment assume there are no seasonal or regional changes in virulence and that all strains of *V. vulnificus* are equally virulent.

5.2 Challenges

International data on *V. vulnificus* levels in molluscan shellfish are growing, with information becoming available from India, Chinese Taipei (Hsueh et al., 2004), Israel, Japan and the Republic of Korea (Park, Shon and Joh*,* 1991). However sufficient data for most of the model inputs shown in Figure 3 were only available for the USA.

Yet, considering these limitations, this risk management framework could be used by other countries to model the risk of *V. vulnificus* illness from raw oysters when the appropriate data are available. The production-process-retail-consumption continuum was modelled using a modular approach, and hence it may also be modified to address other seafood products. However factors that must be considered include:

- The *V. vulnificus*-temperature relationship in oysters at time of harvest may not be applicable to other regions with different environmental conditions, such as high salinities.
- Variations in oyster species.
- Different post-harvest handling practices.
- Different consumption patterns.
- Population dynamics such as higher numbers of at-risk individuals *e.g.* high prevalence of hepatitis C, HIV/AIDS, or high rates of alcoholism.

5.3 Approaches

This reference document is based on information and models found in the "Risk Assessment of *Vibrio vulnificus* in Raw Oysters" (FAO/WHO, 2005) which is derived, in great part, from data in the FAO/WHO "Risk Assessment of *V. parahaemolyticus* in Raw Oysters" (FAO/WHO, 2002) and the USA Food and Drug Administration "Quantitative Risk Assessment on the Public Health Impact of Pathogenic *V. parahaemolyticus* in Raw Oysters" (US FDA, 2005). In the course of considering different process interventions on FSOs and ALOPs, the US FDA generously provided outputs from Analytica© models that were originally developed for the "Risk Assessment of *Vibrio vulnificus* in Raw Oysters" (FAO/WHO, 2005), as well as updated epidemiological information of *V. vulnificus* foodborne infections in the USA. These primary sources of information provided the working groups with the means to develop potential risk management scenarios.

During the course of evaluating various risk management scenarios, the working group identified strengths and weaknesses in the process, including data gaps that may introduce significant uncertainty when the risk assessment and effects of various risk management scenarios are extrapolated to other regions and countries. These include:

- Seawater temperature is a key parameter in the risk assessment. Determining risk management on the basis of harvest season, *i.e.* winter, summer, etc., increases uncertainty since weather patterns may significantly by year.
- In other regions of the world, the density of *V. vulnificus* may be influenced by factors other than salinity and temperature, *e.g.* plankton blooms, nutrients, predation, etc. As such, the models described in the FAO/WHO risk assessment must be validated for other countries.
- *V. vulnificus* survival patterns may vary within different oyster species.
- Dose-response models are likely to vary among different human populations, including the proportion and types of at-risk individuals.
- The effects of on-boat icing to reduce oyster internal temperature to <13°C will vary depending on how the ice is applied.
- The FSOs for different PHPs (*i.e.* <3, <30, <300 MPN/gram) assume that the product will be properly refrigerated and that *V. vulnificus* levels do not increase up to the point of consumption. The survival or growth of *V. vulnificus* in post-PHP treated oysters will depend on storage temperature, the influence of antibacterial factors released in oyster tissues as a result of the PHP, and the inhibitory effects of spoilage flora.
- In the FAO/WHO risk assessment, predictions of illnesses agree with epidemiological data because the epidemiological data were used to develop the dose-response curves.
- Potential differences in strain virulence may be associated with season, region, shellfish species and growth or survival rates during handling and processing.

In addition, during the process of developing this document, the working group identified various efficient procedures to estimate *V. vulnificus* densities and predicted risk for risk management scenarios. Examples include:

- Using satellite remote seawater surface temperature data to estimate *V. vulnificus* levels and risk.
- Using data in Table 9 of the 2005 FAO/WHO *V. vulnificus* risk assessment to estimate changes in *V. vulnificus* numbers at different steps in the process flow and to calculate POs and PCs from hypothetical FSOs.
- Implementing a simple algorithm to estimate risk when the exposure level is known.
- Interrogating Analytica© modules to investigate the effects of different mitigations.

6. CONCLUSIONS AND RECOMMENDATIONS

The risk assessment model for *Vibrio vulnificus* in oysters represents a good starting point for countries wishing to undertake a similar risk assessment on domestic oysters and to develop risk management approaches based on appropriate levels of protection.

The adaptability of the model to other countries and regions is contingent on access to extensive and reliable local data *i.e.* presence and levels of the organisms in the marine environment and oysters, environmental data, handling practices, eating habits and data on vulnerable populations.

The working group demonstrated that good data is an important input into a risk assessment and essential in producing reliable and justifiable risk management approaches. Likewise, the need to review the assessment and revise the risk management was considered highly important as more data and information becomes available.

7. BIBLIOGRAPHY

Amaro, C. & Biosca, E.G. 1996. *Vibrio vulnificus* biotype 2, pathogenic for eels, is also an opportunistic pathogen for human. *Applied and Environmental Microbiology*, 62:1454-1457

Ama, A.-A., Hamdy, M.-K.& Toledo, R.T. 1994. Effects of heating, pH and thermoradiation on inactivation of *Vibrio vulnificus*. *Food Microbiology,* 11:215-227.

Arias, C.R., Aznar, R., Pujalte, M.J. & Garay, E. 1998. A comparison of strategies for the detection and recovery of *Vibrio vulnificus* from marine samples of the Western Mediterranean coast. Systematic and Applied Microbiology. 21:128-134.

Arias, C.R., Garay, E. & Aznar, R. 1995. A nested PCR method for rapid and sensitive detection of *Vibrio vulnificus* in fishes, sediments and water. *Applied and Environmental Microbiology*,61:3476-3478.

Arias, C.R., Macián, M.C., Aznar, R., Garay, E. & Pujalte, M.J. 1999. Low incidence of *Vibrio vulnificus* among *Vibrio* isolates from sea water and shellfish of the western Mediterranean coast. *Journal of Applied Microbiology*, 86:125-134.

Berlin, D.L., Herson, D.S., Hicks, D.T. & Hoover, D.G. 1999. Response of pathogenic *Vibrio* species to high hydrostatic pressure. *Applied and Environmental Microbiology*, 65**:** 2776–2780.

Bisharat, N. & Raz, R**.** 1997. Vibrio infection in Israel due to changes in fish marketing. *Lancet,* 348**:** 1585–1586.

Bisharat, N., Agmon, V., Finkelstein, R., Raz, R., Ben-Dror, G., Lemer, L., Soboh, S., Colodner, R., Cameron, D.N., Wykstra, D.L., Swerdlow, D.L. & Farmer III, J.J. 1999. Clinical, epidemiological, and microbiological features of *Vibrio vulnificus* biogroup 3 causing outbreaks of wound infection and bacteraemia in Israel. *Lancet,* 354**:** 1424.

Chuang, Y.C., Yuan, C.Y., Liu, C.Y., Lan, C.K. & Huang, A.H. 1992. *Vibrio vulnificus* infection in Taiwan: report of 28 cases and review of clinical manifestations and treatment. *Clinical Infectious Diseases,* 15**:** 271–276.

Cook and Ruple. (1992). Cook, D.W. & Ruple, A.D. 1992. Cold storage and mild heat treatment as processing aids to reduce the numbers of *Vibrio vulnificus* in raw oysters. *Journal of Food Protectection*, 55**:** 985–989.

Dalsgaard, A., Frimodt-Moller, N., Bruun, B., Hoi, L. & Larsen, J.L. 1996. Clinical manifestations and molecular epidemiology of *Vibrio vulnificus* infections in Denmark. *European Journal of Clinical Microbiology and Infectious Diseases*, 15: 227-232.

European Commission Health and Consumer Protection Directorate-General. 2001. Opinion of the scientific committee on veterinary measures relating to public health on *Vibrio vulnificus* and *Vibrio parahaemolyticus* (in raw and undercooked seafood).

Food and Agricultural Organization of the United Nations. (2002). Report of the Joint FAO/WHO Expert Consultation on Risk Assessment of *Campylobacter* spp. in broilers chickens and *Vibrio* spp. in seafood, FAO Regional Office for Asia and the Pacific, Bangkok, Thailand, 5 -9 August 2002.

Food and Agricultural Organization of the United Nations /World Health Organization. 2005. Risk assessment of *Vibrio vulnificus* in raw oysters. Microbiological Risk Assessment Series, No. 8. 114p.

Harris-Young, L., Tamplin, M.L., Fisher, W.S. & Mason, J.W. 1993. Effects of physicochemical factors and bacterial colony morphotype on association of *Vibrio vulnificus* with hemocytes of *Crassostrea virginica*. *Applied and Environmental Microbiology,* 59**:** 1012–1017.

Hlady, W.G. & Klontz, K.C. 1996. The Epidemiology of *Vibrio* Infections in Florida, 1983– 1993. *Journal of Infectious Diseases,* 173**:** 1176–1180.

Hsueh P-R, Lin C-Y, Tang H-J, Lee H-C, Liu J-W, Liu Y-C, et al. *Vibrio vulnificus* in Taiwan. *Emerging Infectious Diseases*, [serial on the Internet]. 2004 Aug [*Accessed 29 August 2008*]. Available from: http://www.cdc.gov/ncidod/EID/vol10no8/04-0047.htm

Kaspar, C.W. & Tamplin, M.L. 1993. Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish. *Applied and Environmental Microbiology,* 59**:** 2425–2429.

Kraa, E. 1995. Surveillance and epidemiology of foodborne illness in NSW, Australia. *Food Australia*, 47: 418-423.

McAnulty, J. 1990. Vibrio warning. *New South Wales Public Health Bulletin,* 1:12–13

McCoubrey, D.J. 1996. Risk of *Vibrio vulnificus* infection following consumption of raw commercially harvested North Island oysters. MPH Thesis. The University of Auckland, New Zealand. 86 p.

Mead, P.S., Slutsker, L., Dietz, V., McGaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. & Tauxe, R.V. 1999. Food-related illness and death in the USA. *Emerging Infectious Diseases,* 5: 607–625.

Mertens A., Nagler J., Hansen W. & Gepts-Friedenreich E. 1979. Halophilic, lactose-positive Vibrio in a case of fatal septicaemia. *Journal of Clinical Microbiology*, 9: 233-235.

Montanari, M.P., Pruzzo, C., Pane, L., & Colwell, R.R. 1999. Vibrios associated with plankton in a coastal zone of the Adriatic Sea (Italy). *FEMS Microbiology Ecology*, 29:241-247.

Motes, M.L. & DePaola, A. 1996. Offshore suspension relaying to reduce levels of *Vibrio vulnificus* in oysters (*Crassostrea virginica*). *Applied and Environmental Microbiology,* 62: 3875–3877.

Motes, M.L., DePaola, A., Cook, D.W., Veazey, J.E., Hunsucker, J.C., Garthright, W.E., Blodgett, R.J. & Chirtel, S.J. 1998. Influence of water temperature and salinity on *Vibrio vulnificus* in 72 *Technical report – Risk characterization* northern Gulf and Atlantic Coast oysters (*Crassostrea virginica*). *Applied and Environmental Microbiology,* 64: 1459–1461.

Myatt, D.C. & Davis, G.H. 1989. Isolation of medically significant *Vibrio* species from riverine sources in south east Queensland. *Microbios*, 60, (243):111–23.

Nell, J. A. 2001. The history of oyster fanning in Australia. *Marine Fisheries Review*. 63:14-25.

Park, S.D., Shon, H.S. &, Joh, N.J. 1991. *Vibrio vulnificus* septicemia in Korea: clinical and epidemiologic findings in seventy patients. *Journal of the American Academy of Dermatology,* 24: 397–403.

Strom, M.S. & Paranjpye, R.N. 2000. Epidemiology and pathogenesis of *Vibrio vulnificus*. *Microbes and Infection,* 2: 177–188.

Tamplin, M. L. & Capers, G. M. 1992. Persistence of *Vibrio vulnificus* in tissues of Gulf Coast oysters, *Crassostrea virginica*, exposed to seawater disinfected with UV light. *Applied and Environmental Microbiology,* 58:1506-1510.

Tamplin, M.L., Rodrick, G.E., Blake, N.J. & Cuba, T. 1982. Isolation and characterization of *Vibrio vulnificus* from two Florida estuaries. *Applied and Environmental Microbiology,* 44: 1466–1470.

Tamplin, M.L. 1994. The seasonal occurrence of *Vibrio vulnificus* in shellfish, seawater, and sediment in United States coastal waters. Final report to Salstonstall-Kennedy Grant Program, US Department of Commerce.

USDHSS. [US Department of Health and Human Services]. 1999. National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish. Washington, DC: U.S. Department of Health and Human Services.

US Food and Drug Administration. 2005. Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters. http://www.cfsan.fda.gov/~dms/vpra-toc.html

Veenstra J., Rietra P.J., Goudswaard J, Kaan J.A., van Keulen P.H. & Stoutenbeek C.P. 1993. Extra-intestinal infections caused by *Vibrio* spp. in The Netherlands. *Ned Tijdschr Geneeskd*, Mar 27;137(13):654-7.