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Harvesting and handling practices used to mitigate *Vibrio parahaemolyticus* illness

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Overview

MPI commissioned this report to inform risk management options in relation to the development of good harvesting and handling practices for the summer harvesting of Pacific oysters (*Crassostrea gigas)* to manage the risk from *Vibrio parahaemolyticus* (Vp). This report expands on the control measures outlined in an ESR report commissioned by MPI, ESR Risk profile: "*Vibrio parahaemolyticus* in bivalve molluscan shellfish".

This report compiles risk management controls applied by other regulatory authorities from around the world. Each approach has been evaluated/assessed with respect to efficiency and practicality, and ease of implementing in the New Zealand environment.

This discussion document should enable MPI and the oyster industry to determine which approaches to recommend, where or when needed, for inclusion in a future MPI guidance document for the summer harvesting of Pacific oysters.

Harvesting and handling practices used to mitigate *Vibrio parahaemolyticus* illness

Prepared by Dorothy-Jean & Associates Ltd

December 2017

Contents

LIST OF ABBREVIATIONS

BMS	Bivalve Molluscan Shellfish
BMSRCS	Bivalve Molluscan Shellfish Regulated Control Scheme
C	Celsius
CDC	Communicable Disease Center
CFIA	Canadian Food Inspection Agency
CT	Connecticut
EU	European Union
EFSA	European Food Safety Authority
F	Fahrenheit
FAO	Food and Agriculture Organization
HACCP	Hazard Analysis Critical Control Point
HPP	High Pressure Processing
ISSC	Interstate Shellfish Sanitation Conference
MA	Massachusetts
MPI	Ministry for Primary Industries
MPN	Most Probable Number
NCCOS	National Center for Coastal Ocean Science
NOAA	National Oceanic and Atmospheric Administration
NSSP	National Shellfish Sanitation Program
NZ	New Zealand
QRA	Quantitative Risk Assessment
USA	United States of America
US FDA	United States of America Food and Drug Administration
VA	Virginia
Vp	*Vibrio parahaemolyticus*
Vv	*Vibrio vulnificus*
WA	Washington

1 INTRODUCTION

V. parahaemolyticus (Vp) is a halophilic (salt-loving), motile bacterium that occurs ubiquitously in tropical and temperate coastal environments throughout the world. Their presence in the marine ecosystem is a natural phenomenon, unrelated to human pollution sources. Vp bacteria are often free living in seawater and sediments, but can also be attached to suspended matter, for example plankton or sediments or embedded in the shells of marine animals (Daniels, 2011). All *Vibrio* species, including Vp, can be transported around the world's marine environments by ship ballast water, migratory bird and fish species, tidal currents and imported and exported seafood (DePaola *et al.*, 1994; Martinez-Urtaza *et al.*, 2013). As a result, Vp is often present in the marine seafood species commonly eaten by humans.

While most Vp strains do not pose a human health risk, some strains occasionally cause foodborne illness. The most common Vp clinical syndrome is gastroenteritis; vomiting, nausea, abdominal pain, and watery (sometimes bloody) diarrhoea (MPI, 2001a,b; Odeyemi, 2016). The incubation period is short (4-96 hours). Vp infection infrequently leads to septicaemia, caused by the multiplication of pathogenic microorganisms and/or the presence of their toxins in circulating blood. Illness is more likely to progress to septicaemia in persons with underlying immunocompromising chronic disease, and the probability of this occurring has been estimated as 0.025 (or 25 in every 1,000 people in this subpopulation) (US FDA, 2005b).

The United States of America (USA) and Canada's epidemiological records show that raw oyster consumption is most commonly linked to Vp illness, whereas in Japan and Europe illness is associated with a wider variety of seafood species. To date, no commercial seafood species has caused Vp illness in New Zealand (NZ). However, it is acknowledged that the factors affecting the risk of Vp illness are constantly changing, including the evolution of new Vp strains, climate anomalies and seafood industry practices. Therefore, it is possible that future Vp illness will be linked to New Zealand seafood.

In 2016 ESR Ltd produced a report *Risk Profile of Vibrio parahaemolyticus in Bivalve Molluscan Shellfish* (BMS). This new report is supplementary to the 2016 Vp risk profile and focuses on Pacific oysters, reviewing the scientific data, food safety governance and industry practices used by other countries to mitigate or eliminate Vp illness. A comparative analysis is undertaken to determine if NZ's Pacific oyster (*Crassostrea gigas*) industry is using appropriate handling and harvesting practices to manage the potential risk of Vp illness.

2 STATUS OF SCIENTIFIC INFORMATION

Japanese scientists first identified Vp in the 1950s and since then there has been much international research focused on understanding Vp's relationship with the biophysical (natural) and human environments. However, to date Vp remains an enigma. While science has an understanding about Vp's life cycle, there remains significant data gaps about the environmental and evolutionary factors that cause Vp to become pathogenic. Further, epidemiologists lack the understanding of which Vp strains, and how many, cause human foodborne vibriosis. As a result, it is not yet possible to provide a predictive and preventative public health programme based on shellfish harvesting practices. The only way to ensure Vp does not pose a food safety risk is to treat shellfish with a validated post-harvest treatment, e.g. pasteurization.

The following sections summarises the available Vp scientific information associated with pre- and post-harvested oysters.

2.1. Vp Pathogenicity

Not all Vp strains are pathogenic to humans. Scientific research has been directed towards identifying genotypic and phenotypic traits that can be relied on as pathogenicity indicators. Yet rather than find specific answers the research over the last decade has identified that the pathogenicity factors are not as well understood or as not as predictable as earlier thought (Bechlars *et al.*, 2015; Jones *et al.*, 2012; Nydam *et al.*, 2014; Saito *et al.*, 2015).

Several virulence factors have been identified, including possession of genes encoding haemolysins (*tdh*, *trh*), Type III secretion systems and urease. An isolate containing one or more of these factors is likely to be pathogenic, but their absence is not necessarily an indication that a strain is not pathogenic. For example, an increasing proportion of clinical isolates possess neither *tdh* or *trh* genes and these isolates have been associated with cases with severe illness requiring hospitalisation (FAO/WHO, 2011). On the other hand, it has been identified that a significant population of *tdh+/trh-* (ST 3) environmental isolates in the USA Pacific North West may not be pathogenic (ISSC, 2017 (Hard)).

The search for reliable pathogenicity markers is further complicated by the finding that there are both evolutionary and ecological forces acting on Vp populations (Loyola *et al.*, 2015; Paranjpye *et al.*, 2012; Raghunath, 2011). This means that Vp strains vary in their behaviour over space and time. For example, in New York and Washington states coastal bays considered to be within a homogeneous water body exhibit different Vp pathogenic risk profiles. It also seems likely that potentially virulent Vp strains may be endemic in the environment but only cause illness under certain (currently undefined) conditions (ISSC, 2017 (Hard)).

Vp can be differentiated by serotyping (based on the O and K antigens) and this is useful for indicating the presence of some recognised pathogenic strains; the so-called “pandemic clones”, for example, 03:K6 and 04:K12.

The best estimation for the human Vp dose-response comes from a model based on data from human clinical feeding studies, anchored to epidemiological data from the USA (US FDA, 2005b). The model predicted a 50% probability of illness for a dose of approximately $1x10^8$ Vp cells, or between 10^7 and 10^{10} cells when uncertainty is considered. At exposure levels of approximately 10^4 cells, the probability of illness is <0.1%.

While scientists believe that some strains, such as 04:K12, may be more virulent than other Vp strains this is as yet unverified due to insufficient epidemiological linkages between clinical biological samples and oyster samples causing illness. It is very difficult to find pathogenic strains in the environment because levels fluctuate constantly. Levels of pathogenic strains are not consistent from animal to animal and illness may be caused by just one shellfish. Determining the risk associated with oysters and growing areas is complex (Oliver, 2017; ISSC, 2017).

2.2. Vp in the Environment

As described Vp are found in marine waters, sediments and marine species. Vp are detected throughout the year in tropical waters (Natarajan *et al*., 1980). In other geographical areas where Vp has been detected, their prevalence and concentration follow a distinct seasonal cycle, with highest concentrations occurring in summer and autumn and lowest counts in the winter.

The available information indicates that Vp are rarely isolated from seawaters below 10°C and they are released from marine sediments into waters at temperatures above 14°C. Conditions become more favourable for Vp growth as temperatures increase, and growth is particularly favoured at temperatures above 20°C. The concentration of Vp can reach 100 cells/ml when seawater

temperatures increase to 25°C (DePaola *et al.*, 1990; Kaneko and Colwell, 1973). However, temperature itself is not a strong predictor of Vp densities in water, for example, in Washington state the highest seawater temperatures were two months prior to the highest Vp levels in the water (ISSC, 2017 (Hard)).

The emergence of cold-tolerant strains has been reported (Vasconcelos *et al.*, 1975; Xu *et al.*, 2015). The ability of some strains to tolerate cooler water temperatures has been supported by a study of New Hampshire waters in the USA, which found that the genetic diversity of Vp strains isolated from colder waters (1-11°C) was less than the overall collection of isolates (Ellis *et al.*, 2012). The diversity increased with temperature.

The relationship between salinity and Vp prevalence and levels (concentration) in the environment appears to be variable and complex (Johnson *et al.*, 2012). The relationship between water salinity and Vp has even been found to vary within a region. For example, the concentration of Vp in oysters collected at two sites in the Gulf of Mexico showed that in one site salinity was positively correlated, but not at the other (Zimmerman *et al.*, 2007). Other environmental factors, including salinity and turbidity, have also been linked to Vp environmental prevalence but the correlations are inconsistent. Any relationships are likely to be specific to a region or site.

Nor is the relationship well established between Vp and other environmental parameters such as suspended particulate matter, chlorophyll *a* and dissolved organic carbon. As with salinity, any relationship is probably specific to an area. Recently it has been noted that the number of Vp in the marine environment may be associated with the type of ocean floor substrate; with notable differences between gravel, mud and mixed substrates (ISSC 2017 (Hard)). A seasonal difference was noted, but also harder substrates had higher levels of Vibrio bacteria (ISSC 2017 (Jones)).

The influence of water temperature over the prevalence and concentration of pathogenic (*tdh*+/*trh*+) strains is not well established, but there is some evidence to suggest a positive correlation (ESR, 2016). Numbers are highest during summer months and lowest during winter months. However, higher background Vp numbers and higher ambient temperatures do not necessary equate to a greater risk of Vp illness. For example, the Gulf of Mexico consistently has higher seawater and air temperatures, along with higher Vp background levels compared with those of the higher northern latitudes in the USA. Yet, there is a lower incidence of Vp illness associated with oysters harvested from the Gulf (Scallan, 2011).

Likewise, when evaluating temperature trends in Massachusetts Vp illness is often not associated with temperature peaks, rather during periods of transition. For example, in 2015 five illnesses in Massachusetts occurred during a period of rapid cooling as opposed to the season's highest temperature. Evaluating individual growing area information helps narrow the temperature thresholds when the risk of illness is highest for that area. As an example, in Massachusetts most illnesses from Katama Bay occur between water temps of 76-72°F, (24-22°C) whereas in Duxbury illnesses have primarily occurred with water temperatures between 75 and 68°F (24-20°C) (ISSC 2017 (Schillaci)).

Indeed, there is a growing body of evidence indicating an inverse relationship between exposure and risk when comparing Vp levels in shellfish from warmer and cooler regions. The greatest threat appears when an outbreak strain invades higher latitudes during climate anomalies, such as occurred in Chile and Alaska in 2004 (DePaola, ICMSS 2017).

While knowledge gaps remain about the Vp relationship (particularly pathogenic Vp) with environmental parameters, there is a general concern about the ocean-warming effects of climate change on the distribution and abundance of Vp. Climate change will also affect the salinity of coastal

and estuarine systems due to changes in precipitation and stream flow patterns (Marques *et al.*, 2010). Warmer temperatures appear to be the cause of Vp extending its geographical range into areas such as Alaska, Europe and Chile (Gonzalez-Escalona *et al.*, 2005; Ma and Su, 2011; Martinez-Urtaza *et al.*, 2013; McLaughlin *et al.*, 2005).

Rising water temperatures in shellfish growing areas have been associated with the increasing incidence of Vp and *V. vulnificus* (Vv) cases in the USA (Buenaventura, 2017; Morris, 2003). There are also concerns in Europe and other parts of the world that the increasing numbers of *Vibrio* spp. infections may be linked to rising ocean temperatures (Baker-Austin *et al.*, 2013; Gonzalez-Escalona *et al.*, 2005; McLaughlin *et al.*, 2005; Paz *et al.*, 2007; Sims *et al.*, 2011).

2.3. Environmental Modelling of Vp

Several agencies in various countries have attempted to model where and when Vp illness will occur. Predictive modelling using water quality parameters (temperature, salinity) is being investigated to predict the presence, abundance and potential virulence of Vp, with the intention that such models can be used to identify harvest days with potentially increased human health risks (Froelich *et al.*, 2013). However, such models need to be site specific and well validated, and do not appear to have been used as part of regulatory controls in any country.

In 2005 the United States of America Food and Drug Administration (US FDA) published a Quantitative Risk Assessment (QRA) considering foodborne illness caused by Vp in raw oysters harvested from different regions of the USA during different seasons (US FDA 2005a, 2005b). The model made predictions for 24 region/season combinations, where the regions were separated based on geography (Gulf and Atlantic coasts) and harvest methods (Pacific coast – separated into intertidal and dredged oysters, since intertidal oysters are exposed to higher temperatures before refrigeration). The model only considered pathogenic Vp which was defined as strains that were *tdh*+.

In 2011, the FAO and WHO jointly published a QRA considering Vp in raw oysters (FAO/WHO, 2011), which was based on the US FDA's QRA, but adapted to estimate illness in Australia, Canada, Japan and NZ. However, surrogate data from the USA were used for many of the inputs for these countries. The endpoint modelled was gastrointestinal illness from Vp because of eating raw oysters.

It is now recognized that neither the USA nor FAO models were accurate or could accurately predict the regional illness prevalence. FAO modelling work continues, with the organization currently updating and regionalizing their risk assessments. FAO's next modelling attempts will use local data reflecting the diversity of shellfish species cultured globally; differences in production and handling practices; climate and indigenous or introduced vibrio populations. It is FAO's goal to provide science based advice which serves as the basis for management measures, aimed at protecting consumer health and supporting the establishment of risk based food safety standards which can serve as the basis for fair trade practices.

The European Food Safety Authority (EFSA) hosts "Vibrio viewer". This is a real-time map incorporating daily remote sensing data (e.g. water temperature, salinity) into a model to predict the environmental suitability for *Vibrio* spp. in coastal waters.[1] The model driving the mapping software has been calibrated to the Baltic Region in Northern Europe. Similarly, the US FDA is working with NOAA (National Oceanic and Atmospheric Administration) to find the optimum way of using remote sensing data to predict Vp behaviour in the various environments of the Pacific, Atlantic and Gulf Coasts.

[1] https://e3geoportal.ecdc.europa.eu/SitePages/Vibrio%20Map%20Viewer.aspx (accessed 9th October 2017).

Individual USA state authorities are also working to determine their own predictive modelling tools. For example, Connecticut state regulators are working with the University of Connecticut, NOAA and National Center for Coastal Ocean Science (NCCOS) to develop hydrodynamic models and tools for hindcasting and forecasting the Vp risk associated with Connecticut growing areas.

2.4. Vp in Oysters

Once an oyster ingests Vp during filter feeding, the bacteria are found in the gills, digestive glands (including stomach, digestive ducts and digestive diverticula), adductor muscle and mantle cilia (Wang *et al.*, 2010a).

Oysters will naturally bioaccumulate Vp to concentrations higher than the surrounding waters. The concentration of Vp in the oysters is primarily influenced by water temperature and salinity, but also by the level of dissolved oxygen, the amount of zooplankton in the shellfish growing area and the rate of tidal flushing, since these factors influence both Vp populations and the feeding behaviour of oysters (Kaneko and Colwell, 1977; Venkateswaran *et al.*, 1990). The natural processes such as shellfish immunity, predatory bacteria and bacteriophages, affect the presence and concentration of Vp in BMS. Increased concentrations of Vp have also been measured in oysters experiencing one or more causes of stress, e.g. heat (Aagesen and Hase, 2014).

The US FDA has found that vibrio levels differ based on shellfish type, as well as production and storage conditions. For example, wild oyster harvests generally have higher levels than aquaculture oysters, but still higher levels than wild or aquaculture mussel species. (ISSC 2017 (Jones)). A recent study found that oysters grown suspended in the water had generally lower concentrations of Vp and Vv than oysters grown on the bottom and in contact with sediments (Cole *et al.*, 2015). Thus, stocks of oysters harvested from sediments (commercially or non-commercially) will possibly have higher concentrations of *Vibrio* spp. than those harvested from aquaculture operations in the same water body.

Vp are naturally depurated from the oyster but the depuration rates from oysters living in growing waters are complex and variable, depending on many environmental and oyster physiology factors. The length of time any Vp cell remains inside an individual shellfish residing in its growing area is not well defined, and is probably difficult to predict. The pili and flagellar systems of Vp were found to contribute to bacterial persistence in naturally depurating Pacific oysters (*C. gigas*) (Aagesen *et al.*, 2013). Laboratory experiments found the Vp was retained better in the gills and digestive glands of oysters undergoing depuration, compared with the adductor muscle and mantle cilia (Wang *et al.*, 2010a).

Vp will grow and multiply in oysters when they are out of the water if the temperature is suitable. Summer conditions permit Vp multiplication in oysters exposed by the receding tide as the temperatures of the exposed shellfish can be up to 10°C above that of the air temperature. Studies of oysters growing in the intertidal zone found that the concentration of total and potentially pathogenic (*tdh*+, *trh*+) Vp increased when oysters were exposed on the sunny mudflats by a receding tide, then decreased when the tidal waters covered the shellfish and filter-feeding recommenced (Jones *et al.*, 2016). Jones (2016) found in Washington State, the mean levels of Vp increased 1.38 log MPN/g following intertidal exposure and dropped 1.41 log MPN/g after re-immersion for 1 day, but the levels were dependent upon the container type utilized. Another study measured Vp concentrations 4-8 times higher at maximum intertidal exposure than at the beginning (Nordstrom *et al.*, 2004).

Re submerging to reduce Vp levels

The US FDA Gulf Coast Seafood laboratory has undertaken research to determine how long it takes to reduce Vp growth following intertidal exposure. It was found that vibrio levels returned to background levels after the re-immersion for one tidal cycle (ISSC 2017, (Jones)).

Re-submerging is used in some states, for example Washington, as a Vp mitigation process. In this process intertidal oysters are harvested, culled and then placed in larger cages for re-submerging in either deeper water within the same growing area or for re-submersion by the tide. (See Figure 1). Work done by the US FDA has validated this process (ISSC, 2017 (Jones)).

Figure 1: Photo of the type of cages used to re-submerge oysters

In the USA it is a common industry practice to hold shellstock out of the water for an extended period to control biofouling. The number of days the oysters are kept out of the water varies upon the region, but Hopkins *et al*., (2016) found that Pacific oyster can survive well out in the water for 34 days. However, most industry operators use a much shorter desiccation period to remove shell biofouling, often around 7- 10 days. The US FDA has found that Vp returns to background levels within 14 days or re-submerging in seawater after extended desiccation (Jones, ISSC 2017).

Relaying to reduce Vp levels

The practice of 'relay' is internationally recognised as the transfer of BMS from a growing area to another growing area for reducing pathogens or other contaminants by using the ambient coastal marine area environment as the treatment process (BMSRCS, 2006).

There is limited information on the success of relaying as a treatment step to remove Vp from BMS. The concentration of Vp in *Crassostrea commercialis* oysters was shown to reduce from 18 cells per gram to 5 cells per gram after being relayed from a harvest area to a pollution free waterway. After an increase in Vp levels upon initial re-submergence, Vibrio bacteria decreased after 7 days (Son and Fleet, 1980). However, recent studies by the US FDA show that relaying to higher salinity and/or cooler waters shows promise for reducing Vp levels (Jones, ISSC, 2017).

Cold deep- water purging

Cold deep-water purging means placing oysters in deeper water (with a lower temperature)

This option has been successfully used in Alaska. In May – July 2004, there was an unexpected Vp outbreak (Serotype O6:K18) associated with commercial oysters from Alaska. This outbreak expanded the range of epidemiologically confirmed Vp illness to a latitude higher than 60 degrees north — more than 1000 km north of British Columbia, previously the northernmost area reported to have locally acquired illness (McLaughlin *et al*., 2005). At the time the mean daily water temperatures exceeded

15.0°C on 38 days. 2004 was unusual because mean temperatures were above 15.0°C for a much longer period and were almost 2°C warmer than during any of the previous years. Following this event, the Alaskan industry identified moving oysters into cooler water as an effective Vp mitigation process.

Similarly Taylor Oysters Ltd., who operate in the USA Pacific Northwest, found that identifying oysters with elevated levels during vibrio season, relaying them in to growing trays stocked at grow out densities and hanging them below rafts in water deep enough to reach the 12 – 15°C range, reduces the Vp levels. Taylor's sampling programme has verified regardless of the Vp loadings at the time of relay, within seven days all oysters show a significant reduction; sufficient to meet the Canadian standard of <100 Vp MPN/g.

2.5. Vp Growth in Oysters Post-Harvested

International research has identified that after harvesting Vp within BMS has the potential to survive and multiply, depending on the ambient temperature (ESR, 2016). This research information can be summarised:

- Vp will multiply in shellstock BMS stored at 20°C or above. The concentration can increase by as much as 1 log per gram in one day at 20°C, and more at higher temperatures. Growth to stationary phase[2] occurs within 1-2 days.

- Vp will multiply in BMS at 15°C, increasing by approximately 2 log over two days of storage. No data were located for temperatures in the range 11-14°C.

- Vp will not grow in BMS stored unfrozen at 10°C or lower. The concentration has been observed to remain stable or decrease at these cool temperatures. Survival for up to three weeks has been reported.

- Vp dies under frozen storage but can survive for up to six months. The data suggests that death is more rapid at -10°C or -18°C compared with -30°C. This has been attributed to the formation of larger intracellular ice crystals at the higher temperatures, causing greater cell damage (Shen *et al.*, 2009).

  Lydon *et al.,* (2015) found ice slurries were effective for rapidly cooling freshly harvested oysters (24°C to 10°C) within 12 minutes, but repeated dipping of oysters caused the ice to become contaminated with faecal coliforms, *Clostridium perfringens*, *Vv* and total Vp. However, the concentrations of Vp and Vv. were unchanged in the flesh of the oysters after 15 minutes submersion in the contaminated ice slurry. Another study found that on-board and dockside icing did not predictably reduce the concentration of Vp in oysters, and icing significantly and negatively affected oyster survival (Melody *et al.*, 2008).

[2] During the **stationary phase**, the rate of bacterial cell growth is equal to the rate of bacterial cell death.

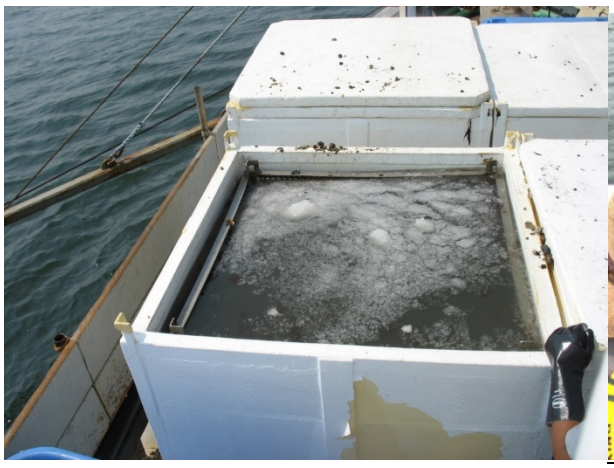


Figure 2 & 3: Photos of ice water slurry systems on harvesting vessels

2.6. Post-Harvest Technologies

Science has validated post-harvest food technologies capable of eliminating viable Vp in oysters, though it is acknowledged there are strain-dependant differences in resistance to control methods, and the level of resistance may also change depending on other stressors the cells were exposed to prior to a control intervention (Burnham *et al.*, 2009; Calik *et al.*, 2002; Drake *et al.*, 2007; Wong *et al.*, 2004a). For example, studies have identified that the pandemic Vp strain O3:K6 is more resistant to controls such as low temperature pasteurisation and High Pressure Processing (HPP) (Andrews *et al.*, 2003b; Cook, 2003). Even so, the US FDA now recognise HPP, individual quick freezing (IQF) with extended storage, and irradiation as processes capable of reducing Vp to non-detectable levels in oyster species (defined as <30 MPN/g) (US FDA, 2011). See Appendix I for the specific technical details on these approved processes.

A 2016 NZ study carried out under the Ministry of Business, Innovation and Employment funded Safe NZ Seafood Programme by Plant and Food Research, evaluated the behaviour of Vp in Pacific oysters (naturally contaminated) after flash freezing followed by frozen storage. The aim of this study was to compare the inactivation of Vp in Pacific oysters with that observed in the USA study of Liu *et al*. (2009), using the end-point of a 3.52 log MPN/g reduction in 30 samples. The results of the NZ study were very similar to the USA study.

Vibrio bacteria are readily destroyed by cooking even when the oysters are highly contaminated (Codex Alimentarius, 2010). Experiments with oysters artificially contaminated with Vp found that treatment of 50°C for 10 minutes was needed to reduce the concentration by >5 log MPN/g (Ye *et al.*, 2012). Treatment at 50°C for only 5 minutes or treatment at 45°C for 20 minutes only achieved reductions of 3.9 and 2.6 log MPN/g, respectively. An earlier study (Andrews *et al.*, 2000) had measured a 5-log reduction of Vp cfu/g in oysters after 5 minutes at 50°C. The difference may be due to different strains or methods (e.g. Andrews *et al.* used a kettle at 55°C to initially heat the oysters to 50°C, while Ye *et al*. used a water bath at 50°C). The commercial practice of heat shocking oysters in boiling water (three minutes) to facilitate opening also reduced counts of Vp to "undetectable" levels (Hackney *et al.*, 1980. However, such a temperature can affect the quality of oyster meat, so a low temperature pasteurisation of 10 minutes at 50°C is a more favoured method for eliminating Vp from shellstock oysters (Andrews *et al.*, 2000).

As demonstrated by data in Section 2.5, Vp is susceptible to freezing, but freezing alone cannot be relied upon to eliminate this pathogen without process validation.

Several chemical controls have been investigated for reducing Vp in BMS. Citric acid and lactic acid effectively reduced Vp in shucked, pre-sterilised oysters, but the effect of these organic acids on Vp in non-sterilised oysters was not investigated (Mahmoud, 2014). Other treatment agents that have demonstrated antimicrobial activity towards Vp in BMS include green tea extract (Xi *et al.*, 2012) and chlorine dioxide (Wang *et al.*, 2010b).

Antimicrobial photodynamic therapy (aPDT) treatment involves delivering visible light of an appropriate wavelength to a photosensitive additive, and exciting this additive to undertake a photochemical reaction with oxygen to produce radicals (type 1 reaction) or singlet oxygen (type 2 reaction) (Wu *et al.*, 2016). The reaction destroys bacterial cells. When oysters were submerged in a solution of the photosensitive additive curcumin and Vp, then opened and exposed to a light source for 60 seconds, the concentration of Vp was reduced by approximately 5 log cfu/g (Wu *et al.*, 2016).

Artificial depuration in tanks can reduce the concentration of Vp inside oysters but is not a reliable method for eliminating these bacteria from oysters (Croci *et al*., 2002). Research has shown that depuration with clean seawater was not effective in reducing certain persistent bacteria including *Vibrio* spp. in BMS because of the colonization of those bacteria in the intestinal tracts. A study undertaken by Eyles and Davey (1984) observed no significant differences in mean counts of naturally occurring Vp between depurated and non-depurated oysters. Therefore, while depuration can reduce the concentration of Vp inside oysters this is not a reliable method for eliminating these bacteria. Depuration can also cause cross-contamination of Vp to other oysters (Ramos *et al.*, 2012a).

To increase the efficacy in reducing bacterial contamination in oysters, depuration in conjunction with chlorine, ultraviolet light, ozone or iodophors were studied (Fleet, 1978). However, none of them could effectively eliminate Vp from BMS. Ren and Su (2006) examined the effects of electrolyzed oxidizing (EO) water depuration on reducing Vp in laboratory-contaminated oysters and found that both species could only be reduced by approximately 1.0 log unit after 8 hours at room temperature. The effectiveness of depuration on removing bioaccumulated Vp can be improved by using UV light and chlorine to control microbes in the water (Ramos *et al.*, 2012a). Even so enforced depuration after harvest does not reliably eliminate all Vp from oysters.

Biological controls offer alternative treatments for Vp. Predatory bacteria are naturally present in seawaters and experiments have demonstrated how even trace amounts of these bacteria can reduce the concentration of Vp in seawater (Richards *et al.*, 2012). Several strains of a small marine predatory bacterium, *Halobacteriovorax*, were shown to be predatory against *Vp* (Richards *et al.*, 2016). Two *Bdellovibrio* and like proteobacteria were effective against Vp in oysters (Li *et al.*, 2011). Bacteriophages are also being investigated (Jun *et al.*, 2014) as well as extracts from marine algae (Fatima *et al.*, 2016; Genovese *et al.*, 2012; Pradhan *et al.*, 2012). It is possible that in the future such biological controls might be used in conjunction with tank depuration processes.

3 INTERNATIONAL GOVERNANCE

Foodborne vibriosis is a globally recognized food safety problem. The following section reviews the Vp epidemiology status of countries, along with the food safety governance strategies used by international, national and state food safety agencies.

3.1. Codex Alimentarius Commission

In 2010, Codex published *Guidelines on the application of general principles of food hygiene to the control of pathogenic Vibrio species in seafood (CAC/GL 73-2010)* (Codex, 2010). The Codex guidelines provide generic food safety principles that should be applied to most food safety pathogens but they do not provide the formulas necessary to predict or eliminate Vp from marine waters or in raw oysters.

The guidelines recognise that general food hygiene controls (e.g. cooling and measures to minimise cross-contamination) will control all Vibrio species, while also recommending that water temperature and salinity levels are established for harvesting areas to indicate increased risk of *Vibrio* spp. contamination. Good Hygienic Practices and the application of Hazard Analysis Critical Control Points principles are recommended for post-harvest operations, along with validating the effectiveness of any treatments (e.g. freezing and high pressure) and monitoring such treatments.

The Annex in the 2010 Codex Vibrio guideline sets out specific control measures for Vp in bivalve molluscs intended for consumption in a live, raw or partially treated state.[3] Controls include environmental monitoring (monitoring human illness, predictive modelling, prevalence studies), temperature control during handling, storage and transport (supported by microbiological data) and education of industry workers.

3.2. European Union

The European Union (EU) comprises 28 different countries, governed using universal BMS food safety directives and the underpinning support of the EFSA (www.efsa.eu).

The EU does not require the formal reporting of cases of vibriosis, but it is recognised that there are sporadic Vibrio illnesses and occasional Vp outbreaks have been reported over the past couple of decades. These outbreaks have occurred primarily in the Galicia region of Spain and have been linked to strains indigenous to Europe and the pandemic O3:K6 strain. Sporadic illnesses have also been reported in France and Italy. There is also evidence of increasing Vp illness linked with BMS harvested from the Mediterranean and the Baltic Sea (Baker-Austin, 2014).

In 2001, the European Commission commissioned a report to review the need for specific vibriosis food safety regulations. The subsequent report, *Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on Vibrio vulnificus and Vibrio parahaemolyticus (in raw and undercooked seafood)* was adopted by the European Commission in September 2001. This report concluded the need for on-going epidemiological monitoring of Vp (and Vv) infections and for the enforcement of good hygienic practices, including the cold chain maintenance at all stages from BMS harvest to the consumer. However, the report also concluded that the currently available scientific data did not support setting specific standards or microbiological criteria for pathogenic Vp (or Vv) in seafood. To date EFSA has maintained this stance, even though a 2012 study by the European Centre

[3] "**Partially treated**" is where a bactericidal treatment has been applied with the intention to reduce *V. parahaemolyticus* and/or *V. vulnificus*, but not eliminate these bacteria.

for Disease Control produced a framework to rank infectious disease agents according to their potential severity to society, and their link to climate change (Lindgren *et al.* 2012, *Science*). This study concluded that vibriosis represents a potential significant risk in Europe.

In summary, in the EU there are general regulatory requirements for the provision of safe food (852/2004 and 853/2004 and general food law etc) but there are no specific microbiological limits or measures for the control of Vibrio bacteria in seafood.

3.3. Canada

Commercial BMS harvesting occurs on both the Canadian Atlantic and Pacific Coasts. The two coasts have different ecological and oyster industry profiles with respect to the prevalence and Vp concentration, and associated illness. Oysters from the Pacific Coast have been linked to Vp outbreaks while those from the Atlantic Coast only to sporadic, occasional Vp cases.

Prior to 1997 there was a low incidence of Vp (1.3 cases per 100,000 population) associated with Pacific coast oysters, but that year there was an illness outbreak of 111 reported cases linked to oysters harvested from British Columbia and Washington State, USA. After the 1997 outbreak the Canadian Food Inspection Agency (CFIA) established environmental indicator stations in six major oyster growing areas. The data from these stations showed an annual presence of Vp from June to September (rarely from October to May). During the period 1998-2008 the Vp incidence dropped to 0.3-0.7 cases per 100,000 population, but increased to 0.8-0.9 cases per 100,000 population in 2009-2010. In the summer of 2015, Canada experienced its largest reported Vp outbreak with 82 cases linked to raw oyster exposure harvested from British Columbia growing areas. It should be noted that during 2015 the spring and summer seawater temperature was on average 2^{o}C higher than usual due to a strong *El Niño* event (Buenaventura, 2017).

The Canadian food safety programme is administered by two government agencies: Health Canada establishes the national standards, while the CFIA implements and verifies these standards. In 2000, the CFIA stopped their environmental monitoring programme and the industry were required to implement their own harvesting management programmes to prevent Vp illnesses.

The 2015 Vp outbreak resulted in a review of the Canadian Vp control programme, using a multi-jurisdictional approach by federal, provincial, regional authorities in partnership with stakeholders in the BMS industry. Recommendations and subsequent actions provided guidance on triggers for implementing additional Vp controls during the high-risk periods. Such controls include tightening of time to temperature controls and industry practices and processes intended to ensure that will meet a final product microbiological criterion. The criterion, n=5, c=0, m=100 Vp MPN/g, is applied to live oysters in the shell and intended for the raw market in Canada. Further, during the summer months, oysters harvested from Canadian waters and intended for live sale should only be harvested from sites where the concentration of Vp in the oysters is ≤100 MPN/g, unless a validated post-harvest processing step is applied that will reduce Vp to this level (FAO/WHO, 2016).

The triggers used to activate the harvesters' summer Vp management plan are any of the following:

1st May;

Seawater temperature of ≥15^{o}C;

Oysters harvested with or near 100 Vp MPN/g.

The seasonal control programme is stepped down once the seawater is less than 15°C and the live oysters at the point of harvest have < 3 Vp MPN/g. The season for Vp management can be extended in situations where there are climate anomalies, illness outbreaks or consistently elevated Vp levels in shellstock.

Because of these requirements to implement a seasonal control programme the Canadian oyster industry have implemented pre-harvest, harvest and post-harvest practices to ensure compliance with the summer time microbiological limits. Examples include a change from intertidal to suspended cultures during high risk periods, transitioning to deeper waters in suspended cultures or re-submersion of shellstock prior to final harvest and live processing. See Section 4 for more discussion.

While these Vp guidelines have been actively implemented the Canadian Shellfish Sanitation Program currently does not include specific Vibrio regulations, but consideration is being given to the need for such regulatory controls (Buenaventura, 2017).

3.4. Japan

The Japanese population consume a high amount of seafood (Hara-Kudo, Kumai, 2014). Therefore, Vp is a dominant cause of foodborne infections in Japan. Indeed, of 3,955 Vp outbreaks from 1989-1999, 2,392 were food-related outbreaks with 92% associated with seafood (Kumagai, 2000). Following a Vp epidemic in 1997 the Japanese Ministry of Health, Labour and Welfare instituted regulations for seafood in 1999/2001 including:

- Use disinfected or artificial seawater, or potable water, for washing and processing seafood;
- Maintaining seafood temperature at or below 10°C during distribution and storage;
- Microbiological standards for Vp: ≤100 MPN/g for seafood intended for raw consumption, not detected/25 g for ready-to-eat boiled seafood;[4] and
- Advice that consumers should consume seafood within 2 hours of it being removed from the fridge, and restaurants should serve it immediately.

The microbiological standards (n=5, c=0, m=100 Vp MPN/g) were based on the assumptions that an infectious dose of TDH-producing Vp is 100 cells/serving and a raw seafood serving is 100 g, and informed by outbreaks and studies of the ratio of total *Vp /tdh*+ Vp in seawater. A substantial reduction in incidence of reported Vp in Japan was credited to these regulations (Hara-Kudo and Kumagai, 2014).

3.5. USA

Vibriosis has been a notifiable disease in the USA since 2007. The incidence of Vp infections (including wound infections) for 2014 was an estimated 0.2 per 100,000 (approximately 600 cases reported during the year). The majority of vibriosis cases are reported from coastal states and peak in summer. Raw oysters are most often implicated as the vehicle of infection.

In 1988 a new Vp serotype, O4:K12, was first identified on the Pacific Coast and 1997 and 2004 this serotype caused large outbreaks linked to Pacific Northwest oysters. It was first identified outside of

[4] Note that the standard for "seafood for raw consumption" is reported as "not detectable in 25 g" in FAO/WHO (2016).

Pacific NW in 2012 at Oyster Bay, New York. During 2013, at least 76 of the 104 isolates collected by Communicable Disease Center were determined to be O4: K12. It is possible that this strain is more virulent than other pathogenic Vp strains.

ISSC Vp regulations - The USA BMS food safety regulations are established under a co-operative programme, administered by the Interstate Shellfish Sanitation Conference. The regulations are published in the *National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish* (www.iss.org) (NSSP) and implemented by the state shellfish regulatory authorities.

The NSSP (Chapter VIII – Control of Shellfish Harvesting) has time/temperature requirements for all harvested shellstock not under the specific jurisdiction of a Vp Control Plan (See Table 1). In this situation, the Authority establishes the water or air temperature to be applied for each growing area by averaging the previous five (5) years maximum monthly water or air temperatures. For 'time to temperature control' the time begins once the first shellstock harvested is no longer submerged.

Table 1: Time Temperature requirements for shellstock based on average monthly maximum air temperature.

Action Level	Average Monthly Maximum Air Temperature	Maximum Hours from Exposure to Receipt at a Dealer's Facility
Level 1	<50 °F (10 °C)	36 hours
Level 2	50 °F - 60 °F (10 °C - 15 °C)	24 hours
Level 3	>60 °F - 80 °F (15 °C - 27 °C)	18 hours
Level 4	>80 °F (≥27 °C)	12 hours

The NSSP lists Vp regulations, including; outbreak management, epidemiological monitoring, annual risk evaluations and when illnesses occur implementing a state Vp Control Plan. The risk evaluation considers:

- The number of Vp cases epidemiologically linked to the consumption of oysters commercially harvested from the State;
- Levels of total Vp and *tdh*+ Vp in the area;
- The water and air temperatures in the area and the water salinity; and
- Harvesting techniques, the quantity harvested, its uses i.e. shucking, half-shell, post-harvest processing (PHP).

A Vp Control Plan is implemented for a State if the annual risk evaluation concludes that:

- Vp infection from the consumption of oysters from that State is "reasonably likely to occur" (i.e. the risk constitutes an annual occurrence).
- State has a BMS growing area that was the source of oysters that were epidemiologically linked to an outbreak of Vp within the prior five years.

If a Vp Control Plan is required, then each state must:

a) Establish one or more triggers for when control measures are needed.

b) Implement one or more control measures to reduce the risk of Vp illness at times when it is likely to occur.

Control measures may include closing the harvest area; restricting oysters to product labelled for shucking (it is assumed in the USA that shucked oysters are always cooked); limiting time from harvest to refrigeration; and post- harvest processing.

The NSSP, Chapter II Risk Management and Risk Assessment describes the actions required when there are epidemiologically confirmed Vp illnesses linked to a growing area. It should be noted that the definition of a Vp illness problem is based on a specific number of cases over a defined time period (See Appendix II for details). The actions, including harvest area closures times, are linked to both the number of cases and the spatial frequency between cases. It should be noted that the ISSC definition of a Vp illness problem has changed several times over the years depending on the policy approach taken by the conference.

State Vp Control Plans - Vp control plans vary considerably, which is appropriate given the USA's wide variance in biophysical parameters and industry operations. Connecticut, Massachusetts and Virginia and Washington states provide examples of this diversity.

i) Connecticut State

Connecticut's (CT) commercial oyster (*Crassostrea virginica*) industry is based on ranch-style[5] habitats on the ocean floor), subtidal aquaculture operations, generally in 10-30 feet (3 -9 m) depth at Mean Low Water. Harvest methods, include traditional oyster dredging or hauling up aquaculture cages.

The introduction of a virulent Vp strain (O4:K12 or ST36) from the Pacific Northwest in Long Island Sound on the East Coast in 2012 resulted in large-scale closure of CT's most critical oyster production areas. In 2013 there was an outbreak of 23 confirmed illnesses and subsequent closures and recalls severely impacted the industry. Since the 2013 outbreak the State of Connecticut has significantly reduced oyster associated illnesses associated to only one or two confirmed sporadic cases in the subsequent years.

CT Vp Control Plan (VPCP)

The plan categorises the oyster areas into those associated and those not associated with Vp illness. The areas associated with illness must comply with the Rapid Cooling Plan and all other areas with the General Vp Plan. As seen below, the difference relates to time/temperature management.

> **Rapid Cooling VPCP (2013 outbreak area)**: required the rapid cooling of oysters harvested from the waters of Norwalk, Westport and Darien to an internal temperature of 50°F (10°C) within one hour of harvest from June 1st through September 30th, when water temperatures are above 68°F (20°C); shading
>
> **General CT VPCP:** 5 hours from harvest to refrigeration and 5 hours to cool to internal temperature of 50°F (10°C) from June 1st through September 30th shading of exposed product on harvesting vessels.

Industry compliance with Vp plan time temperature requirements is tested using Smart Button data loggers through the chain from harvesting to processor.

ii) Massachusetts State

Massachusetts's (MA) commercial oyster industry is based on subtidal and intertidal harvests of American oysters (*Crassostrea virginica*).

Prior to 2011, cases of Vibriosis linked to the consumption of raw BMS harvested in MA were extremely rare. Although the Vp species had been identified for years in the local waters, the cool and high-salinity waters in most of the State's BMS growing areas were not considered particularly conducive to vibriosis. Since 2011, over 100 cases of Vp illnesses involving oyster consumption have been

[5] R**anching** is a type of shellfish farming in which juveniles are released into the ocean to grow unprotected and unassisted to be subsequently harvested

epidemiologically linked to MA harvest areas. This rapid emergence of Vp in MA has resulted in a significant burden on public health managers and the state's rapidly growing oyster aquaculture industry; and highlighted the ability for pathogenic strains to rapidly change regional risk profiles.

In 2012 MA state implemented harvest restrictions in the growing areas deemed to be of high risk for Vp illness, primarily due to large tidal exposure resulting from Cape Cod Bay's expansive tidal shelf. Following 2012 illness reports, outside of the control area, MA implemented Vp controls state-wide. Since 2012 most sole source cases (where only one growing area is implicated in illness report) in MA have been attributed to two harvest areas (Duxbury and Katama Bays), where illness levels have exceeded the NSSP closure threshold in 3 of the past 5 years.

In MA state-wide trends do fit the predicted model of highest risk during periods of highest average temperatures. An epi-curve of illnesses from 2011-2016 shows state-wide peak occurrence between July 1st and September 15th, when average water and air temperatures are at their annual maximum. However, while it might be expected most of these cases would come from intertidal harvesting this is not the case with subtidal areas causing the major MA illness burden.

Significant differences in environmental conditions between MA implicated harvest areas, along with inter-annual variability within harvest areas, means a wide range of air and water temperatures associated with reported illness. Evaluating this information on an individual growing area level helps narrow the temperature thresholds when the risk of illness is highest for that area. Massachusetts illness occurrence is often not associated with seawater temperature peaks, rather during periods of transition

In 2016, MA enhanced its temperature controls in Duxbury Bay and Katama Bay during the highest risk peak (July 1st -Sept 15th) from 2 hours to 1 hour from harvest. A transplanting program was also initiated in Katama Bay to move oysters out of the warmer Bay waters into cooler open water prior to harvest. Both Duxbury Bay and Katama Bay experienced a rapid decrease in illness occurrence following the reduction in time to ice and initiation of the transplant program.

MA Vp Control Plan

Operates between 21st May and October 16th, 2017 with harvest area conditions predicated on the level of illnesses associated with the area.

All market-bound oysters harvested from May 21st 2017 through October 16th 2017 shall be adequately shaded immediately after harvest and remain adequately shaded until placed in a shellfish icing container and adequately iced. All market-bound oysters, except those described in Section B.6 (B.6 lists nominated MA growing) of this Plan, shall be adequately iced within 2 hours of time of harvest or exposure, or prior to leaving the point of landing, whichever occurs first.

Time of harvest for sub-tidal areas means the time when the first oyster in a harvester lot is taken from the water on a calendar day. Time of harvest for intertidal areas means the time when the first oyster in a harvester lot is exposed during a single low tide cycle or when the first oyster in a harvester lot is taken from the water, whichever occurs first.

All market-bound oysters harvested from growing areas CCB-42, CCB-43, CCB-44, CCB-45, CCB-46, CCB-47 and V-20, between July 1st – September 15th, shall be adequately iced within one (1) hour of time of harvest or exposure, or prior to leaving the point of landing, whichever occurs first.

Oyster culture activities of market-sized oysters conducted on barges, boats, or other floating structures within growing areas CCB-42, CCB-43, CCB44, CCB-45, CCB-46, CCB-47 and V-20, between July 1st – September 15th that exceed the (1) hour requirement for icing at section B.6 of this Plan, but

do not exceed (2) two hours from time of exposure, shall be returned to the original license site and harvested no sooner than the following calendar day.

All oysters received by the original dealer between May 21st, 2017 and October 16th, 2017 shall be cooled in the original dealer's facility to 45°F (7°C) within 10 hours of the time of harvest or tidal exposure before shipment.

iii) Virginia State

Virginia's commercial oyster species is *Crassostrea virginica.* Most of the industry is subtidally grown oysters, but because of the shallow nature of many areas, some harvesters must work on tides.

Virginia (VA) has only had single digit Vp cases each year since 2004. This despite the annual oyster harvest increasing from 20,000 bushels in 2004 to 70,000 bushels[6] in 2016 (ICMSS, 2017).

VA Vp Control Plan

Virginia's state plan manages the risk for both Vp and Vv. As the Vv controls tend to be stricter, these controls drive the plan. The Vp/Vv Control Plan is activated during the period May 1st – October 31st.

Harvesters have four pre-harvest options to choose from. All controls except for the curfew option requires additional permits:

1) Curfews. Harvest must be landed and under refrigeration by:

- May 1st - May 31st: 11am
- June 1st – August 31st: 10am
- September 1st - September 30th: 12pm

2) On-board icing/refrigeration. Harvesters must continuously ice/refrigerate for the duration of harvest.

3) Dock-to-temperature control time limits with GPS tracking. Harvesters must carry a GPS timing device during harvest, and have their catch under temperature control by: May-5 hours; June-3 hours; July-August 2 hours

4) Labelling "For Shucking or PHP Only". The regulation requires that a Certified VA Shucker-Packer obtain the permit and shuck all green tagged product. VA regulations do not allow the product to be shipped into interstate commerce with a green tag.

The post-harvest control is that the shellstock must be cooled to 55°F (13°C) within 5 hours. Most harvesters start icing on the boat. Some operators use mechanical refrigeration, a few use the ice slurry method. HACCP recordkeeping is required to document cooling times.

iv) Washington State

As previously discussed Washington (WA) state has a history of Vp Illness, including the first USA outbreaks caused by the O4:K12 or ST36 serotype.

The WA oyster industry is primarily aquacultured Pacific oysters (*Crassostrea gigas*) but the commercial operations vary. For example, Turners Oysters is the USA's largest commercial operator

[6] A ***bushel of oysters*** is a USA industry measurement. It routinely contains weighs between 45 and 60 pounds (20 - 27 kg) and contains between 100 and 150 oysters.

and WA also has multiple small and self-employed operators. There is also the full range of oyster production methods: subtidal and intertidal harvesting of ranched product on the substrate; aquaculture oysters in bags pinned to the seafloor; oysters grown on racks, baskets, mesh trays, bags attached to racks in the intertidal zone; and subtidal long-lines.

WA Vp Control Plan

The WA plan uses a risk-based system, based on a rolling five-year average of vibriosis cases to calculate the risk categories.

Category 1: 0.2 or fewer cases attributed to the growing area.

Category 2: 0.3 to 1 cases attributed to the growing area.

Category 3: 1 or more cases attributed to the growing area.

The state plan requires all harvesters and dealers, intending to harvest or be an original dealer of shellstock oysters from May through September, to have an approved Vp harvest plan. Harvest plans must be submitted to the WA State officials prior to March 1st for initial approval. Provided the plan is approved and no changes are made in subsequent years, then those companies would just need to keep it on file and sign and date the plan to meet the requirements annually.

Depending on the harvesting area's risk category, there are time/temperature requirements. For example:

Category I Requirements: Time to Cooling: Except as noted below, the time of harvest to cooling requirement from June 1st through September 30th is: 9 hours.

When ambient air temperature at harvest is greater than 90°F (32°C), the time of harvest to cooling requirement is: 7 hours.

When harvest temperature is between 68°F (20°C) and 70°F (21°C) from July 1st through August 31st, the time of harvest to cooling requirement is: 5 hours. Harvest Control: From July 1st through August 31st, harvest is not allowed for twenty-four hours when harvest temperature[7] of product is above 70°F (21°C).

Category 2 Requirements: Time from harvest to cooling May 1st – 30th September is 7 hours.

When ambient air temperature is greater than 85°F (29°C) time to cooling is 5 hours.

When harvest temperature is between 66°F (15°C) and 68°F (20°C) from July 1st through August 31st, time to cooling is 3 hours.

From July 1st through August 31st, harvest is not allowed for twenty-four hours when harvest temperature is above 68°F (20°C).

Category 3 Requirements: time of harvest to cooling requirement from May 1st through September 30th is 5 hours.

When ambient air temperature at harvest is greater than 80°F (27°C, the time of harvest to cooling requirement is 3 hours.

When harvest temperature is between 64°F (17°C) and 66°F (15°C) from July 1st through August 31st, the time of harvest to cooling requirement is I hour.

[7] **Harvest temperature** means the water temperature or internal oyster tissue temperature at the time of harvest.

From July 1st through August 31st, harvest is not allowed for twenty-four hours when harvest temperature is above 66°F (15°C).

4. PRACTICES USED TO MITIGATE Vp FOOD SAFETY ISSUES

Due to the complex evolutionary and environmental variables causing Vp there is scant predictability as to when, and where, Vp will cause food safety issues. As a result, other countries implement specific governance when Vp illnesses exceed national epidemiological thresholds. The suite of management practices implemented depends on national policies and the local environmental variables previously linked to Vp illness. Such practices can be singular or multi-prong, including: harvest area closures; pre-and post-harvest processes to mitigate and/or eliminate viable Vp; and public education about the Vp risks (see Table 2).

Table 2: Mitigation techniques used in regions where Vp illness has occurred.

Control Point in Production Chain	Process
Pre-harvest Controls	• Deep water suspension of cultures. • Relaying. • Re-submerging.
Harvesting controls	• Cease harvesting for raw product market during the high risk Vp season. • Suspend intertidal harvesting for raw product market. • Harvesting curfews based on tidal conditions or time conditions. • Shading of shellstock on harvesting vessels.
Post-harvest controls	• Divert product for shucking (cooked product) market. • Rapid cooling of oysters using ice and ice slurries on board vessels (Rapidly cools products to <10°C in 20 minutes and maintains the cold chain (4°C)). • Other cooling systems on the harvest vessel. • Establishing time/temperature controls for harvested product. • Adequate refrigeration at the distribution, retail and restaurant levels is important. Monitoring of temperature is being performed on shipments of oysters upon arrival at these various stages. • Cooling after landing. Maintaining seafood temperature at or below 10°C during distribution and storage (Japan).
Processing conditions	• Oysters are processed in an environment which is temperature controlled. • Use disinfected or artificial seawater, or potable water, for washing and processing seafood. • Processing companies operate under a HACCP plan which includes Critical Points for incoming product.
End-product micro standard	• Japanese use Vp microbiological standards: ≤100 MPN/g for seafood intended for raw consumption, not detected/25 g for ready-to-eat boiled seafood. (n=5, c=0, m=100 Vp MPN/g).

	• Canada summer microbiological criterion for live oysters in the shell and intended for the raw market in Canada (n=5, c=0, m=100 Vp MPN/g).
Education	• US FDA and ISSC have put significant resources into educating the public and medical practitioners about the risks of vibriosis. See https://www.fda.gov/food/populartopics/ucm341987.htm and http://www.issc.org/vibrio-specific-information
Consumer Advisories **	• US FDA requires that advisory health warnings are provided when selling shellfish.

**The advisory is meant to inform consumers, especially susceptible populations (i.e. elderly, children, pregnant mothers, immunocompromised), about the increased risk of foodborne illness from eating raw or undercooked animal foods. The intent is to have the advisory conveniently displayed for consumer awareness. Therefore, the statement shall be displayed on brochures, deli cases, menus, stickers, table tents, placards, or other effective written means. An example warning advisory is:

> "Consuming raw or undercooked meats, poultry, seafood, shellfish, or eggs, may increase your risk of foodborne illness, especially if you have certain medical conditions."

5. NEW ZEALAND SITUATION

5.1. Environmental Vp prevalence

Vp infection is not notifiable in NZ unless an outbreak is detected, or the sick person has an occupation that puts others at risk of infection. Between January 1998 and July 2016 there were eight sporadic cases of Vp infection reported, where BMS (most likely from NZ) were specifically implicated as the vehicle of infection. The implicated BMS were oysters or mussels, commercially or non-commercially harvested. However, there is no evidence specifically linking these illnesses to commercial harvest areas and post- harvest cross-contamination and time temperature abuse cannot be discounted. The only documented NZ Vp outbreak was associated with a non-commercial harvest of Greenshell mussels (*Perna canaliculus*) taken from the Hauraki Gulf (McCoubrey, 2007). There have been no Vp illness outbreaks associated with commercial BMS harvesting or with Pacific oysters (*Crassostrea gigas*).

However, there is evidence that Vp is prevalent in NZ's marine environment and can be isolated from BMS species. NZ prevalence surveys (1985, 2008/9, 2009-12 and 2013-15) identified Vp concentrations to be higher in commercial BMS harvested from harbours in the north half of the North Island compared with the Marlborough Sounds. Most samples were of Pacific oysters, and Vp was detected in Pacific oysters more often and at higher concentrations during summer months compared with other seasons; when sea surface temperatures were ≥19°C. There was no significant correlation with water salinity (Vp was isolated from Pacific oysters at salinities >35‰). (ESR, 2016).

Up to 100% of Pacific oyster samples from harbours located in the upper half of the North Island yielded Vp at concentrations as high as $4.8x10^4$ MPN/g. Potentially pathogenic Vp were also detected in these samples but at lower prevalence (up to 27%) and concentrations (maximum 933 MPN *trh*+ Vp cells per gram). Vp was also detected in 42% (16/38) of samples of green-lipped mussels from northern North Island harbours in a survey from 2009-2012.

Only two Vp isolates from these three surveys were serotyped (Kirs *et al*., 2010). They were tested against O and K antisera but were not the 03:K6 pandemic strain.

General surface seawater temperature data is collected by the National Institute of Water and Atmospheric Science (NIWA). Sites in northern NZ have an annual mean coastal sea-surface temperature around 17°C, and 12°C in southern NZ.[8] The maximum temperature reported at the northern-most coastal monitoring station (Ahipara) during the period 1953-2014 was 23.8°C, and was 17.2°C in the southern-most coastal monitoring station (Bluff). NZ's climate is affected by the El Niño Southern Oscillation. The La Niña phase of this oscillation brings warmer waters to the NZ coast, generally warmer weather, and increased rainfall to the north-east of the North Island.[9] Over the last decade, the La Niña phase has been present during the summers of 2008, 2009 and 2011.[10] The 2011 phase was particularly prolonged, spanning from mid-2010 to mid-2011 (ESR, 2016).

5.2. Pacific Oyster production and harvesting practices

NZ Pacific oysters (*Crassostrea gigas*) are commercially harvested and also gathered non-commercially. There is evidence to suggest that certain ethnic groups in NZ (Māori, Pacific Islanders, Asians) comprise a greater proportion of the population involved in non-commercial shellfish harvesting (Hay *et al.*, 2000). Kai moana, harvested by Maori, is an important cultural and dietary component. A survey in the upper North Island found that 11% of households reported collecting seafood more than once a week, 31% collected seafood at least weekly, and 52% reported collecting seafood at least fortnightly (Hay *et al.*, 2000).

In 2015, 1,910 tonnes of Pacific oysters were commercially harvested, or approximately 2.4 million dozen from aquaculture operations (C. Johnston, Aquaculture New Zealand, pers. comm.). Most of the commercially harvested Pacific oyster areas are distributed around the northern half of the North Island, as far south as Kawhia on the west coast and Ohiwa (Bay of Plenty) on the east coast. A small proportion (3%, in 2011) are harvested from the Marlborough Sounds region (Aquaculture New Zealand, 2012).

NZ's Pacific aquacultured oysters are grown on racks, or in baskets, mesh trays or bags attached to racks in the intertidal zone, or sometimes on subtidal long-lines (Castinel *et al.*, 2015). The oysters grown in the subtidal zone are usually transferred to the intertidal zone for some time before harvest to harden the shells. The oysters are harvested after 12-18 months, usually during May to November when the oysters are in peak condition. Oysters spawn over the summer months and the subsequent loss in condition means harvesting during this period is limited. Even so non-commercial and commercial summer harvesting occurs, with summer oysters sold within NZ and to export markets. Over the last few years some Pacific oyster industry operators have started farming triploid oysters, which do not spawn or lose condition over the summer. This changing practice is likely to increase the summer harvest of Pacific oysters. For example, since 2015 one company has been harvesting

[8] http://www.stats.govt.nz/browse_for_stats/environment/environmental-reporting-series/environmental-indicators/Home/Marine/coastal-sea-surface-temperature.aspx (page and associated data file accessed 6th November 2017). See also http://www.stats.govt.nz/browse_for_stats/environment/environmental-reporting-series/environmental-indicators/Home/Atmosphere-and-climate/oceanic-sea-surface-temperature.aspx (accessed 6th November 2017).

[9] https://www.niwa.co.nz/climate/information-and-resources/elnino and https://www.niwa.co.nz/climate/information-and-resources/elnino/elnino-impacts-on-newzealand (accessed 6th November 2017)

[10] http://www.bom.gov.au/climate/current/soihtm1.shtml (accessed 6th November 2017). A sustained period of +7 are typical of a La Niña episode.

10,000 dozen per week in the summer period, (live as well as half shell oysters). This company expects to increase this summer harvest to 20,000 dozen in 2018/19.

5.3. BMS Food safety governance

The commercial BMS industry operate under the jurisdiction of the Ministry for Primary Industries (MPI). All commercial BMS production and harvesting must be undertaken in compliance with MPI's food safety legislation (Animal *Products (Regulated Control Scheme—Bivalve Molluscan Shellfish*) *Regulations 2006* and the *Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006)*) (BMSRCS). This BMSRCS is based on sound food safety practices, it is recognised as providing protection against microbiological, marine biotoxin and chemical hazards. NZ's BMSRCS is internationally acknowledged as a robust shellfish sanitation programme and is regularly audited by international agencies.

All BMS harvest lots must be protected from environmental contaminants and excessive sunlight, and must be placed under temperature control within prescribed geographical zone time limits. Appendix III lists the harvest zones' maximum time between harvesting and temperature control, defined as:

- 36 hours where average maximum is ≤18^{o}C;
- 24 hours where average maximum is 19-27^{o}C; and
- 20 hours where average maximum is ≥27^{o}C.

Generally, summer harvests (October – May) must be under temperature control within 24 hours and all other seasonal harvests within 36 hours.

Once placed under temperature control the storage area must be continuously maintained at 7^{o}C or cooler, sufficient to ensure that the internal temperature of the oyster reaches 10^{o}C or cooler so as to minimise all bacterial growth, including that of pathogens.

Part 13 Microbiological risk management of the *Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006* provides the principles for monitoring and managing all microbiological risks, including Vibrio species. Such principles include powers to close harvest areas when implicated in illness, and retaining closures until food safety issues have been addressed.

5.4. Comparative analysis between NZ and other countries who experience Vp illness

Pacific oysters are cultured in many countries, under a variety of natural habitats and using various production and harvesting methods. The consumption of raw Pacific oysters has caused Vp illness in specific regions of some countries, but is not a universal problem.

Vp bacteria are endemic in NZ's marine environment, with the northern region providing optimal climatic conditions suitable for Vp's survival and propagation. The consumption of commercially harvested NZ Pacific oysters has not been associated with cases of Vp illness. Specific protective mechanism/s, which might include NZ's marine biophysical features; lack of endemic pathogenic Vp; insufficient concentrations to cause a pathogenic dose; industry's production and harvesting practices; and post-harvest time-temperature controls.

Table 3 compares NZ with other countries who have experienced vibriosis.

Table 3: Comparison between NZ and other countries

New Zealand's Biophysical Features and Industry Practices	Overseas Biophysical Features and Industry Practices
Climate - Oceanic & temperate climate	Continental climate with significant seasonal and geographical temperature ranges.
Tidal features - New Zealand's semidiurnal tides (two high and low tides per day) are moderate by world standards. The tidal range is 1–2 metres.	Ranges from diurnal (daily), semi-diurnal and mixed semidiurnal (high and low tides differ in height).
Marine substrate – varies from mud, sand and rock.	Varies from mud, sand and rock.
Commercial oyster species - Pacific Oyster (*Crassostrea gigas)*	Predominately American or Eastern oyster *Crassostrea virginica.* Others = *Ostreola conchaphila* and *Crassostrea gigas*
Production methods – Aquaculture, generally inter-tidal, raised above the substrate (racks or wires), using sticks, bags and baskets. Less often grown sub-tidally, then oysters usually 'hardened off' by placing in intertidal zone.	Wild harvesting with dredges & tongs. Aquaculture methods vary from ranching, bags on substrate, racks, bags, subtidal and intertidal systems. USA often uses desiccation to remove biofouling from shellstock.
Harvesting methods – in accordance with the BMSRCS requirements. Barges used to collect aquaculture product in lots, usually when the tide is out.	Wild harvests range from small operations who hand tong a few bags daily, through to large scale dredging several tonnes onto open deck barges. Aquaculture includes dredging ranched product, harvesting of subtidal and tidal cages, trays etc. Some operators use a re-submerging system – gathering & collating stock on one tide and harvesting full lot on another.
Cooling systems –refrigerator units within the temperature control period prescribed in BMSRCS.	Ice, ice-slurries and refrigeration units on board vessels and in land based premises.
Growing area classification system – MPI generally classifies Pacific Oyster leases defined by the map references on Fisheries licence.	USA classifies large water bodies no matter whether all this water space is used for specific shellfish harvesting operations.
Incidence of Vp illness linked to oysters – NZ = Nil	USA - 0.2 per 100,000 population (2014) Canada - 0.8 -0.9 cases per 100,000 (2010) Outbreak of 85 cases associated with oysters (2015).

In summary, there are many variables that make NZ's Pacific oyster industry unique.

5.5. Discussion of International Harvesting Practices Used to mitigate Vp illness

Sections 3 and 4 describe the incidence of Vp illness and Vp management practices in other countries. It is internationally recognised that all raw BMS species provide an elevated food safety risk, therefore countries implement regulated shellfish sanitation programmes to mitigate the microbiological, marine biotoxin and chemical food safety risks.

Currently, countries only mandate specific Vibrio management controls when there is evidence of illness. When illness does occur above a defined threshold management programmes prescribe control practices designed around the seasonal environmental factors linked to illness (see Table 2). Such practices include harvest cessation; shifting shellfish to a different environment; adapted harvesting practices; and post-harvest procedures to reduce or eliminate microbial growth. The following sections discusses these options in the context of NZ Pacific oyster harvesting and handling practices.

5.5.1 Harvest cessation

The BMSRCS has the capacity to quickly close harvest areas when illnesses are epidemiologically associated with shellfish harvest areas. While such regulated closures effectively manage the emerging event, they are a reactive measure; unlike proactive systems that prevent illness occurring in the first instance. However, as NZ Pacific oysters have not yet caused Vp illness it is not currently possible to design a predictive opening/closing system based on associated environmental parameters. Given the current state of knowledge any preventive harvesting measures would need to be broad based, such as the prevention of summer oyster harvesting.

5.5.2 Relaying

There is limited overseas information on the success of relaying as a treatment step to remove Vp from BMS. However, recent US FDA studies confirm that relaying to higher salinity and/or cooler waters shows promise for reducing Vp levels, with around seven days sufficient to reduce Vp levels in oysters (ISSC, 2017 (Jones)). It should be recognised that there is the potential for Vp in the relayed lots to contaminate other shellstock growing in the new water space.

In the NZ context, such relays would be dependent on the availability of classified water space (BMSRCS classification system) exhibiting a lower Vp risk profile. All relays must comply with the BMSRCS relay conditions, including individual permits, relay cleansing studies, identified relay lots, transport conditions and adequate record keeping.

Most of the NZ's Pacific oyster crop is currently grown in the intertidal zone and there are recognised biophysical changes when Pacific oysters are grown sub-tidally, for example, softer shells.

More research is required to verify NZ relay conditions that might appropriately mitigate Vp prevalence in NZ's Pacific oysters.

5.5.3 Re-submerging after harvest

Re-submerging is used in Canada and the USA as a Vp mitigation process. The re-submerging practice is usually defined as harvesting, culling and placing oysters in larger cages for re-submerging in a deeper water body on for re-submersion by the tide. (See Section 2.3). US FDA studies found that oysters grown on both the east and west coast oysters, containing elevated Vp caused by pre-submerging conditions, returned to background levels after one tidal cycle following re-immersion (ISSC 2017 (Jones)).

If re-submerging is to be used in the NZ context, this practice would require availability of classified water space deep enough for re-submerging oyster harvest lots. Industry would need to design systems whereby the intertidal product is harvested, shifted and anchored in deeper water or submerged in the incoming tidal waters on the lease, and in a manner, that they can be directly harvested from this deeper water. For example, Taylor Shellfish Farms in Washington State, use a barge fitted with a crane to harvest the cages of re-submerged oysters.

Re-submerging options in NZ also need to be further investigated to verify Vp reduction in Pacific oysters. Such investigations need to be undertaken in conjunction with the industry, to identify suitable systems for re-submerging and subsequent harvesting. For example, systems for using intertidal leases when tidal water is covering the oysters or large scale vertical re-submersions in deeper waters. Regional water hydrographic patterns might also need to be considered.

5.5.4 Curfews of harvesting operations

Harvesting curfews aim to ensure oysters are harvested under conditions which minimise Vp growth in oysters. Examples include early morning harvests (before the heat of the day) and within specified tidal periods. The latter example is based on the scientific observation that Vp levels increase when oysters are exposed on the sunny mudflats by a receding tide, then decrease when the tidal waters recover the shellfish and filter-feeding recommences (Jones *et al.*, 2016).

However, it needs to be recognised that the climatic and tidal conditions in other countries are often different to those in NZ. Much of the USA tidal/Vp research relates to situations where oysters are grown directly on the substrate; the substrate varies; diurnal temperatures are often higher than NZ's daily maximum; and there are spatial and temporal differences in the tidal cycles.

NZ has yet to assess whether Vp increases in Pacific oysters during the intertidal period of our semi-diurnal tides. Further, research also needs to determine if Vp increases are linked to seasonal or diurnal tidal factors, for example summer or midday low tides.

If instigated in NZ, such curfew practices would need to be factored into the industry harvesting operations, while also ensuring any post-harvest transport arrangements comply the BMSRCS time/temperature requirements.

5.5.5 Post-harvest temperature controls

All shellfish sanitation programmes have specific time/temperature and transport controls. Once the product has been placed under temperature control this must be maintained (aside for short transit periods in the transport chain).

In North American countries (Canada, Mexico and USA) the Vp control plans often prescribe a maximum time outside of time/temperature control with some states requiring immediate cooling with ice or ice-slurries at the time of harvest. This requirement has meant significant changes to industry harvest practices, with engineering innovations needed to establish icing and ice-slurry systems on harvesting vessels. There are significant costs associated with icing/ice slurry cooling on harvesting vessels. These include the harvest vessel modifications necessary for the cooling functions and the availability of potable ice, particularly in remote areas.

However, as discussed in Section 2.3 ice slurries have been associated with increased contamination with faecal coliforms, *Clostridium perfringens*, Vv and total Vp. Another study found that on-board and dockside icing did not predictably reduce the concentration of Vp in oysters, and icing significantly and negatively affected oyster survival (Melody *et al.*, 2008). Such immediate icing/cooling systems should be investigated within the NZ context.

NZ's current BMSRCS time temperature controls have to date, adequately prevented microbial foodborne illness, and met international market access requirements. Unless there is evidence of a NZ Vp illness there is no evidence for the need of stricter time temperature controls. Regulatory adjustments, requiring more restrictive time temperature controls, will escalate industry costs, both the effort to practically implement in the field and the finance needed to re-engineer the current refrigeration facilities.

5.5.6 Post-harvest Processing

Overseas there is the option to divert product, deemed to be Vp contaminated, away from the raw oyster markets. In such cases oysters are shucked for cooking or can be redirected to validated to post harvest treatments. In the USA, the assumption is that all shucked oysters are cooked, and therefore NSSP Vp controls focus on half shell oysters which are anticipated will be eaten raw.

Such culinary assumptions cannot be made in NZ, as shucked oysters are often used in raw oyster recipes. A previous NZ food poisoning incident, involving norovirus contaminated raw oysters from Korea (Simmons, 2007), highlighted handling mistakes can occur even when packaging is clearly labelled with advisories that oysters must be cooked before consumption.

In NZ the Pacific oyster industry prides itself on taking the positive marketing stance that all oysters (raw and processed) can be considered safe and quality products.

6. NZ CONCLUSIONS AND RECOMMENDATIONS

NZ does not currently have an endemic vibriosis problem associated with the consumption of Pacific oysters (or any other commercial BMS species). The reason for the absence of illness is unknown, particularly as Vp is present in NZ's marine environment. However, when compared to those countries experiencing a chronic vibriosis problem, NZ's Pacific oyster industry operates under a different combination of biophysical features and production/harvesting practices.

NZ currently complies with the international 'best management practice' used to prevent Vp illness. Internationally, specific Vp harvest controls around are only implemented, when and where, national food safety authorities deem Vp illnesses have breached illness thresholds. In such situations Vp management controls are crafted for each harvest area because the environmental triggers causing Vp illness vary geographically (and possibly seasonally). Overseas management options include harvest cessation, harvesting curfews, relaying, post-harvest re-submerging, post-harvest temperature controls and post-harvest processing.

While other countries have devised regulatory Vp controls it should not be assumed that such management controls can be quickly transferred to, or are suitable, for NZ's conditions. The above list of overseas management options need to be explored within the NZ context. It should also be noted that while these controls have the potential to mitigate illness, it should not be assumed they will eliminate sporadic Vp cases or outbreaks.

Due to bacterial evolution, climate variance and changing industry practices the risk of Vp illness is constantly changing throughout the world. Therefore, NZ should be prepared to deal with future Vp illnesses.

Given these conclusions it is recommended that the following steps be undertaken:

1) Start the conversation – NZ's Pacific oyster industry does not yet have a Vp problem, but it is possible future illnesses will occur, with the event quickly emerging. Therefore, it is important that a discussion be started prior to emergency events on how all parties (industry and regulators) might best deal with such an episode.

Questions should be posed, for example:

1. Would the best management option be to just close the implicated area until the Vibrio event passes?
2. What defines an event, e.g. illnesses or elevated levels of pathogenic Vp strains?
3. Are there deep-water relay options available?
4. Is it possible to introduce post-harvest quicker cooling systems?
5. Does the NZ industry have their own ideas on what might work or their preferred management options?

2) Consider how science might assist – internationally there has been much Vp research, including NZ's environmental prevalence surveys. In countries experiencing chronic vibriosis problems, specific mitigation steps have been established. These steps need further research within the NZ context. For example:

1. Does a Vp increase/decrease within oysters over the tidal cycle?
2. Are diurnal harvesting curfews necessary in the summer?
3. Does relaying to deeper water have the potential to reduce Vp levels in NZ Pacific oysters?
4. Does NZ have the capacity and capability to differentiate between total Vp and pathogenic strains. Based on what virulence factors?
5. Does NZ have the capacity and capability to determine the Vp strain type?
6. If so, how would such strain information be used for management purposes?

The discussions with industry should assist formulate the priority NZ research areas, so that practical and potentially applied knowledge can be gained.

3) Consider governance options – other countries experiencing vibriosis have undergone much angst over how to manage Vp's complicated and messy factors. Some countries use regulations, others industry guidelines, while some have made an active decision not to use any form of governance.

It is important that the regulatory authority, in conjunction with the Pacific Oyster industry, consider how potential management options might work in NZ. Options such as harvesting curfews, relaying and re-submerging practices, and post-harvest time temperature controls. For example:

1. would relaying or re-submerging need to comply with the BMSRCS relay protocols?
2. How might harvesting curfews work?
3. Are there validated post-harvest treatment systems that Vp contaminated product could be directed towards?

In summary, *Vibrio parahaemolyticus* is a complicated organism which is confounding the world's oyster industry and food safety regulators. If NZ wants to be best prepared to deal with the consequences of any foodborne vibriosis, there needs to be timely investigations and discussions, prior to the event of Vp illnesses.

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APPENDIX I TECHNICAL DETAILS ON POST-HARVESTING PROCESSES

High Pressure Processing - It has been found that HPP inactivates Vp by damaging the cell membrane, cell wall and degrading cellular proteins (Wang *et al.*, 2013). Combining HPP with low temperature pasteurisation has a synergistic effect on killing Vp (Ye *et al.*, 2012).

An HPP of 293 MPa for two minutes at 8°C reduced the concentration of Vp in Pacific oysters by >3.52 log MPN/g (Ma and Su, 2011). Oysters processed in this way had a shelf life of 6-8 days when stored at 5°C or 16-18 days when stored in ice. A treatment of 275 MPa or more for two minutes at 21°C achieved the same *V. vulnificus* reduction (>3.52 log MPN/g) in Atlantic oysters (*Crassostrea virginica*) (Ye *et al.*, 2012). A pressure of 300 MPa was required to achieve a reduction of >5 log MPN/g.

Two studies found that lowering the temperature of the HPP process improved its effectiveness against Vp, but the experimental conditions were not realistic (one study used inoculated oyster homogenates, the other pre-sterilised oysters) (Kural and Chen, 2008; Phuvasate and Su, 2015). Another study, using shucked oysters, did not identify the HPP temperature as being important (Ye *et al.*, 2013).

Irradiation involves exposing BMS to ionising energy, either gamma rays, machine-generated electrons or X-rays. *Vibrio* spp. are among the most radiation-sensitive bacteria. Experiments with oysters have found that the shellfish usually survive low dose irradiation and consumers could not tell the difference between irradiated and non-irradiated oysters (Andrews *et al.*, 2003a; Drake *et al.*, 2007; Jakabi *et al.*, 2003; Thupila *et al.*, 2011). However, irradiation has been reported to decrease shelf-life of oysters (Dixon and Rodrick, 1998).

An ionising irradiation dose of 1.0 kGy reduced Vp artificially bioaccumulated in whole shell oysters by 4-6 log MPN/g (Jakabi *et al.*, 2003). A 4-log reduction of *V. parahaemolyticus* O3:K6 in whole shell oysters was achieved with an ionising irradiation dose of 1.5 kGy (Andrews *et al.*, 2003a).

An X-ray dose of 1.5 kGy was needed to generate a 5-log reduction in the concentration of artificially bioaccumulated *V. parahaemolyticus* in oysters treated as half-shells, but the dose had to be increased to 5.0 kGy to achieve the same reduction in whole shell oysters (Mahmoud and Burrage, 2009). The oysters were able to survive a treatment of 3 kGy followed by storage at (5°C) for up to seven days.

Thermal processes - Thermal processes such as cold storage, freezing, and low temperature pasteurization have been reported capable of achieving certain reductions of *Vibrio* species in oysters. Thompson and Vanderzant (1976) reported that populations of *V. parahaemolyticus* in shucked oysters decreased from >11,000 to 0.36 MPN/g after 7 days of storage at 3°C. Muntada-Garriga *et al.* (1995) reported that viable cells of *V. parahaemolyticus* (10^{5-7} cfu/g) in oyster homogenates were completely inactivated by freezing at −18°C and −24°C for 15–28 weeks depending on initial populations of the microorganism and freezing temperatures. Andrews *et al.*, (2000) developed a low-temperature pasteurization for shellstock oysters by placing the oysters in 55°C water to achieve an internal temperature of 48–50°C for 5 min. The authors reported that the process reduced *V. parahaemolyticus* in oysters (1.2×10^5 MPN/g) to non-detectable levels (<3 MPN/g). An added benefit of the mild heat treatment is that oysters are often killed and shucked automatically during the treatment. Therefore, oysters need to be banded before being processed to prevent loss of juice during treatments. A major disadvantage of the pasteurization process is that it may cause changes in oyster texture due to protein denaturation occurred during the heat treatment.

APPENDIX II: USA Vp food safety regulations

NSSP Chapter II Risk Management and Risk Assessment

@.02 Shellfish Related Illnesses Associated with *Vibrio parahaemolyticus* (V.p.)

A. When the investigation outlined in Section @.01 A. indicates the illness(es) are associated with the naturally occurring pathogen *Vibrio parahaemolyticus* (V.p.), the Authority shall determine the number of laboratory confirmed cases epidemiologically associated with the implicated area and actions taken by the Authority will be based on the number of cases and the span of time as follows.

(1) When sporadic cases do not exceed a risk of one (1) illness per 100,000 servings or involves at least two (2) but not more than four (4) cases occurring within a thirty (30) day period from an implicated area in which no two (2) cases occurred from a single harvest day, the Authority shall determine the extent of the implicated area. The Authority will make reasonable attempts to ensure compliance with the existing Vibrio Management Plan.

(2) When the risk exceeds one (1) illness per 100,000 servings within a thirty (30) day period or when cases exceed four (4) but not more than ten (10) over a thirty (30) day period from the implicated area or two (2) or more cases but less than four (4) cases occur from a single harvest day from the implicated area, the Authority shall:

(a) Determine the extent of the implicated area; and

(b) Immediately place the implicated portion(s) of the harvest area(s) in the closed status; and

(c) As soon as determined by the Authority, transmit to the FDA and receiving States information identifying the dealers shipping the implicated shellfish.

(3) When the number of cases exceeds ten (10) illnesses within a thirty (30) day period from the implicated area or four (4) or more cases occurred from a single harvest date from the implicated area, The Authority shall:

(a) Determine the extent of the implicated area; and

b) Immediately place the implicated portion(s) of the harvest area(s) in the closed status; and

(c) Promptly initiate a voluntary industry recall consistent with the Recall Enforcement Policy, Title 21 CFR Part 7 unless the Authority determines that a recall is not required where the implicated product is no longer available on the market or when the Authority determines that a recall would not be effective in preventing additional illnesses. The recall shall include all implicated products.

(d) Issue a consumer advisory for all shellfish (or species implicated in the illness).

(4) When a growing area has been closed as a result of V.p. cases, the Authority shall keep the area closed for the following periods of time to determine if additional illnesses have occurred:

(a) The area will remain closed for a minimum of fourteen (14) days when the risk exceeds one (1) illness per 100,000 servings within a thirty (30) day period or cases exceed four (4) but not more than ten (10) cases over a thirty (30) day period from the implicated area or two (2) or more cases but less than four (4) cases occur from a single harvest date from the implicated area.

(b) The area will remain closed for a minimum of twenty-one (21) days when the number of cases exceeds ten (10) illnesses within thirty (30) days or four (4) cases occur from a single harvest date from the implicated area

(5) Prior to reopening an area closed as a result of the number of cases exceeding ten (10) illnesses within thirty (30) days or four (4) cases from a single harvest date from the implicated area, the Authority shall:

(a) Collect and analyze samples to ensure that tdh does not exceed 10/g and trh does not exceed 10/g; or other such values as determined appropriate by the Authority based on studies.

(b) Ensure that environmental conditions have returned to levels not associated with V.p. cases.

(6) Shellfish harvesting may occur in an area closed as a result of V.p. illnesses when the Authority implements one or more of the following controls:

(a) Post-harvest processing using a process that has been validated to achieve a two (2) log reduction in the levels of total *Vibrio parahaemolyticus* for Gulf and Atlantic Coast oysters and/or hard clams and a three (3) log reduction for Pacific Coast oysters and/or hard clams;

(b) Restricting oyster and/or hard clam harvest to product that is labeled for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing;

(c) Other control measures that based on appropriate scientific studies are designed to ensure that the risk of V.p. illness is no longer reasonably likely to occur, as approved by the Authority.

@. 03 Annual Assessment of *Vibrio vulnificus* and *Vibrio parahaemolyticus* Illnesses and Shellfish Production.

A. The Authority shall assess annually *Vibrio vulnificus* and *Vibrio parahaemolyticus* illnesses associated with the consumption of molluscan shellfish. The assessment will include a record of all *Vibrio vulnificus* and *Vibrio parahaemolyticus* shellfish-associated illnesses reported within the State and from receiving States, the numbers of illnesses per event, and actions taken by the Authority in response to the illnesses.

B. The Authority shall collect by month and report annually to the ISSC the volume of shellfish harvested in the State. The report shall include the volume of shellfish harvested for each species. Where available the volume breakdown of the production data will be reported by utilization type (raw, shucked, PHP, etc.).

@.07 *Vibrio parahaemolyticus* Control Plan

The goal of the Control Plan is to reduce the probability of occurrence of *Vibrio parahaemolyticus* (V.p.) illness during periods that have been historically associated with annual illnesses. The Plan is to be implemented as part of a comprehensive program which includes all the time and temperature requirements contained in the Model Ordinance.

A. Independent Species-Specific Risk Evaluation. Every State from which oysters or hard clams (*Mercenaria mercenaria*) are harvested shall conduct a Vibrio parahaemolyticus risk evaluation annually. The evaluation shall consider each of the following factors, including seasonal variations in the factors, in determining whether the risk of Vibrio parahaemolyticus infection from the consumption of oysters or hard clams harvested from an area (hydrological, geographical, or growing) is reasonably likely to occur: (For the purposes of this section, "reasonably likely to occur" shall mean that the risk constitutes an annual occurrence)

(1) The number of *Vibrio parahaemolyticus* cases epidemiologically linked to the consumption of oysters or hard clams commercially harvested from the State; and

(2) Levels of total and tdh+ *Vibrio parahaemolyticus* in the area, to the extent that such data exists; and

(3) The water temperatures in the area; and

(4) The air temperatures in the area; and

(5) Salinity in the area; and

(6) Harvesting techniques in the area; and

(7) The quantity of harvest from the area and its uses i.e. shucking, half-shell, PHP.

B. Independent Species Specific Control Plan

(1) If a State's *Vibrio parahaemolyticus* risk evaluation determines that the risk of *Vibrio parahaemolyticus* illness from the consumption of oysters or hard clams harvested from a growing area is reasonably likely to occur, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan; or

(2) If a State has a shellfish growing area in which harvesting occurs at a time when average monthly daytime water temperatures exceed those listed below, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan. The average water temperatures representative of harvesting conditions (for a period not to exceed thirty (30) days) that prompt the need for a Control Plan are:

(a) Waters bordering the Pacific Ocean: 60 °F (15°C).

(b) Waters bordering the Gulf of Mexico and Atlantic Ocean (NJ and south): 81°F (27°C).

(c) Waters bordering the Atlantic Ocean (NY and north): 60 °F (15°C).

(d) However, development of a Plan is not necessary if the State conducts a risk evaluation, as described in Section A. that determines that it is not reasonably likely that *Vibrio parahaemolyticus* illness will occur from the consumption of oysters or hard clams harvested from those areas.

(i) In conducting the evaluation, the State shall evaluate the factors listed in Section A. for the area during periods when the temperatures exceed those listed in this section;

(ii) In concluding that the risk is not reasonably likely to occur, the State shall consider how the factors listed in Section A. differ in the area being assessed from other areas in the state and adjoining states that have been the source of shellfish that have been epidemiologically linked to cases of *Vibrio parahaemolyticus* illness; or

(3) If a State has a shellfish growing area that was the source of oysters or hard clams that were epidemiologically linked to an outbreak of Vibrio parahaemolyticus within the prior five (5) years, the State shall develop and implement a Vibrio parahaemolyticus Control Plan for the area.

(4) For States required to implement *Vibrio parahaemolyticus* Control Plans, the Plan shall include the administrative procedures and resources necessary to accomplish the following:

(a) Establish one or more triggers for when control measures are needed. These triggers shall be the temperatures in Section B. (2) where they apply, or other triggers as determined by the risk evaluation. (b) Implement one or more control measures to reduce the risk of *Vibrio parahaemolyticus* illness at times when it is reasonably likely to occur. The control measures may include:

(i) Post-harvest processing using a process that has been validated to achieve a two (2) log reduction in the levels of total *Vibrio parahaemolyticus* for Gulf and Atlantic Coast oysters and hard clams and a three (3) log reduction for the Pacific Coast oysters;

(ii) Closing the area to oyster and/or hard clam harvest;

(iii) Restricting oyster and/or hard clam harvest to product that is labeled for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing;

(iv) Limiting time from harvest to refrigeration to no more than five (5) hours, or other times based on modeling or sampling, as determined by the Authority in consultation with FDA;

(v) Limiting time from harvest to refrigeration such that the levels of total *Vibrio parahaemolyticus* after the completion of initial cooling to 60 °F (internal temperature of the oysters or hard clams) do not exceed the average levels from the harvest water at time of harvest by more than 0.75 logarithms, based on sampling or modeling, as approved by the Authority;

(vi) Other control measures that based on appropriate scientific studies are designed to ensure that the risk of V.p. illness is no longer reasonably likely to occur, as approved by the Authority.

(c) Require the original dealer to cool oysters and/or hard clams to an internal temperature of 50 °F (10 °C) or below within ten (10) hours or less as determined by the Authority after placement into refrigeration during periods when the risk of *Vibrio parahaemolyticus* illness is reasonably likely to occur. The dealer's HACCP Plan shall include controls necessary to ensure, document and verify that the internal temperature of oysters and/or hard clams has reached 50 °F (10 °C) or below within ten (10) hours or less as determined by the Authority of being placed into refrigeration. When deemed appropriate by the Authority an exception may be permitted for hard clams to allow for tempering. Oysters and/or hard clams without proper HACCP records demonstrating compliance with this cooling requirement shall be diverted to PHP or labeled "for shucking only", or other means to allow the hazard to be addressed by further processing.

(d) Evaluate the effectiveness of the Plan.

(e) Modify the Control Plan when the evaluation shows the Plan is ineffective, or when new information is available or new technology makes this prudent as determined by the National Shellfish Sanitation Program (NSSP) Guide for the Control of Molluscan Shellfish: 2015 Revision.

f) Optional cost benefit analysis of the Vibrio parahaemolyticus Control Plan. C. The Time When Harvest Begins For the purpose of time to temperature control, time begins once the first shellstock harvested is no longer submerged.

NOTE: Implementation will be delayed until June 1, 2015, for States not involved with V.p. outbreaks in clams to allow adequate time for States to work with industry to develop enforceable clam tempering plans.

APPENDIX III: NZ Mean daily maximum temperature statistics

3 **Table 4B: Mean daily maximum temperature statistics in degrees Celsius**

(Based on data for the period 1999 – 2003)

Location	J	F	M	A	M	J	J	A	S	O	N	D
Kaitaia	24	24	23	21	19	17	16	16	18	19	20	22
Kerikeri	24	24	23	21	19	17	16	16	18	19	20	23
Dargaville	25	25	25	22	20	18	17	17	19	21	21	23
Whangarei Airport	24	24	23	20	19	17	15	16	18	19	20	23
Warkworth	22	23	22	20	18	16	14	15	17	18	19	20
Port Fitzroy	24	24	22	20	19	17	16	16	17	19	20	na
Coromandel	23	23	22	20	18	15	14	15	16	18	19	22
Whakatane Airport	24	24	23	20	18	16	15	15	17	19	20	23
Port Taharoa (Kawhia)	23	24	23	20	18	16	15	15	16	18	19	21
Gisborne Airport	24	24	22	20	18	16	14	15	18	19	20	23
Napier Airport	23	23	22	19	18	16	14	15	17	19	20	23
Nelson Park, Hastings	24	24	23	20	18	16	14	16	18	20	20	24
Palmerston North Airport	23	24	23	19	16	14	13	14	16	17	18	21
Riwaka, Motueka	23	23	22	18	17	14	13	14	16	18	19	22
Crail Bay, Pelorous Sound	22	23	22	18	16	14	13	14	16	17	18	21
Akaroa, Bank's Peninsula	22	22	21	17	16	14	12	13	16	17	18	22
Musselburgh, Dunedin	18	19	18	15	14	12	10	11	14	15	15	18
Oban, Stewart Island	17	18	16	14	13	11	10	11	13	14	14	17