New Zealand Food Safety
Haumaru Kai Aotearoa

RISK PROFILE:

VIBRIO VULNIFICUS IN BIVALVE MOLLUSCAN SHELLFISH

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Prepared for the Ministry for Primary Industries
By Nicola King, Dorothy-Jean McCoubrey and Peter Cressey ESR

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Scientific Interpretative Summary

This SIS is prepared by MPI risk assessors to provide context to the following report for MPI risk managers and external readers

Risk Profile: Vibrio vulnificus in bivalve molluscan shellfish

ESR Report FW16032

Vibrio vulnificus is present in New Zealand bivalve molluscan shellfish (BMS), in the areas where it survives in New Zealand's coastal waters higher temperatures and lower salinity.

While susceptible consumers of raw BMS harvested from these waters, particularly during summer and from the northern half of the North Island, may be at risk of V. vulnificus foodborne infection, it is currently impossible to quantify the risk of foodborne infection with V. vulnificus as major uncertainties remain regarding the ability to identify pathogenic from non-pathogenic strains, the population susceptibility and the dose-response relationship.

Until mid-2016, there have been few reported cases possibly reflecting (a) low prevalence of pathogenic strains compared to the overall concentration of vibrios, (b) consumption of BMS from southern locations and during colder months, (c) the small proportion of the NZ population consuming raw BMS, and (d) effective cool-chain requirements for industry.

Unfortunately, this Risk Profile concludes that there are insufficient data to accurately estimate the risk to New Zealand consumers of V. vulnificus from BMS and other seafood harvested in New Zealand. These include prevalence data from mussels and non-commercially gathered species and those harvested from regions other than the north, and the effect of environmental factors and time/temperature profiles from harvest to point-of-sale. The incidence of gastroenteritis in New Zealand as a result of V. vulnificus infection is poorly understood, as are the determinants of pathogenicity of V. vulnificus and its dose-response.

MPI concludes that the current risk to New Zealanders of V. vulnificus infection from commercially harvested BMS appears low considering the current small number of cases being reported. MPI will, however, reassess this risk if the number of reported cases increases.

However, the risk may rise as coastal water temperatures increase as a result of climate-change. The concentration of V. vulnificus in the marine environment is highly correlated with water temperature (to a lesser extent salinity and turbidity), especially during summer months, although the correlation is less for pathogenic strains of V. vulnificus.

MPI intends to monitor changes in temperature and salinity of New Zealand's coastal waters and reassess the risk to New Zealand consumers of V. vulnificus from BMS should these environmental determinants change. The effect of any changes on the risk of Vibrio parahaemolyticus and ciguatoxins, will also be considered in any further projects on climate change.

RISK PROFILE:

VIBRIO VULNIFICUS IN BIVALVE MOLLUSCAN SHELLFISH

With a specific focus on
V. vulnificus in Pacific oysters

October 2016

Prepared by:

Nicola King, Dorothy-Jean McCoubrey and Peter Cressey

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REVIEWED BY: **Dr Rob Lake and Dr Lucia Rivas**

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Manager	Peer reviewers	Authors
		
Dr Rob Lake	Dr Rob Lake[1] Dr Lucia Rivas[2]	Nicola King[1] DJ McCoubrey[2] Peter Cressey[1]
Manager, Risk and Response Group, ESR Christchurch	[1]Risk and Response Group, ESR Christchurch [2]Microbiology Group, ESR Christchurch	[1]Risk and Response Group, ESR Christchurch [2]Dorothy-Jean & Associates Ltd.

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SUMMARY

This Risk Profile considers *Vibrio vulnificus* in New Zealand bivalve molluscan shellfish (BMS). The risk is considered for BMS harvested in New Zealand from wild stocks (commercial or non-commercial harvesting) and from aquaculture (commercial production) and consumed raw. The risk from imported BMS is also discussed. Consumption of Pacific oysters (*Crassostrea gigas*) that have been harvested from New Zealand waters during the summer months has been identified as a potential risk for *Vibrio* spp. infection, so this Risk Profile includes a specific focus on this food.

V. vulnificus live naturally in coastal marine environments and their presence is not related to faecal contamination or discharges from human activities. These microorganisms can become concentrated inside BMS as the shellfish filter water to obtain food. The most important environmental parameters influencing the presence and concentration of *V. vulnificus* in the marine environment and in BMS are water temperature and water salinity. The highest concentrations of *V. vulnificus* have been detected in waters and BMS when the water temperature was ≥20ºC. The relationship with salinity is more complex; the highest concentrations of *V. vulnificus* are usually measured at salinities of 5-25‰, but there is considerable regional variability. Changes in water temperatures and salinities caused by climate change will influence the distribution and abundance of *V. vulnificus*.

There are data on *V. vulnificus* in BMS harvested from New Zealand waters. Most data comes from Pacific oyster samples. Surveys during the period 2008-2016 detected *V. vulnificus* in Pacific oysters sampled from harbours located in the Northland, Auckland and Coromandel regions (prevalence up to 43%), but in only one sample from the Marlborough Sounds region. The concentration of *V. vulnificus* in most positive samples was ≤10 MPN/g. The highest concentration measured was 9,300 MPN/g, in 2011. Molecular analyses of 30 *V. vulnificus* isolates suggested that the majority of strains of *V. vulnificus* found in New Zealand Pacific oysters are of a type less associated with human disease. *V. vulnificus* were detected in Pacific oysters more often and at higher concentrations during summer months compared with other seasons, when sea surface temperatures were ≥20ºC. There was no correlation with salinity. *V. vulnificus* were isolated from Pacific oysters at salinities up to 37‰. Preliminary data from one study suggested that rainfall events increased the numbers of *V. vulnificus* in Pacific oysters in one harbour. *V. vulnificus* were not isolated from 21 samples of commercially-grown dredge oysters nor from 55 samples of green-lipped mussels in a survey from 2009-2012. A 1995/96 study found *V. vulnificus* in non-commercially harvested pipi and cockles.

V. vulnificus causes three types of human infection. Wound infections, which can lead to primary septicaemia, are not foodborne and not considered in this Risk Profile. Foodborne exposure to can lead to gastroenteritis, or to primary septicaemia in people with underlying health conditions. Primary septicaemia is a serious condition and approximately half of infected patients die. Internationally, gastrointestinal infections without septicaemia appear to be rare, but will be underreported because patients usually do not require medical care. In New Zealand, infection with *Vibrio* spp. is not notifiable unless an outbreak is detected or the sick person has an occupation that puts others at risk of infection. This will also contribute to underreporting in this country. No cases of *V. vulnificus* infection were reported to New Zealand's public health surveillance system, EpiSurv, during the period January 1998 to July 2016. Five cases of *V. vulnificus* infection have been reported in other records since 1989 but none were identified as foodborne. It is notable that cases of gastrointestinal disease caused by *Vibrio parahaemolyticus* have been reported in New Zealand, despite similar challenges with underreporting. It appears that cases of *V. vulnificus* are rare in New Zealand, and that gastroenteritis in people without underlying health conditions occurs infrequently.

Uncertainty in the science of *V. vulnificus* means that three assumptions are necessary for this assessment of risk:

- All strains of *V. vulnificus* are equally virulent and potentially able to cause disease (gastroenteritis or primary septicaemia): Work to date indicates that potentially pathogenic strains of *V. vulnificus* can be identified from those less likely to cause human illness, but there is no single or suite of markers that can predict the ability of an isolate to cause disease, with certainty.
- All members of the susceptible New Zealand population (those with underlying health conditions, particularly liver disease and immunosuppression) are equally susceptible to primary septicaemia and gastroenteritis. Some members of the general New Zealand population may be susceptible to gastroenteritis.
- The presence of *V. vulnificus* in BMS at any concentration has the potential to cause illness: The dose of *V. vulnificus* required to cause gastroenteritis or septicaemia is not known. Estimates of 10^3 and 10^4 cells have been made for people with pre-existing health conditions that make them more susceptible to infection, but the actual dose may be lower, or higher.

These assumptions mean that the following assessment overestimates risk.

This Risk Profile answers three Risk Management Questions (RMQs).

RMQ1: What is the risk to human health from *V. vulnificus* in BMS consumed in New Zealand?

Based on the available information, and the assumptions above, those in the susceptible population are at risk of foodborne *V. vulnificus* infection (gastroenteritis or primary septicaemia) from BMS harvested from New Zealand waters and consumed raw. The risk is greatest when the BMS consumed raw are those harvested during the summer months from waters in the northern half of the North Island.

This assessment of risk suggests that there should be cases of *V. vulnificus* infection reported regularly in New Zealand, but there is currently no evidence of this. The extent to which *V. vulnificus* cases are underreported is not known, but other factors may be contributing to this apparent discrepancy. The concentration of *V. vulnificus* measured in most positive samples of Pacific oysters harvested from New Zealand waters was ≤10 MPN/g and an analysis of 30 isolates found the majority to be those less likely to cause human disease (data on other BMS species, and on BMS from other geographical regions are scarce). Population exposure is low; BMS are consumed by only a small proportion of New Zealanders on a daily basis (estimates of 1.5% adults in 2009, 0.5% children in 2002) and the shellfish are cooked in approximately two-thirds of servings. Of the oysters consumed raw, we predict from available data that the majority are likely to be dredge oysters sourced from southern, Foveaux Strait waters, rather than Pacific oysters. Furthermore, the size of the susceptible population and their BMS consumption patterns have not been properly estimated for New Zealand.

There are insufficient data to determine the risk to New Zealand consumers of *V. vulnificus* infection from imported BMS.

RMQ2: Does the commercial harvest of Pacific oysters in New Zealand during the summer months pose a public health risk for consumers of this food with respect to *V. vulnificus*?

The commercial harvest of Pacific oysters in New Zealand during the summer months poses a public health risk for consumers of this food with respect to *V. vulnificus*, particularly BMS consumers in the susceptible population. Pacific oysters harvested from New Zealand waters

during the summer months are more likely to contain *V. vulnificus* than oysters harvested at other times of the year. The available data suggest that Pacific oysters harvested from farms located in the northern half of the North Island are more likely to contain *V. vulnificus* compared with those harvested from the Marlborough region.

RMQ3: Is there currently scientific justification for additional risk management controls over commercial harvests of Pacific oysters in New Zealand during the summer months, to protect consumers of this food from *V. vulnificus*?

The primary purpose of the current BMS monitoring and testing regimes is to prevent illness from contamination by faecal pathogens or biotoxins. There are no requirements for monitoring or controlling *V. vulnificus* in BMS or BMS growing areas unless specific monitoring is included in a Risk Management Programme or a case or outbreak of *V. vulnificus* infection is linked to BMS. Currently the concentration of *V. vulnificus* is being indirectly controlled through post-harvest cooling requirements, which reduces the opportunity for *V. vulnificus* to multiply.

While there is evidence that consumers of Pacific oysters harvested during the summer months in New Zealand could be exposed to *V. vulnificus*, at this time additional risk management controls would be difficult to justify from a scientific perspective. The current uncertainties over dose response and pathogenicity markers make it difficult to quantify the risk of *V. vulnificus* infection should this pathogen be detected in oysters. In addition, BMS consumption has not yet been linked to any cases of *V. vulnificus* infection in New Zealand so there is little evidence to support this food/hazard combination as an important contributor to the overall burden of foodborne disease in this country at this time.

There are important data gaps that impact on this assessment of risk. Aside from internationally-recognised data gaps around pathogenicity and dose-response, the assessment of risk for New Zealand would be improved with additional data on *V. vulnificus* in BMS harvested from New Zealand waters other than Pacific oysters (including at the point-of-sale), and the incidence of acute gastroenteritis in New Zealand as a result of *V. vulnificus* infection.

1. INTRODUCTION

Risk Profiles provide scientific information for risk managers relevant to a food/hazard combination and describe potential risk management options.[1] This document provides a Risk Profile considering *Vibrio vulnificus* in New Zealand bivalve molluscan shellfish (BMS). The risk is considered for BMS consumed raw, although the impact of controls on risk are discussed (e.g. cooking). The risk is considered for BMS harvested in New Zealand from wild stocks (commercial or non-commercial harvesting) and from aquaculture (commercial production). Discussion of the risk from imported BMS has been included.

Consumption of Pacific oysters (*Crassostrea gigas*) that have been harvested from New Zealand waters during summer months has been identified as a potential risk for *Vibrio* infection, so this Risk Profile includes a specific focus on this food/hazard combination.

The purpose of this Risk Profile is to critically review available information to answer the following Risk Management Questions (RMQs):

1. What is the risk to human health from *V. vulnificus* in BMS consumed in New Zealand?

2. Does the commercial harvest of Pacific oysters in New Zealand during the summer months pose a public health risk for consumers of this food with respect to *V. vulnificus*?

3. Is there currently scientific justification for additional risk management controls over commercial harvests of Pacific oysters in New Zealand during the summer months, to protect consumers of this food from *V. vulnificus*?

The *Vibrio* species are metabolically very diverse and not all of them cause disease in humans or other animal species (Sims *et al.*, 2011; USFDA, 1994). Of the 78 species identified so far, 12 have been reported to be pathogenic to humans (European Commission, 2001). Their interactions with humans are opportunistic, since *Vibrio* species are ubiquitous around the world in marine and estuarine environments where they play chemical, physical, symbiotic and commensal roles (Tamplin, 1994). Indeed, vibrios are one of the most common organisms in surface waters in the world (Veenstra *et al.*, 1994).

Of the 12 *Vibrio* species pathogenic to humans, nine are associated with foodborne disease. However, only three *Vibrio* species represent a serious and growing public health hazard: *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* (European Commission, 2001).[2] Other pathogenic *Vibrio* species can cause skin and ear infections when humans are exposed to marine waters (Daniels and Shafaie, 2000; Pien *et al.*, 1977).

A Risk Profile considering *V. parahaemolyticus* in BMS (King *et al.*, 2016) has been prepared alongside this current document. Both *V. vulnificus* and *V. parahaemolyticus* may also infect wounds when existing wounds are exposed to sea water containing these bacteria, or when the bacteria are carried into a fresh penetration wound (e.g. caused through handling seafood).

[1] http://foodsafety.govt.nz/elibrary/industry/RMF_full_document_-_11604_NZFSA_Risk_Management_Framework_3.1.pdf (accessed 5 July 2016)

[2] The other six species are *Vibrio alginolyticus*, *Vibrio mimicus*, *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio hollisae*, and *Vibrio damsela* (Crim *et al*., 2015; NC Division of Public Health, 2012).

2. HAZARD AND FOOD

2.1 THE PATHOGEN: *VIBRIO VULNIFICUS*

KEY FINDINGS

Work to date indicates that potentially pathogenic strains of *V. vulnificus* can be identified from those less likely to cause human illness, but there is no single or suite of markers that can predict the ability of an isolate to cause disease with certainty. Currently, all *V. vulnificus* should be considered potentially pathogenic.

2.1.1 The microorganism

Appendix A.1 contains further information on the characteristics of *V. vulnificus*.

V. vulnificus are halophilic (salt-loving), motile bacteria that occur naturally in estuarine and coastal marine environments. Since first being identified in 1976, *V. vulnificus* have been found in tropical and temperate estuarine and coastal marine environments throughout the world. Their presence is not due to faecal pollution or human contamination (e.g. domestic or industrial discharges to water). They can be free living (planktonic) but are frequently attached to suspended matter or sediments, or form biofilms on marine biotic surfaces (e.g. on BMS shells or zooplankton). *V. vulnificus* are able to break down chitin so can embed themselves in the shells of marine animals (Daniels, 2011). *Vibrio* spp., including *V. vulnificus* 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). Changes in the distribution of plankton species may also affect *Vibrio* spp. distribution (Vezzulli *et al.*, 2016). Sediments serve as a reservoir for *Vibrio* spp. (Huehn *et al.*, 2014).

Three biotypes are recognised based on a combination of phenotypic, serologic, and host range characteristics (Chase and Harwood, 2011; Drake *et al.*, 2007). Biotype 1 is found worldwide and is most commonly associated with clinical infections. Biotype 2 is an eel pathogen found in saltwater used for eel farming in the Far East and Western Europe, and has rarely been isolated from human cases. Biotype 3 has so far only been reported from wound infections amongst fishery workers in Israel, and is a hybrid of biotypes 1 and 2 (Horseman and Surani, 2011; Phillips *et al.*, 2014; Zaidenstein *et al.*, 2008). Despite the frequently lethal consequences of *V. vulnificus* infections, the growth rates of the various biotypes and their response to environmental changes are not well characterised (Chase and Harwood, 2011).

2.1.2 Disease and pathogenicity

V. vulnificus are opportunistic human pathogens since they do not require the human host to replicate (Daniels, 2011). Three different clinical syndromes have been documented associated with *V. vulnificus*: Wound infections, primary septicaemia and gastroenteritis (USFDA, 1994). Foodborne exposure results in gastrointestinal infection in a minority of cases. In most foodborne cases, exposure leads to primary septicaemia and the mortality rate for this condition is high (approximately 50% is commonly reported).

A large number of *V. vulnificus* strains have been identified, which emphasises that *V. vulnificus* are genetically diverse. A single oyster can contain over 100 different strains, and 118 *V. vulnificus* strains were isolated from just three oyster samples taken from Apalachicola Bay, Florida, in the United States of America (USA) (Buchrieser *et al.*, 1995). It is likely that not all *V. vulnificus* strains are pathogenic, but at this stage the specific virulence factors remain unclear so there are no reliable indicators of *V. vulnificus* pathogenicity (Johnson *et*

al., 2010). In 2005 the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) reviewed the literature about the potential factors necessary to cause human virulence. This review concluded that virulence appears to be multifaceted and is not well understood, thus all strains should be considered virulent (FAO/WHO, 2005).

Biotype 1 strains of *V. vulnificus* can be classified into two distinct genotypes. A common molecular-based approach targets the virulence-correlated gene (*vcg*) (Rosche *et al.*, 2005; Warner and Oliver, 2008). Using this method, the genotypes are referred to as the Clinical (C) and Environmental (E) genotypes, since most clinical isolates possess the *vcgC* sequence variant and most environmental (non-clinical) isolates possess the *vcgE* sequence variant. For example, in one study 28/30 (93%) isolates from seawater, BMS and sediment were E-type, and 18/25 (72%) of isolates from human cases of *V. vulnificus* infection were C-type (Rosche *et al.*, 2005). This grouping has been supported by other molecular and phenotypic studies, including typing of 16S rRNA (Oliver, 2015).[3] However, as the example above shows, E-genotypes have been isolated from human cases, so the C/E grouping only indicates the likelihood of an isolate being pathogenic (Oliver, 2015; Yokochi *et al.*, 2013). Efforts are being directed towards generating information on genes unique to the C or E groups, but the reasons for the ability of C-genotype strains to more readily cause disease require more study. It has been suggested that the C-genotype is better at coping with the stressful transition from seawater or BMS to humans compared with the E-genotype (Rosche *et al.*, 2010).

The available information suggests that, in addition to being biotype 1 C-genotype, isolates expressing the following virulence factors are better able to initiate human disease (Horseman and Surani, 2011; Oliver, 2015):

- Expression of capsular polysaccharide (CPS): The ability for an isolate to form a capsule is detected on routine laboratory media where encapsulated cells appear opaque and those lacking the capsular polysaccharide appear translucent. CPS expression enhances survival in the human host by providing resistance to phagocytosis by macrophages, resistance to the bactericidal effects of serum and by masking immunogenic structures that would normally activate non-specific host immune responses.

- Production of a lipopolysaccharide (LPS) endotoxin: This toxin is believed to cause the endotoxic shock associated with severe disease.

V. vulnificus are also known to produce a haemolysin (VvhA) that facilitates the release of iron from haemoglobin, and an enzymatic toxin (VvpE), but while both may contribute to virulence the importance of these toxins is still unclear (Horseman and Surani, 2011). Mutant *V. vulnificus* strains without the *vvhA* or *vvpE* genes were still cytotoxic, and evidence points to another toxin, RtxA1 (VvRtxA), which may be responsible for the severe disease caused by *V. vulnificus*.

Other virulence factors that have been identified include possession of the *viuB* gene, which encodes a siderophore (iron-chelating compound) and production of membrane pili, which are essential for cytotoxicity (Bogard and Oliver, 2007; Horseman and Surani, 2011).

[3] The small subunit of the 16S rRNA gene is sequenced and nucleotide differences separate strains into type A (associated with non-clinical isolates) and type B (associated with clinical isolates), but isolates can possess both A and B alleles (Nilsson *et al*., 2003; Vickery *et al*., 2007).

2.2 THE FOOD: BIVALVE MOLLUSCAN SHELLFISH

KEY FINDINGS

New Zealand's Ministry for Primary Industries manages the commercial harvest of wild stocks of most BMS species. Data from the 2014/15 fishing year shows that cockles and Foveaux Strait dredge oysters were harvested in the highest amounts by commercial harvesters from wild stocks, by weight (approximately 1,000 tonnes each). Recreational harvesters collect more scallops and mussels (by number) than other types of shellfish.

New Zealand green-lipped mussels and Pacific oysters are commercially farmed in New Zealand. The majority of Pacific oysters are farmed in the upper half of the North Island (Northland, Auckland and Coromandel). In 2011, 1,804 tonnes of Pacific oysters were harvested. Most harvesting is during winter and spring but harvesting at other times also occurs. Oysters are usually sold live or raw. The available data indicates that the majority of Pacific oysters harvested in New Zealand are exported, mostly as frozen half shells.

Scallops make up the majority of imported BMS products, by weight. Approximately 22 tonnes of frozen, shucked Pacific oysters were imported during 2015.

For the year 2011, an estimated 13,000 tonnes of shucked BMS were available to New Zealand consumers, with green-lipped mussels accounting for 96% of this amount.

The shellfish considered in this Risk Profile are marine- or estuarine-dwelling bivalve molluscan shellfish (BMS; phylum Mollusca, class Bivalvia) that filter water through their gills to capture food (mainly phytoplankton). This feeding mechanism also captures and concentrates microorganisms (some of which may be pathogenic to humans), which are moved into the digestive organs along with food and are not necessarily excreted. BMS that live in freshwater (e.g. kākahi) are not considered because *V. vulnificus* are not naturally found in freshwater.

A variety of BMS are harvested from New Zealand marine and estuarine environments (wild stocks) or are farmed (aquaculture). These include clams (e.g. cockles, pipi, toheroa, tuatua), oysters, mussels and scallops. A list has been provided in Appendix A.4.1 and FIGURE 1 explains the sources of BMS available to New Zealand consumers.

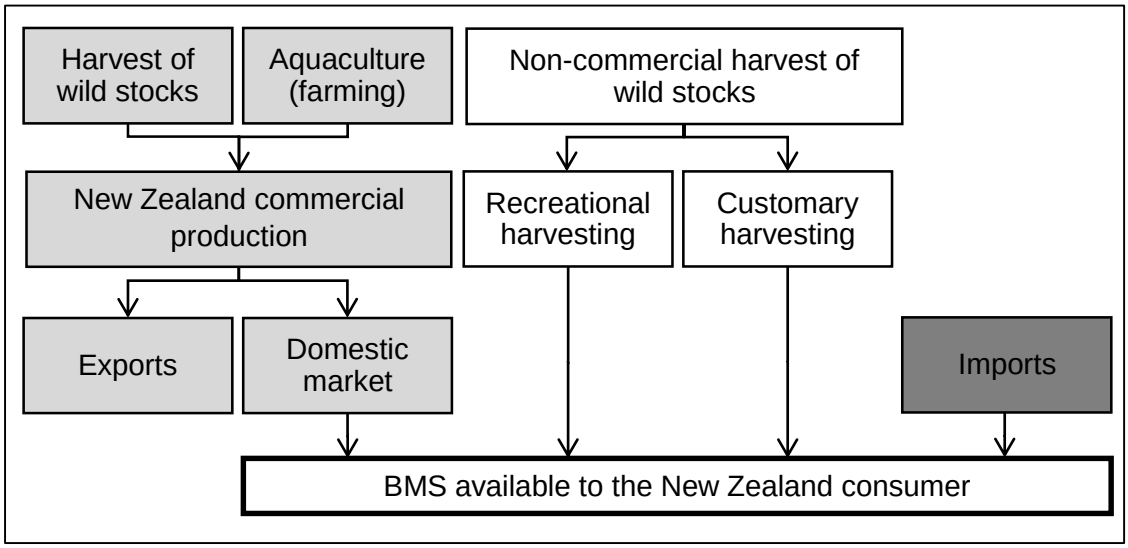

FIGURE 1: Sources of BMS available to New Zealand consumers (reproduced from King and Lake 2013)

2.2.1 BMS production and harvesting in New Zealand

Harvesting of many wild BMS stocks is managed under the Quota Management System (QMS) for New Zealand. TABLE 1 lists the weight of reported commercial shellfish landings for the 2014/15 fishing year and the permitted landings (quota) under the QMS.[4] The amounts listed represent a summation of data for specific areas (Quota Management Areas) around New Zealand.[5] As well as managing the QMS, the New Zealand Ministry for Primary Industries (MPI) sets limits on the number and size of BMS that can be gathered by individuals under customary or recreational allocations.[6]

TABLE 1: Reported commercial landings and quota management amounts for BMS managed under the QMS (2014/15 fishing year)[1]

BMS SPECIES (QMS CODE)	REPORTED COMMERCIAL LANDINGS (TONNES)	PERMITTED LANDINGS (TONNES)		
		TOTAL ALLOWABLE COMMERCIAL CATCH (TACC)	CUSTOMARY ALLOWANCE	RECREATIONAL ALLOWANCE
Cockle (COC)	1,078	3,214	161	221
Dredge oyster Foveaux Strait (OYU)[2]	1,020	1,526	0	0
Scallop (SCA)[2]	360	4,576	652	652
Triangle shell (SAE)	307	2,437	10	0
Green-lipped mussel (GLM)	207	1,720	467	310
Deepwater tuatua (PDO)	131	890	69	68
Large trough shell (MMI)	69	744	10	0
Ringed dosinia (DAN)	8	384	10	0
Deepwater clam (PZL)	4	32	0	0
Dredge oysters (OYS)	3	623	13	13
Frilled venus shell (BYA)	2	16	0	0
Queen scallop (QSC)	2	380	0	0
Tuatua (TUA)	2	43	137	137
Pipi (PPI)	0	204	242	242
Trough shell (MDI)	0	160	0	0
Horse mussel (HOR)	0	29	9	9
Silky dosinia (DSU)	0	8	0	0
TOTAL	3,192	16,985	1,780	1,652

[1] Data extracted from shellfish catch data provided by MPI and available from http://fs.fish.govt.nz/Page.aspx?pk=87&tk=287&ey=2015 (accessed 31 May 2016).
[2] OYU are reported as number of individual shellfish landed, SCA are reported as meatweight (shucked). Conversion factors to standardise values to greenweight in tonnes were: 1 dredge oyster = 102 g (MPI, 2016b) and a multiplier of 8.00 for scallops (MPI, 2014).

New Zealand green-lipped (Greenshell™) mussels and Pacific oysters are farmed commercially as aquaculture in New Zealand. Data on Pacific oyster aquaculture are included in Section 2.2.2. Green-lipped mussels are grown on ropes permanently submerged in

[4] Quota are the same for the 2015/16 fishing year but full data on reported landings are not available until October 2016.
[5] Not all quota management areas for a single species are managed under the QMA so additional harvesting may have occurred that was not reported.
[6] http://www.mpi.govt.nz/travel-and-recreation/fishing/fishing-rules/ (accessed 31 May 2016).

subtidal waters. During 2015, 80,000 tonnes of New Zealand green-lipped mussels were harvested (C. Johnston, Aquaculture New Zealand, pers. comm.). Most green-lipped mussel production takes place in the Marlborough/Tasman region (59% of total production in 2015) and the Waikato/Coromandel region (30% of total production in 2015).

The most recent recreational fisher survey was completed in 2012 and estimates for the number of shellfish harvested by recreational gatherers during the 2011/12 year have been published (Wynne-Jones *et al.*, 2014). Scallops were harvested in the largest amount (an estimated 1.7 million), followed by mussels (approximately 1 million), tuatua (0.9 million), cockles (0.7 million) and pipi (0.6 million). The total estimated harvest for oysters for the 2011/12 fishing year was 303,190 (figures for separate oyster species were not reported).

Using conversion factors from King and Lake (2013), the weights non-commercially harvested BMS can be roughly estimated, although the size and weight of non-commercially harvested BMS will vary greatly, and will also differ by species (e.g. green-lipped mussels vs. blue mussels). Estimates are 174 tonnes of scallops, 23 tonnes of tuatua, 17 tonnes of mussels, 7 tonnes of pipi and 6 tonnes of cockles.

2.2.2 Pacific oyster production and harvesting in New Zealand

Pacific oysters grow best at temperatures between 15 and 18ºC and an adult oyster (80-100 mm in length) may filter up to 240 litres of water a day (Aquaculture New Zealand, 2012).

All commercially harvested Pacific oysters are from aquaculture stocks. There is no commercial harvesting of wild stocks, although oyster spat are collected from the wild. The majority of Pacific oysters are harvested from areas distributed around the north 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). Harvesting of wild stocks of Pacific oysters is not managed under the QMS.

In 2015, 1,910 tonnes of Pacific oysters were harvested, or approximately 2.4 million dozen (C. Johnston, Aquaculture New Zealand, pers. comm.). A large proportion of this amount is exported but there are no robust data on the tonnage available to New Zealand consumers (estimates have been calculated, see Section 2.2.4).

Most oysters are grown from naturally-harvested spat, but spat is also available from a commercial hatchery located at the top of the South Island (Castinel *et al.*, 2015). 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. The oysters grown in the subtidal zone are usually transferred to the intertidal zone for some time before harvest to harden the shells. Oysters are harvested after 12-18 months, usually during May to November when the oysters are in peak condition, but harvesting at other times also occurs. Oysters spawn over the summer months and the subsequent loss in condition means harvesting during this period is limited. However, triploid oysters are now available from hatcheries. These are sterile so do not spawn or lose condition over the summer.[7] Data on the amount of triploid oysters harvested from New Zealand waters for human consumption are not available.

The temperature control requirements after harvest are explained in Section 5.1.1. Cooling to 7ºC is required. Raw oysters are commonly sold live in their shell, as half shell oysters (with one shell removed) or as fresh shucked oyster meat (both shells removed). The first post-harvest step for raw oysters is washing to remove the external marine debris from the shell. Once the half shell or shells have been removed the oysters are usually washed again in freshwater.

[7] http://www.cawthron.org.nz/aquaculture-park/pacific-oyster-spat-sales/ (accessed 9 August 2016).

The New Zealand Pacific oyster industry has been affected by the Ostreid herpesvirus-1 since 2010, which causes mass mortality amongst oyster stocks during the summer months (Castinel *et al.*, 2015). *Vibrio* spp. (not *V. vulnificus* or *V. parahaemolyticus*) have been isolated from oysters infected with this virus, and co-infection as a cause of mortality has been suggested (Keeling *et al.*, 2014).

2.2.3 International trade

New Zealand imports some BMS and BMS meat. In the year ending December 2015, 2.4 million Pacific oysters were imported and all were shucked and frozen. This is approximately 22 tonnes meatweight and 160 tonnes greenweight.[8] Most (73%) of these imported oysters were from the Republic of Korea and the remainder were from China. Importation of Pacific oysters has fluctuated between 0.5 and 5.3 million oysters each year over the last decade (see Appendix A.4.2).

During the year ending December 2015 there were also 465 tonnes of scallops, mussels, cockles and other clams imported, in the form of meat, half-shell or whole shell. The majority by weight was frozen scallops (97%), thus importation of other BMS species is very small in comparison. In 2015 most (86%) of these frozen scallops came from China. Frozen scallops are traded as adductor muscle only (eviscerated and with the guts and roe removed) so present a lower risk for *Vibrio* spp. contamination compared with non-eviscerated BMS.[9]

Export data for the year ending December 2015 shows exports of approximately 28,000 tonnes of mussel products, which made up 92% of BMS exports by weight (Seafood New Zealand, 2015).[10] Smaller weights of product from oysters (1,900 tonnes), cockles (192 tonnes), tuatua (93 tonnes), scallops (39 tonnes) and other clams (318 tonnes) were also exported. Together, these shellfish products represent approximately 10% of the total 290,000 tonnes of seafood product exported from New Zealand in the year ending December 2015.

Of the 1,900 tonnes of oyster products exported, 1,760 tonnes were from Pacific oysters (Seafood New Zealand, 2015). The majority (71%, by weight) was exported in the form of frozen half-shells. Just over a quarter (26% by weight, or 462 tonnes) was exported live and chilled. The main markets for Pacific oysters are (in decreasing order by weight exported and by value): Australia, Hong Kong, Japan and French Polynesia.[11]

2.2.4 Amount available to the New Zealand consumer

An estimated 68,000 tonnes greenweight (13,000 tonnes meatweight) of BMS were available to New Zealand consumers for the year 2011 (King and Lake, 2013). This analysis took into account commercial production and harvesting, non-commercial harvesting and international trade. Most (99%, by weight) of the available BMS were commercially harvested. Mussels, mostly New Zealand green-lipped mussels, accounted for 96% of the total available BMS by meatweight. Oysters (mostly dredge and Pacific) accounted for 0.6%. The estimate requires updating now that more recent data are available (e.g. 2012 recreational fisher survey).

[8] Greenweight is the weight of the whole, unshucked shellfish. Meatweight is the weight of the shucked shellfish (minus the shell and any liquid in the shell). Conversion factors applied were those reported in King and Lake (2013) and are for New Zealand, so may not be suitable for Pacific oysters produced in other countries.
[9] Imported Food Requirements: Bivalve Molluscan Shellfish (March 2015). Kindly provided by the New Zealand Ministry for Primary Industries.
[10] Data are weight of exports in all forms – fresh, frozen, processed.
[11] Oyster export statistics, provided by Aquaculture New Zealand, September 2016.

2.3 CONTAMINATION OF BMS BY *V. VULNIFICUS*

KEY FINDINGS

The concentration of *V. vulnificus* in estuarine waters is usually low (<10 CFU/ml) but these bacteria can be concentrated inside BMS as the shellfish filter water to obtain food. There is no evidence that *V. vulnificus* cause adverse health effects to shellfish themselves, or affect their organoleptic qualities.

The presence of *V. vulnificus* in the marine environment is not related to faecal contamination. The most important environmental parameters influencing the presence and concentration of *V. vulnificus* in the marine environment and in BMS are water temperature and water salinity.

The highest concentrations of *V. vulnificus* have been detected in waters and BMS when the water temperature was ≥20ºC. The relationship with salinity is more complex. The majority of studies found that water salinity was either negatively correlated with the concentration of *V. vulnificus*, or not significant. The highest concentrations of *V. vulnificus* are usually measured at salinities of 5-25‰,[12] but there is considerable regional variability. Changes in water temperatures and salinities caused by climate change will influence the distribution and abundance of *V. vulnificus*.

V. vulnificus can enter a viable but non-culturable state at temperatures ≤13ºC but there is limited evidence to support this occurring under natural marine conditions or inside BMS. Studies using mice suggest that the ability of these non-culturable *V. vulnificus* to cause infection is lost over time.

V. vulnificus can be accumulated inside oysters to concentrations 1-2 log higher than found in surrounding waters and it is assumed that all BMS species have the ability to bioaccumulate *V. vulnificus*. *V. vulnificus* spreads from the intestinal tract to the rest of the oyster's tissues. The number of *V. vulnificus* accumulated in oysters depends on the oyster's general environmental conditions and on its health and natural microflora. It appears that oyster uptake or colonisation strongly favours the E-genotype of *V. vulnificus* but not all studies support this. *V. vulnificus* can be depurated by oysters, but not completely under normal environmental conditions.

The concentration of *V. vulnificus* in estuarine waters is usually low (<10 CFU/ml) but these bacteria can be concentrated inside BMS as the shellfish filter water to obtain food (Oliver, 2015). There is no evidence that *V. vulnificus* cause adverse health effects to shellfish themselves. *V. vulnificus* inside BMS may be better protected from adverse environmental conditions (Desmarchelier, 2003). The presence of *V. vulnificus* has no effect on the appearance, taste or odour of BMS (Horseman and Surani, 2011).

2.3.1 Distribution and prevalence of *V. vulnificus* in coastal marine environments

As an autochthonous (naturally occurring) bacteria, the presence of *V. vulnificus* in the marine environment is not related to faecal contamination, and so common indicators of water quality such as coliforms or *E. coli* are not useful (Desmarchelier, 2003). Their distribution in the environment is determined by a number of factors; most important are water temperature and salinity. They have not been detected in open ocean waters where the salinity is higher than 35‰. Sediments serve as a reservoir for *Vibrio* spp. (Chase *et al.*, 2015; Huehn *et al.*, 2014). A recent study measured higher concentrations of *V. vulnificus* in the intestines of fish

[12] Note that salinity in seawater is usually presented as ‰ i.e. parts per thousand rather than parts per hundred.

compared with sediment, water and oysters from the same environment, suggesting fish also have an important role in the environmental cycling of *V. vulnificus* (Givens *et al.*, 2014).

The two environmental parameters that appear to be most important for influencing the distribution and concentration of *V. vulnificus* in the environment are water temperature and water salinity (Oliver, 2015). A number of studies have been undertaken to assess the relationship of *V. vulnificus* with other environmental variables, including dissolved organic carbon, chlorophyll *a*, sea surface height, suspended particulate matter, turbidity and plankton (e.g. Chase and Harwood, 2011; Johnson *et al.*, 2012; Johnson *et al.*, 2010; Julie *et al.*, 2010; Montanari *et al.*, 1999). However, these studies concluded that any linkage to environmental parameters, other than temperature and salinity, is ecologically and regionally specific.

Temperature

While both biological and physicochemical factors are important to the survival of *V. vulnificus* in the environment, the prevalence and density *V. vulnificus* in the environment and in seafood products is shown to be highly dependent on the ambient temperature, with the largest numbers occurring at warm sea water temperatures (Appendix A.3; European Commission, 2001). *V. vulnificus* may grow in the marine environment at temperatures as low as 13ºC, and has been isolated from waters at 7.6ºC, but its numbers generally remain low (i.e. close to or below the limit of detection) at temperatures below 20ºC (FAO/WHO, 2005; Fletcher, 1985; Johnson *et al.*, 2012; Kaspar and Tamplin, 1993; Kelly, 1982; O'Neill *et al.*, 1992; Wright *et al.*, 1996). Surveys have found that *V. vulnificus* becomes easy to isolate from oysters from waters at 15-17ºC or above (Froelich and Noble, 2016).

Highest concentrations have been detected when the water temperature was between 20 and 30ºC (Desmarchelier, 2003; FAO/WHO, 2005; Kaspar and Tamplin, 1993; Wetz *et al.*, 2014). Epidemiological data and environmental surveys suggest the largest risk to human health comes when BMS are harvested from waters ≥20ºC. For example, a study in Germany found that at water temperatures in the range 15-20ºC, an increase of 1ºC increased the probability of *V. vulnificus* presence in the water and sediment by approximately 4% (Boeer *et al.*, 2013). At water temperatures above 20ºC, every 1ºC increase led to a tenfold increase in the probability of *V. vulnificus* presence.

V. vulnificus are rarely isolated from marine waters at temperatures below 10ºC (Desmarchelier, 2003; Strom and Paranjpye, 2000; USFDA, 1994), although the organisms can be cultured from sediment and oysters at those temperatures. Temperatures ≤13ºC can induce the viable but non-culturable state (see Section 2.3.3).

Salinity

Evidence for salinity being an important influence over the presence of *V. vulnificus* is less clear compared with temperature. The effect of salinity on *V. vulnificus* in oysters has been calculated to be either negatively associated or insignificant in the majority of studies (Appendix A.3; Froelich and Noble, 2016).

Environmental surveys indicate that *V. vulnificus* can be found at salinities ranging from 0.8 to 35‰ (FAO/WHO, 2005; Kaysner *et al.*, 1987; Tamplin, 1990). Highest numbers are usually measured at salinities of 5-25‰ (Desmarchelier, 2003; Kelly, 1982; Motes *et al.*, 1998; O'Neill *et al.*, 1992; Tamplin *et al.*, 1982; Tamplin, 1990; Wetz *et al.*, 2014). An analysis conducted as part of the FAO/WHO 2005 risk assessment estimated an optimal salinity for growth of 17‰ (FAO/WHO, 2005), based on data from the USA.

Studies of New Zealand isolates suggest *V. vulnificus* have adapted to higher salinities in New Zealand waters. Twenty strains of *V. vulnificus* isolated from New Zealand-grown Pacific oysters were grown in minimal medium with a base salinity of 5‰ or salinities ranging 25-40‰, at 15ºC for four days (Cruz *et al.*, 2016). The general trends as measured by optical density were:

- The highest concentration at the end of stationary phase was measured at 25‰.
- Growth was observed at 35 and 40‰, but growth was delayed and the growth rate was lower than that measured at 25 and 30‰.

There were differences between isolates, e.g. the maximum concentration was not affected by salinity for six isolates.

Overseas studies have found that marine waters of salinities >30‰ are generally associated with low or non-detectable levels of *V. vulnificus* regardless of the temperature (this is not the case in New Zealand, see above and Section 2.5.1). A USA study (Apalachicola Bay) linked increasing salinity with decreasing recovery of *V. vulnificus* from oysters (Motes and DePaola, 1996). When North Carolina drought conditions caused salinity levels to reach 35‰, *V. vulnificus* could no longer be isolated from the water (Froelich *et al.*, 2012). Conversely, when heavy rainfall caused flash floods to impact coastal lagoons in Southern France, reducing the salinities within the range 2.2-16.4‰, there was an associated increase in *V. vulnificus* in the lagoons (Esteves *et al.*, 2015). The positive effect of salinity-lowering storm events on survival of *V. vulnificus* was also observed in another North Carolina study (Wetz *et al.*, 2014).

Because the optimum salinity for *V. vulnificus* appears to vary from area to area, it is difficult to estimate the joint effect of temperature and salinity over the entire range of both moderate and high salinities (FAO/WHO, 2005). This opinion has recently been confirmed by an intensive three-year study that measured concentrations of *V. vulnificus* in water, sediment and oysters collected from four coastal locations in the USA (Johnson *et al.*, 2012). Using regression analysis, surface seawater temperature was significantly associated with the concentration of *V. vulnificus* in water, oysters and sediment and accounted for 28% of the variability in oysters. This study did not identify salinity as being an important predictor of *V. vulnificus*, despite a large salinity range being measured. The authors concluded, upon review of a number of other studies, that the relationship between salinity and *V. vulnificus* may be variable and complex.

2.3.2 Climate change

Due to the relationship between warm ambient temperatures and the presence of vibrios in the marine environment there is concern about the ocean-warming effects of climate change on the distribution and abundance of *V. vulnificus*. 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). Rising water temperatures in shellfish growing areas have been associated with the increasing incidence of *V. parahaemolyticus* and *V. vulnificus* cases in the USA (Morris, 2003). There are also concerns in Europe and other parts of the world that the increasing numbers of *Vibrio* illnesses may be linked to rising ocean temperatures (Baker-Austin *et al.*, 2010; Baker-Austin *et al.*, 2013; Gonzalez-Escalona *et al.*, 2005; McLaughlin *et al.*, 2005; Paz *et al.*, 2007; Sims *et al.*, 2011).

A recent paper has provided strong evidence of a linkage between climate change, the abundance of *Vibrio* spp. and the incidence of human *Vibrio* spp. infections (foodborne and wound infections) for the North Atlantic region (Vezzulli *et al.*, 2016). Using DNA extracted from 133 plankton samples taken from nine sites across the North Atlantic during the period 1985-2011, the researchers found a positive correlation between the abundance of *Vibrio* spp. (relative to total bacteria) and sea surface temperature in 8/9 sites. Both increased over the time period studied. The long-term climatic drivers of *Vibrio* spp. abundance were identified as the Northern Hemisphere Temperature (a measure of atmospheric and ocean temperature over the northern half of the globe) and Atlantic Multidecadal Oscillation (a natural oscillation of the Atlantic Ocean thermohaline). The researchers also identified a correlation between the abundance of *Vibrio* spp. and diatom phytoplankton and hypothesised that changes in plankton populations and distribution as a result of global warming will also affect *Vibrio* spp.

Importantly, Vezzulli *et al.* (2016) found a positive correlation between human *Vibrio* spp. infections reported during the period 1973-2011 in Northern Europe and the USA Atlantic coast, and *Vibrio* spp. abundance. This correlation was particularly evident during heat waves. They also found that the highest number of reported *Vibrio* spp. infections were correlated with the presence of the *Vibrio* species, *cholerae*, *parahaemolyticus* and *vulnificus* in the phytoplankton samples. They cite an example from a 2006 heat wave in Northern Europe during which "an unprecedented number" of human *V. vulnificus* infections were reported, and a plankton sample from the southern North Sea yielded one of the highest relative abundances of *Vibrio* spp. and was also positive for the *V. vulnificus* gene *vvhA*. This work demonstrates a link between increased *Vibrio* spp. concentration in seawater as a result of ocean warming and increased incidence of human *Vibrio* spp. infections.

There is evidence that climatic events can affect *V. vulnificus* concentrations in market oysters. The strong La Niña event over continental USA during 1998/99 resulted in higher than normal air and water temperatures (4°C above average) and this correlated with higher than usual concentrations of *V. vulnificus* in oysters taken from the Gulf Coast (Cook *et al.*, 2002). A case of primary septicaemia was linked to oysters harvested during winter 1999, which was unusual (Cook *et al.*, 2002).

2.3.3 The viable but non-culturable (VBNC) state

Bacterial cells are said to be in a VBNC state when they remain alive and metabolically active, but are unable to be cultured using standard laboratory methods. The VBNC state is induced in response to stress (e.g. temperature, osmotic stress, starvation). *V. vulnificus* cells entering the VBNC state were first reported in 1989, when Linder and Oliver observed that *V. vulnificus* exhibited a stress response to low ambient temperatures (Linder and Oliver, 1989).

There is evidence to show that *V. vulnificus* can enter the VBNC state in response to low temperatures and can be resuscitated by a temperature upshift (Ayrapetyan *et al.*, 2014; Nilsson *et al.*, 1991; Oliver and Bockian, 1995). Water temperatures ≤13ºC can induce *V. vulnificus* to enter the VBNC state (Oliver, 2015). Although entry into the VBNC state in estuarine waters has been reported (Oliver and Bockian, 1995), molecular analyses to detect *V. vulnificus* in estuarine waters during the winter months suggest that populations of these bacteria are absent, rather than present in the water as VBNC cells (Wetz *et al.*, 2014). In addition to moving into the sediments, lower temperatures in marine waters may inactivate the majority of *V. vulnificus* cells and only some may transition to a VBNC state. Laboratory experiments have demonstrated that VBNC *V. vulnificus* are more resistant to environmental stresses (Nowakowska and Oliver, 2013).

In vivo studies of VBNC *Vibrio* spp. are few. Successful resuscitation of naturally present, VBNC *Vibrio* spp. inside oysters has been reported (Ayrapetyan *et al.*, 2014; Froelich and Oliver, 2013a). However, *V. vulnificus* were rarely isolated amongst the resuscitated colonies (other *Vibrio* spp. were dominant) (Froelich and Oliver, 2013a).

Thus the available evidence shows that there is potential for *V. vulnificus* to be present in BMS in the VBNC state, but further studies are needed to confirm this phenomenon in BMS growing under normal environmental conditions. It is also important to establish whether *V. vulnificus* in the VBNC state remain virulent. Laboratory studies using mice suggest that *V. vulnificus* maintains its infectious ability upon entry to the VBNC state (i.e. can resuscitate and cause illness) but this ability is lost over time (Oliver and Bockian, 1995).

2.3.4 Uptake, retention and depuration of *V. vulnificus* by BMS

Scientists have focused their attention on understanding the relationship between oysters and *V. vulnificus*, since most foodborne *Vibrio* illnesses are linked to raw oyster consumption (see Section 3). *V. vulnificus* can be accumulated inside oysters to concentrations 1-2 log higher than found in in surrounding waters (Johnson *et al.*, 2010; O'Neill *et al.*, 1992). Selective retention in oysters was also demonstrated under laboratory conditions by the isolation of *V.*

vulnificus from oysters when no *V. vulnificus* were detected in the surrounding waters (Harris-Young *et al.*, 1995). It is assumed that the higher concentrations within the shellfish are largely the result of bioaccumulation, but multiplication within the shellfish can also occur. Since *V. vulnificus* have also been isolated from non-oyster BMS species (Appendix A.3) it is assumed that all BMS species have the capacity to accumulate *V. vulnificus* from the aquatic environment.

The number of *V. vulnificus* accumulated in oysters depends on both the general environmental conditions and on the health of the oyster itself. There is also evidence that established microbial populations within the oyster can prevent uptake of further bacterial cells from the surrounding water (Froelich and Oliver, 2013a; Froelich and Noble, 2014). The concentration of *V. vulnificus* 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 *V. vulnificus* populations and the feeding behaviour of oysters (FAO/WHO, 2005; USFDA, 1994).

Once ingested by filter feeding, vibrios are found in the intestinal tracts, haemolymph (invertebrate fluid), adductor muscle, gills and mantle ("muscle") of oysters (FAO/WHO, 2005; Froelich and Oliver, 2013b; Staley *et al.*, 2011). There is also some evidence that *V. vulnificus* can persist within oyster haemocytes (phagocytes of invertebrates), but survival appears to be dependent on the type of haemocyte cell and whether the *V. vulnificus* are encapsulated (Froelich and Oliver, 2013b). They are more likely to be killed by these haemocytes (Harris-Young *et al.*, 1995).

A recent study found that oysters grown suspended in the water had generally lower concentrations of *V. vulnificus* and *V. parahaemolyticus* than oysters grown on the bottom and in contact with sediments (Cole *et al.*, 2015). Thus stocks of BMS harvested from sediments (commercially or non-commercially) will possibly have higher concentrations of *Vibrio* spp. than those harvested from aquaculture operations in the same area.

Because *V. vulnificus* resides in, and adheres to marine sediments, it has been hypothesised that *Vibrio* spp. concentrations increase in oyster tissue when sediments are resuspended, such as during storm events. However, when the concentration of *V. vulnificus* was monitored in aquacultured oysters (*Crassostrea virginica*) in the Chesapeake Bay estuary before and after a hurricane, the concentrations were not significantly different (Shaw *et al.*, 2014). The researchers suggested other factors were at play, such as the oysters reducing filter-feeding during periods of high suspended solids.

A 12-month study monitoring the C-genotype and E-genotype variants of *V. vulnificus* in 100 individual oysters and surrounding waters collected from two sites on the East coast of the USA found (Warner and Oliver, 2008):

- Levels within oysters were strongly skewed towards the E-genotype: Of 743 *V. vulnificus* isolates from oysters, only 16% possessed the C-genotype. The prevalence and concentration of *vcgC* isolates in oysters was lower than *vcgE*. Only 2/85 oysters containing *V. vulnificus* had populations of the C genotype at higher concentrations than the E genotype and both of these oysters were taken from waters >30ºC.
- Levels of E- and C-genotypes in water were approximately equal and equally affected by water temperature: Of 292 *V. vulnificus* isolates from waters, just over half (155, 53%) were *vcgC*. The concentration of *vcgC* and *vcgE* changed over the sampling period, but the ratio between the two genotypes remained similar (approximately 1:1).
- Oysters living adjacent to one-another contained variable concentrations of *V. vulnificus* in their tissues, e.g. not detected and >10^3 CFU/g in adjacent oysters.

These results suggest that oyster uptake or colonisation strongly favours the E-genotype, and that uptake of *V. vulnificus* varies between individual oysters. Recent research has also

identified that E-genotype strains integrate into marine aggregates more efficiently than C-genotype strains, leading to a greater uptake of E-genotype strains by oysters feeding on these aggregates (Froelich *et al.*, 2013a; Phippen and Oliver, 2015; Williams *et al.*, 2014). However, a study in Japan found that C-genotypes dominated in seafood and other environmental samples taken in 2/3 harbours studied, suggesting that geographical location was important (Yokochi *et al.*, 2013). It has been proposed that *V. vulnificus* strains carrying virulence markers occupy a niche different from that of the species as a whole, and that the C- and E-genotypes represent different ecotypes that are possibly in the process of diverging into separate species (Froelich and Noble, 2016; Rosche *et al.*, 2010). It is important to note that the presence of E-genotypes inside BMS does not appear to prevent C-genotypes from also being present. The relationship between these two types within individual shellfish requires further study.

V. vulnificus will grow in oysters when they are out of the water if the temperature is suitable. It has been demonstrated that the concentration of *V. vulnificus* increases in oysters exposed to the warm air between tides, then decreases when the tidal waters cover the shellfish and filter-feeding recommences (Jones *et al.*, 2016). The same has been reported for oysters subjected to "dry storage" (an antifouling practice) then resubmergence (Kinsey *et al.*, 2015).

V. vulnificus have been detected in BMS sampled at retail, demonstrating that these bacteria are retained well in harvested shellfish. It is clear that *V. vulnificus* are depurated by BMS, but the length of time any *V. vulnificus* cell remains inside an individual shellfish still residing in its growing area is not well defined, and is probably difficult to predict. While laboratory studies have monitored depuration, depuration rates from naturally contaminated BMS were far lower than those that were artificially contaminated (Froelich and Noble, 2014). It has been shown that standard depuration methods are not effective for removing *V. vulnificus* from contaminated oysters, although depuration of *V. vulnificus* is improved by relaying these shellfish to high salinity waters (see Section 5.2.1).

2.4 BEHAVIOUR OF *V. VULNIFICUS* IN BMS

KEY FINDINGS

V. vulnificus can multiply in harvested oysters at temperatures of 15ºC or above. Chilling (≤10ºC) and freezing will reduce the concentration of *V. vulnificus* in oysters over time but *V. vulnificus* naturally contaminating oysters have survived 21 days at 5ºC and 12 weeks at -20ºC. Data on the behaviour of *V. vulnificus* in other BMS species are lacking.

V. vulnificus inside BMS meat are readily killed by cooking but can be protected from acidic sauces or marinades.

2.4.1 The behaviour of *V. vulnificus* in harvested BMS

Studies of the behaviour of *V. vulnificus* in BMS after harvesting have focussed on oysters, mostly *Crassostrea virginica*.

Temperature is critical to controlling the growth of *V. vulnificus* in oysters (Kaspar and Tamplin, 1993). Oysters are not fragile and can live out of water for several days (or weeks) under refrigeration, e.g. Sydney rock oysters can remain alive for up to two weeks at ambient air temperatures after harvesting (Nell and Holliday, 1988). Oysters are often shipped live to markets for raw consumption. Any initial washing and shucking steps do not significantly reduce the levels of vibrios in oysters (Ruple and Cook, 1992).

V. vulnificus will grow in harvested oysters if the temperature is suitable. Data assembled in TABLE 2 generally show that the concentration of culturable *V. vulnificus* increases in oysters held at 15ºC or above, and decreases in oysters held at 10ºC or below. No studies of the

survival of *V. vulnificus* in oysters at 10-14ºC were located. The available data indicate that the growth/no growth boundary for *V. vulnificus* in oysters lies somewhere in this range, although growth in laboratory media has been measured at 10ºC (see Section A.1). Published growth models (DaSilva *et al.*, 2012; FAO/WHO, 2005) did not consider growth in this temperature range (the FAO/WHO model assumed zero growth at 13ºC).

The increased concentration of naturally-present *V. vulnificus* at 6ºC observed by Wood & Arias (2005) is anomalous. Reasons suggested by the authors include cold-adaptation and/or an enrichment method that may help recover cold-stressed or VBNC cells. A period of cold acclimatisation has been shown to increase the ability of *V. vulnificus* to survive subsequent cold storage (Limthammahisorn *et al.*, 2009). However, these factors also apply to other studies in TABLE 2, and perhaps their results arise from only testing one batch of oysters at each time point (1, 7 and 14 days). It is possible that, by chance, the oysters sampled in the first batch contained lower concentrations of *V. vulnificus* so the two subsequent measurements appear as growth. Growth experiments using the *V. vulnificus* isolates from this study would be informative.

TABLE 2 clearly shows that the concentration of culturable *V. vulnificus* decreases in frozen oysters but freezing cannot be relied upon as a control method since *V. vulnificus* are able to survive long periods in a frozen state. *V. vulnificus* in naturally contaminated oysters were still detected after three months of frozen storage at -20ºC (Cook and Ruple, 1992).

A New Zealand study has evaluated the behaviour of *V. vulnificus* in Pacific oysters as a result of flash freezing followed by frozen storage (G. Fletcher, Plant & Food Research, pers. comm.).[13] Pacific oysters were contaminated with *V. vulnificus* using bioaccumulation tanks (starting concentration approximately 4.5 $\log_{10}$ MPN/g), vacuum-packed, held at -55°C until the internal temperature was -18ºC (19-23 min), then stored at -18ºC. The experiment was continued until tests showed that the concentration of *V. vulnificus* in 30 (or more) oysters had reduced by 3.52 $\log_{10}$ MPN/g (approximately 90 days). This was the end-point measured in an equivalent study for *V. parahaemolyticus* (Liu *et al.*, 2009).

The results have not been fully analysed. Preliminary analysis shows that the concentration of *V. vulnificus* initially decreased one day after freezing in 5/6 batches tested, then continued to decrease at a slower, log-linear rate over time (G. Fletcher, Plant & Food Research, pers. comm.). The initial decrease was not observed in a sixth batch of oysters. The difference was possibly because the sixth batch were smaller oysters that would have frozen faster, causing less damage to the *V. vulnificus* cells. Work is continuing to look at the differences in *V. vulnificus* survival between oysters of different sizes.

An initial decrease in *V. vulnificus* concentration followed by a slower, linear decrease was also observed when contaminated oysters were frozen by blast freezing, carbon dioxide or liquid nitrogen, before frozen storage (Mesty and Rodrick, 2003). Reductions of 2 $\log_{10}$ MPN/g *V. vulnificus* were observed in whole oysters immediately after freezing with CO_2 and after 14 days of storage at -10ºC there were no *V. vulnificus* recovered. In whole oysters frozen with NO_2, it was 21 days before *V. vulnificus* became non-detectable. *V. vulnificus* were more rapidly inactivated when oysters were treated as half-shells.

[13] Work carried out under the Seafood Safety Programme by Plant & Food Research.

TABLE 2: Change in the concentration of *V. vulnificus* in raw shellfish held under different post-harvest conditions

SHELLFISH TESTED	SHELLFISH SOURCE	INOCULUM	CONDITIONS	CHANGE IN CONCENTRATION (log_{10} MPN/g or log_{10} CFU/g)[1]	REFERENCE
Storage ≥13ºC					
Oyster (*C. virginica*)	Alabama waters, USA	Naturally present (910-5,400 MPN/g)	Ambient air temperature (24-33ºC), 3.5-14 h	↑ 1.9 (mean at 14 h)	(Cook, 1997)
Oyster (*C. virginica*)	Alabama waters, USA	Naturally present (~4 log_{10} MPN/g)	Ambient air temperature (28-32ºC), 5 or 24 h	*vvh*A PCR: ↑ 1.4 (mean at 5 h) ↑ 1.2 (mean at 24 h)	(Kinsey *et al.*, 2015)
Oyster (*C. virginica*)	University of Delaware, USA	Accumulated in inoculated seawater (initial counts ~8.4 log_{10} MPN/g)	21ºC or 35ºC, 5 h, air	NC	(Ye *et al.*, 2013)
Oyster (*C. virginica*)	Alabama waters, USA	Naturally present (330-5,100 MPN/g)	18ºC, 30 h	↑ ~0.7	(Cook, 1994)
Oyster (shucked) (*C. gigas*)	Markets, Seoul, Korea	Naturally present (~1.2 log_{10} CFU/g)	16, 18 or 24ºC, 40 h 30 or 36ºC, 20 h	↑ 1.5-2.7 ↑ 2.7	(Kim *et al.*, 2012)
Oyster (*C. virginica*)	Chesapeake Bay, VA, and Mobile Bay, AL, USA	Naturally present (means of samplings range 10^1-10^4 MPN/g)	15 or 20ºC, 14 d 25 or 30ºC, 7 d	↑ 1-3 after 24 h[2]	(DaSilva *et al.*, 2012)
Cool storage (<13ºC, unfrozen)					
Oyster (*C. virginica*)	University of Delaware, USA	Accumulated in inoculated seawater (initial counts ~8.4 log_{10} MPN/g)	10ºC, 1 d, seawater (salinity 15-20‰)	↓ 1.9	(Ye *et al.*, 2013)
Oyster (*C. virginica*)	Alabama waters, USA	Naturally present ($1.1x10^5$ MPN/g)	6ºC, 14 d	NC	(Wood and Arias, 2012)
Oyster (*C. virginica*)	Florida waters (retail), USA	Naturally present ($1.0x10^4$ MPN/g)	6ºC, 14 d	↑ 1.0	(Wood and Arias, 2012)

SHELLFISH TESTED	SHELLFISH SOURCE	INOCULUM	CONDITIONS	CHANGE IN CONCENTRATION (log_{10} MPN/g or log_{10} CFU/g)[1]	REFERENCE
Oyster (*C. virginica*)	Chesapeake Bay, USA	Naturally present (means of 3 samplings: 6, 10^3, 10^4 MPN/g)	5ºC, 21 days 10ºC, 21 days	↓ 2.2 ↓ 1.8	(DaSilva *et al.*, 2012)
Oyster (*C. virginica*)	Mobile Bay, AL, USA	Naturally present (means of 3 samplings: 10^1, 10^1, 10^2 MPN/g)	5ºC, 21 days 10ºC, 21 days	↓ 1.3 ↓ 1.2	(DaSilva *et al.*, 2012)
Oyster (*C. virginica*, *C. gigas*)	Retail, USA	Naturally present (370 lots with <0.2-10^5 MPN/g)	Chilled retail conditions (average below 5ºC), average storage time 7.7 d	↓ 10% per day	(Cook *et al.*, 2002)
Oyster (*C. virginica*)	Louisiana waters, USA	Naturally present (3.4 log_{10} CFU/g)	3-5ºC, 21 d	↓ 2.8	(Andrews *et al.*, 2003)
Oyster (*C. virginica*)	Louisiana waters, USA	Accumulated in inoculated seawater (10^7 log_{10} CFU/g)	3-5ºC, 21 d	↓ 6.0	(Andrews *et al.*, 2003)
Oyster (*C. virginica*)	University of Delaware, USA	Accumulated in inoculated seawater (initial counts 8.4 log_{10} MPN/g)	4ºC , 1-2 d, seawater (salinity 15-20‰)	↓ 3.0	(Ye *et al.*, 2013)
Oyster (*C. virginica*)	Louisiana waters, USA	Naturally present (2,300 MPN/g)	4ºC, 7 d	↓ 0.4	(Cook and Ruple, 1992)
Oyster (*C. virginica*)	Alabama waters, USA	Accumulated in inoculated seawater (1x10^6 CFU/g)	35ºC, 10 h, chilled, then 4ºC, 168 h	↓ 1.5	(Limthammahisorn *et al.*, 2009)
Oyster (*C. virginica*)	Alabama waters, USA	Accumulated in inoculated seawater (1x10^6 CFU/g)	25ºC, 12 h, chilled, then 4ºC, 168 h	↓ 1.2	(Limthammahisorn *et al.*, 2009)
Oyster (*C. virginica*)	Alabama waters, USA	Accumulated in inoculated seawater (1x10^6 CFU/g)	15ºC, 36 h, then 4ºC 168 h	NC	(Limthammahisorn *et al.*, 2009)

SHELLFISH TESTED	SHELLFISH SOURCE	INOCULUM	CONDITIONS	CHANGE IN CONCENTRATION (log_{10} MPN/g or log_{10} CFU/g)[1]	REFERENCE
Oyster (*C. virginica*)	Alabama waters, USA	Accumulated in inoculated seawater (~5 log_{10} MPN/g)	2-3ºC, 14 d	↓ 2	(Kaysner *et al.*, 1989)
Oyster (*C. virginica*)	Alabama waters, USA	Naturally present (~2-3 log_{10}/g)	0.5ºC, 7 d 10ºC, 7 d	Detected	(Kaysner *et al.*, 1989)
Oyster (*C. virginica*)	Louisiana waters, USA	Naturally present (2,300 MPN/g)	Containers packed on ice (0ºC), 7 d	↓ 1.2	(Cook and Ruple, 1992)
Oyster (*C. virginica*)	Louisiana waters, USA	Naturally present (2,300 MPN/g)	-1.9ºC, 7 d	↓ 2.7	(Cook and Ruple, 1992)
Frozen storage					
Oyster	Seafood wholesaler	Naturally present[3]	Immersion in liquid nitrogen, then: -10ºC, 1 d -10ºC, 21 d	Means of three lots: 1 day: ↓ 0.4-2.5 21 days: ↓ 1.2-3.8	(Wright *et al.*, 2007)
Oyster (*C. virginica*)	University of Delaware, USA	Accumulated in inoculated seawater (initial counts 8.4 log_{10} MPN/g)	-18ºC, 14 d	↓ 2.9	(Ye *et al.*, 2013)
Oyster (shucked; *C. virginica*)	Louisiana waters, USA	Naturally present ($1.5x10^5$ MPN/g)	-20ºC, 12 weeks	↓ 3-5	(Cook and Ruple, 1992)
Oyster (*C. virginica*)	Gulf coast waters, USA	Naturally present ($1x10^4$ CFU/g)	-20ºC, 70 d -20ºC, 70 d (vacuum packaged)	↓ 3-4, both treatments at 70 d	(Parker *et al.*, 1994)

[1] ↑ increase in concentration, ↓ decrease in concentration; NC, no change in concentration (<0.5 log_{10} change); ND, not detectable. For naturally contaminated BMS the change in concentration is that measured against other naturally contaminated BMS prior to the storage conditions. Where data are not specified, estimates have been made based on graphs.

[2] Average growth rates were (log MPN/h): 0.022, 0.042, 0.087 and 0.093 for storage at 15, 20, 25 and 30ºC, respectively. The change in concentration was not reported for the experiments continued for 14 days at 20ºC and 7 days at 30ºC (only data at 24 hours were reported).

[3] The natural concentration (2-4 log_{10} MPN/g) was increased by temperature abuse (24h, 26ºC) prior to freezing.

2.4.2 The effect of shellfish preparation

V. vulnificus are sensitive to low pH so acidic marinades or sauces may affect them. Marinating half-shell oysters (*C. virginica*) in lemon juice or white vinegar for 30 minutes reduced the concentration of naturally present *V. vulnificus* by 3.4 and 2.5 $\log_{10}$ MPN/g, respectively (Borazjani *et al.*, 2003). While there was some belief that "hot sauces", such as Tabasco sauce or horseradish-based seafood cocktail sauce, killed *V. vulnificus* in BMS, scientific investigations measured no significant reduction in the concentration of *V. vulnificus* inside the meat of raw oysters treated with sauce (10 minutes) compared with control oysters to which no sauce was added (Sun and Oliver, 1995). The Tabasco sauce significantly reduced the concentration of *V. vulnificus* on the surface of the oysters. This suggests that *V. vulnificus* within the oyster tissue are partially protected from acidic hot sauces.

Vibrios are readily destroyed by cooking even when the oysters are highly contaminated (Codex Alimentarius, 2010). *V. vulnificus* is considered to be more sensitive to heat than other *Vibrio* species and other foodborne pathogens. *V. vulnificus* (4.3×10^3 CFU/g) in naturally contaminated shellfish was reduced to non-detectable levels by exposing oysters to a temperature of 50ºC for 10 minutes (Cook and Ruple, 1992). This treatment, often called low temperature pasteurisation, does not impart a noticeable cooked appearance or taste to oysters and may be employed as a strategy to improve the safety of oysters (see Section 5.2).

2.5 EXPOSURE ASSESSMENT

KEY FINDINGS

V. vulnificus have been detected in Pacific oysters sampled from harbours located in the Northland, Auckland and Coromandel regions. The concentration of *V. vulnificus* in most positive samples was ≤10 MPN/g. The highest concentration measured was 9,300 MPN/g, in 2011. Molecular analyses of 30 *V. vulnificus* isolates suggested that the majority of strains of *V. vulnificus* found in New Zealand Pacific oysters are of a type less associated with human disease.

V. vulnificus were detected in Pacific oysters more often and at higher concentrations during summer months compared with other seasons, when sea surface temperatures were ≥20ºC. There was no correlation with salinity (*V. vulnificus* were isolated from Pacific oysters at salinities up to 37‰). Preliminary data from one study suggests that rainfall events increased the numbers of *V. vulnificus* in Pacific oysters in one harbour, but using environmental indicators to predict the presence of *V. vulnificus* is both difficult and site-specific.

V. vulnificus were not isolated from 21 samples of commercially-grown dredge oysters nor from 55 samples of green-lipped mussels in a survey from 2009-2012. A 1995/96 study found *V. vulnificus* in non-commercially harvested pipi and cockles.

No consumer level recalls were issued in New Zealand during the period January 2001 to August 2016 for BMS potentially contaminated with *Vibrio* spp.

Water temperature and salinity do not represent barriers to the occurrence of *V. vulnificus* during the summer months in New Zealand, particularly in northern areas of New Zealand and/or during La Niña. Over the last decade, the La Niña phase has been present during the summers of 2008, 2009 and 2011. The 2011 phase was particularly prolonged.

A comparison of data from two National Nutrition Surveys suggests that shellfish are being consumed less often by adults in 2009 compared to 1997. Data from a survey of children (2002) indicate that children consume lower quantities of shellfish, less often than adults.

V. vulnificus will not grow in BMS post-harvest if the shellfish are kept at temperatures ≤10ºC. There is potential for growth to occur during the period between harvest and first

cooling, and after retail sale. Retail and food service storage temperatures for BMS are not readily available.

2.5.1 New Zealand prevalence studies

There are six studies of *V. vulnificus* in BMS freshly harvested from New Zealand waters.[14] All of these studies included samples of Pacific oysters and the results for this species are summarised in TABLE 3; the details from each study follow the table. *V. vulnificus* were only detected in one sample of Pacific oysters from the Marlborough region (TABLE 3), and were not detected in samples of dredge oysters from the South Island (n=21) or green-lipped mussels from the North and South Islands (n=55). *V. vulnificus* have been isolated from non-commercially harvested pipi and cockles. There are no surveys for *Vibrio* spp. in BMS sampled at New Zealand retail or food service outlets.

TABLE 3: Prevalence and maximum concentration of *V. vulnificus* measured in pooled samples of Pacific oysters taken from New Zealand harbours

YEAR OF SURVEY	LOCATION OF HARBOUR(S) SAMPLED	LIMIT OF DETECTION (MPN/g)	*V. VULNIFICUS* PREVALENCE	MAXIMUM CONCENTRATION OF *V. VULNIFICUS* MEASURED
1995-1996	Upper half North Island	NR	0/12	NA
2008-2009	Upper half North Island	3	10/58 (17%)	20 MPN/g
2009-2012	Upper half North Island	0.36	32/217 (15%)	9300 MPN/g
2010-2012	Marlborough Sounds	0.36 or 3	0/18	NA
2013	Upper half North Island	0.31	0/48	NA
2014	Upper half North Island	0.31	6/91 (7%)	1.6 MPN/g
2015	Upper half North Island	0.31	1/22 (5%)	0.74 MPN/g
2015	Marlborough Sounds	0.31	1/19 (5%)	0.92 MPN/g
2015	Upper half North Island	NR	14/150 (9%)	15 MPN/g
2016	Upper half North Island	0.36	13/30 (43%)	424 MPN/g
2016	Marlborough Sounds	0.36	0/10	NA

NR, not reported; NA, not applicable.

1995/96 summer survey of Pacific oysters (McCoubrey, 1996)

V. vulnificus were not detected in 12 samples of farmed Pacific oysters collected from four harbours in the upper half of the North Island, from December 1995 to March 1996. This was despite sampling being targeted to areas and times where seawater conditions were optimal for the presence of these bacteria. Sampling times after rainfall that might reduce salinity were chosen, as otherwise the salinity was suboptimal for the organism. Parallel sampling and testing (using the same methods) of wild shellfish from recreational sites in the Bay of Plenty did find *V. vulnificus* in four samples (pipis, cockles, and snails). This sampling was undertaken by the Bay of Plenty Regional Council and the total number of samples taken was not given.

[14] The laboratory method used for the 2015 industry testing was not reported. The laboratory methods and microbiological media used for the other five studies were similar but had varied detection limits (see table). McCoubrey (1996) did not use an MPN.

2008/09 summer survey of Pacific oysters (Kirs *et al.*, 2011; Kirs *et al.*, 2010)

From December 2008 to April 2009, a total of 58 pooled Pacific oyster samples (each containing 10-12 oysters) were taken from six aquaculture farms located in the upper half of the North Island. Oysters had been grown in plastic netting bags, racks or on sticks attached to racks located in the intertidal zone, and most (70%) were sampled during intertidal exposure.

V. vulnificus were detected in 10/58 (17.2%) samples (95% Confidence Interval (CI) 8.6-29.4%) and positive samples were found from all farms. The concentrations were generally low (<3-10 MPN/g, apart from one sample at 20 MPN/g). *V. parahaemolyticus* was also detected during this survey but there was no significant correlation between the concentrations of these two *Vibrio* species.

Molecular analysis of the 16S rRNA gene of eighteen *V. vulnificus* isolates was conducted. Identification of a type was only successful for 10 of these: Eight of Type A and one of each of Types B and AB. Type A is associated with non-clinical isolates of *V. vulnificus* and Type B is associated with clinical isolates. These data suggest that the majority of strains of *V. vulnificus* found in New Zealand are of a less virulent type, but the correlation between these types and pathogenicity is not yet definitive.

The mean water temperature during the study was 20.6ºC (range 16.0-24.8, with one measurement at 31.5ºC (cause not known)). The mean water salinity was 34‰ (range 28-37‰, with one measurement at 13‰, which was associated with a rainfall event). There was no significant correlation between the concentration of *V. vulnificus* and water temperature, salinity, or any of the other environmental parameters measured. The absence of correlation with environmental parameters may have been due to lack of variation in these parameters, or else the fact that they were not measured continuously.

2009-2012 survey of oysters and green-lipped mussels (Cruz *et al.*, 2016)

Between December 2009 and June 2012, a total of 235 pooled Pacific oyster samples, 21 pooled dredge oyster samples and 55 pooled green-lipped mussel samples were taken from eight aquaculture farms in the Northland, Auckland, Coromandel and Marlborough Sounds regions. Each pooled sample included the meat and liquor from 12 individual shellfish. The Pacific oysters from the North Island were grown in intertidal racks. The dredge oysters, mussels and Pacific oysters from the South Island were grown subtidally. Sampling from the Marlborough Sounds did not commence until 2010.

V. vulnificus were detected in 32/217 (15%) of Pacific oyster samples from the North Island, with concentrations in the range 0.31-9300 MPN/g. *V. vulnificus* were not detected in Pacific oyster samples from the South Island (n=18), dredge oysters from the South Island (n=21), or green-lipped mussels from the North and South Islands (n=55).

The frequency of *V. vulnificus* positive samples was significantly higher in the summer months (December to May) than the winter months (June to November). The highest concentrations were recorded in the summer of 2011 when surface sea temperatures remained elevated for longer than was observed in 2010 and 2012. Significant mortality from Ostreid herpesvirus-1 was also noted during this year (G. Fletcher, Plant & Food Research, pers. comm.).

V. vulnificus were not detected when the surface sea temperature was <20ºC, except in one sample (0.62 MPN/g, harvested in June 2010 from water at 13ºC and 21‰ salinity). *V. vulnificus* were detected from BMS harvested from waters with salinities in the range 21-37‰, and no correlation between concentration and salinity was evident.

Of 20 *V. vulnificus* isolates, all were biotype 1, and all but one were classified as the E-genotype and 16S rRNA type A (16S rRNA types were not identified for two isolates). The single C-genotype isolate was also found to be a 16S rRNA type B.

2013-2015 oyster farming method experiments[15]

During the summer of 2013, the concentration of *V. vulnificus* was measured in Pacific oysters grown in a Northland harbour for the purpose of investigating whether there was a relationship between water depth and *Vibrio* spp. contamination (G. Fletcher, Plant & Food Research, pers. comm.; publication pending). *V. vulnificus* were not detected in 48 samples. The experiment was repeated in a different Northland harbour during the 2014 summer, and *V. vulnificus* were detected in 6/91 (7%) of samples, at a maximum concentration of 1.6 MPN/g.

During the 2015 summer, *V. vulnificus* were also measured in samples taken from commercial growing areas in the Coromandel and Marlborough regions, for comparative purposes. *V. vulnificus* were detected in 1/22 (5%) samples from Coromandel (0.74 MPN/g) and 1/19 (5%) samples from Marlborough (0.92 MPN/g).

The sporadic pattern of detection meant that it was not possible to correlate the presence or concentration of *V. vulnificus* with water depth or growing method (intertidal, subtidal). The available data on seawater temperature shows temperatures in all four harbours fluctuating between approximately 15 and 25ºC, but the authors report that the temperatures in the Marlborough site were, on average, lower than those recorded at the North Island sites.

2015 industry testing

MPI required that if Pacific oysters were being commercially harvested from a growing area during the period 15th January to 15th April 2015, weekly samples of oysters from the area had to be tested for *V. vulnificus* (Fletcher and Wei, 2016). If the concentration was ≥30 MPN/g *V. vulnificus* the growing area had to be closed. No samples exceeded this limit.

Samples were tested from sites located in the Northland, Auckland and Coromandel regions. From 13th January 2015 to 16th April 2015, 150 samples were tested and *V. vulnificus* were detected in 14 samples (9%).[16] The concentrations of *V. vulnificus* in the positive samples were low: 3 MPN/g (1 sample), 4 MPN/g (9 samples), 9 MPN/g (1 sample), 11 MPN/g (1 sample) and 15 MPN/g (2 samples). For the dates when these positive samples were taken, the average daily temperature was always >20ºC, and the salinity was always ≥32‰, although temperature and salinity data were not available for all positive samples.[17] Salinity readings of <32‰ were not recorded during the entire sampling period.

2016 survey of Pacific oysters

During the period January-June 2016, Pacific oysters were sampled fortnightly from four harbours and tested for *V. vulnificus* and *V. parahaemolyticus* (Fletcher and Wei, 2016). Three of the harbours were in Northland and one was in Marlborough. The oysters were tested in batches of 12.

Of 40 samples tested, *V. vulnificus* were detected in 13 (33%). The limit of detection was 0.36 MPN/g. None of the 10 samples from Marlborough were positive, so the prevalence for the three North Island sites was 13/30 (43%). The concentration of *V. vulnificus* ranged from 0.36 MPN/g (four samples) to 424 MPN/g (one sample).

Full analysis of these data has not been completed. The raw data show:

- Six samples, all from one harbour, contained *V. vulnificus* at 42 MPN/g or higher. Concentrations of 231 MPN/g (2 samples) and 424 MPN/g (1 sample) were measured

[15] Research supported through the MBIE-funded Safe New Zealand Seafood Programme.
[16] As calculated from data provided to MPI by the oyster industry.
[17] Temperature data were available for 10/14 positive samples, salinity data for 11/14 positive samples.

from samples taken when the harbour was closed due to high rainfall events. These events had decreased seawater salinity (down to 0‰) during the two weeks prior to measurement of these peak *V. vulnificus* concentrations.

- *V. vulnificus* were detected from oysters collected from waters of salinities >30‰ in all North Island harbours.
- The last positive sample was detected at the end of April (3.6 MPN/g). Water temperatures from a nearby buoy showed a diurnal pattern developing during April, but afternoon/evening temperatures were still ≥20ºC.

The weight of the meat and liquor tested was available for four pooled samples from one harbour. Estimates for the number of *V. vulnificus* per oyster can be calculated from these data:

- For the maximum concentration detected in oysters from this harbour (424 MPN/g): The estimated concentration per oyster is 1.4×10^4 *V. vulnificus*, assuming that the *V. vulnificus* were evenly distributed amongst the 12 pooled oysters. If all the *V. vulnificus* were present in only 1/12 oysters, the estimated concentration in this oyster would be 1.6×10^5 *V. vulnificus*.
- For the minimum concentration detected in oysters from this harbour (3.57 MPN/g): The estimated concentration per oyster is 114 *V. vulnificus*, assuming that the *V. vulnificus* were evenly distributed amongst the 12 pooled oysters. If all the *V. vulnificus* were present in only 1/12 oysters, the estimated concentration in this oyster would be 1.4×10^3 *V. vulnificus*.

Data are not available on virulence markers for these isolates.

2.5.2 Product recalls

No consumer level recalls were issued in New Zealand during the period January 2001 to August 2016 for BMS potentially contaminated with *Vibrio* spp. FSANZ did not issue any recalls for BMS/*Vibrio* spp. during the same period.

2.5.3 New Zealand marine conditions

Available surface seawater temperature and salinity data gathered during the New Zealand BMS surveys are included in Section 2.5.1. These data show that, during the summer and autumn months, BMS are commercially harvested from waters at temperatures most favourable for *V. vulnificus* growth (≥20ºC). These data also show that *V. vulnificus* have been detected in Pacific oysters harvested from New Zealand waters with salinities of >30‰, a level considered to be suboptimal for this pathogen in environmental surveys from other countries. Moreover, laboratory studies of *V. vulnificus* isolates from New Zealand-grown Pacific oysters suggest that *V. vulnificus* have adapted to higher salinities in New Zealand waters.

More general surface seawater temperature data are collected by the National Institute of Water and Atmospheric Science (NIWA). Sites in northern New Zealand have an annual mean coastal sea-surface temperature around 17ºC, compared to a mean of 12ºC in southern New Zealand sites.[18] The maximum temperature reported at the northern-most coastal monitoring station (Ahipara) during the period 1953-2014 was 23.8ºC, and was 17.2ºC in the southern-most coastal monitoring station (Bluff). However, temperatures in oyster growing harbours can exceed this for short times, e.g. 28.2°C recorded in Whangaroa Harbour by the

[18] 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 15 August 2016). 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 15 August 2016).

monitoring buoy when the tide was in during the 2016 survey of Pacific oysters (G. Fletcher, Plant & Food Research, pers. comm.).

New Zealand's climate is affected by the El Niño Southern Oscillation (ENSO). The La Niña phase of this oscillation brings warmer waters to the New Zealand coast, generally warmer weather, and increased rainfall to the north-east of the North Island.[19] Over the last decade, the La Niña phase has been present during the summers of 2008, 2009 and 2011.[20] The 2011 phase was particularly prolonged, spanning from mid-2010 to mid-2011.

Based on these data, temperature and salinity do not represent barriers to the occurrence of *V. vulnificus* during the summer months in New Zealand, particularly in northern areas of New Zealand and/or during La Niña. Spring and autumn periods may also support the presence of *V. vulnificus* in New Zealand coastal waters, but probably only in warmer, northern areas. Extended analyses of available temperature and salinity data from all of the New Zealand studies (Section 2.5.1) may indicate the New Zealand coastal water conditions that favour the presence of *V. vulnificus* in BMS, particularly if northern sites are compared with Marlborough, where *V. vulnificus* have rarely been isolated from BMS. However, studies from other countries show that using environmental indicators to predict the presence of *V. vulnificus* is both difficult and site-specific.

2.5.4 BMS consumption by New Zealanders

The following information is taken from analyses (Cressey, 2013; Cressey *et al.*, 2006) of data from the 24-hour dietary recall components of the New Zealand adult nutrition surveys conducted in 1997 (1997NNS; Russell *et al.*, 1999) and 2008-2009 (2009ANS; University of Otago and Ministry of Health, 2011), plus the 2002 Children's National Nutrition Survey (2002CNS; Ministry of Health, 2003). It should be noted that these data do not distinguish between commercial or non-commercial sources of shellfish, and that 'paua' and 'paua fritters' were included in these analyses. Prawns and lobsters were excluded.

Proportion of the population consuming shellfish

For the adult New Zealand population, 1.5% of survey respondents reported consuming shellfish in the previous 24-hour period, compared to 2.4% in 1997 (TABLE 4). Those aged over 65 years of age are approximately as likely (1.3%) to consume shellfish than those aged under 65 years of age (1.5%). This is a change from the 1997NNS, which found that those aged over 65 years of age were less likely (1.7%) to consume shellfish than those aged under 65 years of age (2.6%). None of the pregnant participants in the 2009ANS (*n*=64) reported consuming shellfish.

A FSANZ assessment of the 1997NNS data, using a series of standard recipes to determine quantities of commodities in compound food, estimated the proportion of respondents consuming mussels, oysters and scallops as 1.9, 0.6, and 0.3% per day respectively (ANZFA, 2001). In the 2009ANS these proportions were 1.0, 0.3 and 0.1% per day, respectively.

Children aged 5-15 years are infrequent consumers of shellfish, with only 0.5% of respondents in the 2002CNS reporting consumption of shellfish in the previous 24-hour period.

[19] 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 17 August 2016)

[20] http://www.bom.gov.au/climate/current/soihtm1.shtml (accessed 17 August 2016). A sustained period of +7 are typical of a La Niña episode.

TABLE 4: Consumption of shellfish by New Zealanders (national nutrition surveys)

STATISTIC	ADULT (1997NNS)	ADULT (2009ANS)	CHILD (2002CNS)
Number of respondents	4636	4721	3275
Number of servings	128	74	16
Number of consumers (percentage of total respondents)	112 (2.4%)	69 (1.5%)	16 (0.5%)
Servings/consumer/day (average)	1.1	1.1	1.0
Consumer mean (g/person/day)	105.5	85.1	49.4
Respondent mean (g/person/day)*	2.5	1.2	0.2
Mean serving size (g)	92.3	79.3	49.4
Median serving size (g)	64.0	65.5	43.5
95th percentile serving size (g)	276.0	164.4	108.0
Number of consumers above 95th percentile serving size point (percentage of consumers)	7 (6.1%)	4 (5.9%)	(not reported)

* The total amount of shellfish consumed during the 24-hour recall period divided by the total number of survey respondents. This is an estimate of the ongoing mean daily consumption of the food across the whole population.

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

More recently, a study lead by the National Institute of Water and Atmospheric Research (NIWA) investigated the kai moana consumption patterns in two Māori populations; Te Arawa, living around Lake Rotorua in the North Island, and Arowhenua, living in the South Canterbury region of the South Island (NIWA, 2011). In the Te Arawa cohort, 21% of respondents reported eating mussels at least weekly, with half of those respondents eating mussels 3-4 times each week. In the Arowhenua cohort, a similar proportion of respondents (20%) reported consuming mussels at least weekly, but none reported consuming mussels more frequently than twice per week.

Mean daily consumption of shellfish

Analysis of all (raw and cooked) shellfish serving data from the adult nutrition surveys indicates that the mean amount (g/person/day) of shellfish consumed has decreased over time (TABLE 4), for both those who reported eating shellfish (consumers) and all survey respondents (respondents). In the 2009ANS, daily consumption by consumers less than 65 years (91 g/person/day) is markedly higher than consumers 65 years and older (66 g/person/day). The amount consumed per day by a child (5-15 years) is less than for an adult (TABLE 4).

The FSANZ assessment of the 1997NNS data reported a mean amount eaten by consumers of 69.2, 92.0, and 69.7 g/day respectively for mussels, oysters and scallops (ANZFA, 2001). In the 2009ANS, the mean amounts of mussels, oysters and scallops reported as eaten by consumers were 85.2, 121 and 57.6 g/day, respectively.

A 2011 analysis of the amount of raw, shucked shellfish available to New Zealanders estimated 8 g/person/day for the total New Zealand population, and 407 g/person/day for

shellfish consumers (King and Lake, 2013). These values were compared with data from the 1997NNS and 2002CNS because results from the 2009ANS were unavailable at the time. These values are around three times that reported in the nutrition surveys for adults and children combined. However, the figures of King and Lake (2013) represent an estimate of the raw shucked shellfish 'available for consumption', while the nutrition survey figures represent shellfish reported to have been consumed. The differences between these two figures are not unusual, particularly considering the weight lost with cooking prior to consumption.

Analyses of data from the adult nutrition surveys suggest Māori consumers, on average, consume larger amounts of shellfish. From the 1997NNS, the average daily consumption of shellfish by Māori was 139 g as compared to 99 g for non-Māori. These figures from the 2009ANS were 135 g and 69 g, respectively, suggesting decreased daily consumption by non-Māori. These data represent a national average; consumption is likely to vary between regions and be influenced by access to kai moana harvesting areas (rohe moana). The NIWA study derived estimates for mussel consumption of 16.9 g/person/day for the Te Arawa cohort and 11.1 g/person/day for the Arowhenua cohort (NIWA, 2011). These are lower than the FSANZ estimate (38.4 g/day), but not directly comparable since the survey populations, methods and timeframes differ.

Serving sizes of shellfish

A comparison of serving sizes between the 1997NNS and 2009ANS shows that mean and 95th percentile serving sizes have decreased, but the median serving sizes are similar (TABLE 4). The difference in mean serving sizes between 1997 and 2009 is not statistically significant (Cressey, 2013).

Child servings (2002CNS) are smaller than those of adults. These values are derived from all shellfish servings, whether raw or cooked. There are insufficient data to differentiate raw versus cooked servings.

In deriving daily consumption estimates for kai moana mussels in the Te Arawa cohort, NIWA used a 'meal size' of 144 g for kākahi (freshwater mussels), mussels and pipi (NIWA, 2011).

In an assessment of heavy metal contaminant exposure from consumption of green-lipped mussels in the Bay of Islands, a mean serving size of 78 g was used (Whyte *et al.*, 2009). While the source for this figure was not identified, it is very close to the mean adult serving size derived from the 2009ANS.

Types of shellfish consumed and cooking method used

Of 74 servings of shellfish identified in the 2009ANS 24-hour dietary recall records, 45 (61%) were mussels, 12 (16%) were oysters and 5 (7%) were scallops. The balance was paua, pipis, tuatua or recipes in which the shellfish was not specifically identified.

Compared to the 1997NNS, a greater proportion of shellfish servings were mussels (61% compared to 46%), about the same proportion were oysters (16% compared to 17%) and fewer servings were scallops (7% compared to 12%).

Oysters were the shellfish most commonly consumed raw (6/12 – 50% of servings). Mussels were consumed raw (7/45) or marinated (11/45) for 40% of servings. These results are proportionally similar to those from 1997NNS (59% of oyster servings and 47% of mussel servings eaten raw or marinated).

There is a data gap concerning exposure assessment from shellfish, in that while recreational gathering of wild shellfish is acknowledged to be widespread, there are few quantitative consumption data specifically focussing on non-commercial BMS consumption. The NIWA study has provided some information. A full analysis of data from the 2012 recreational fisher survey (Wynne-Jones *et al.*, 2014) using the weight conversion methods of King & Lake (2013) would provide additional information.

2.5.5 Potential for growth of *V. vulnificus* along the food chain

Growth of *V. vulnificus* in harvested BMS is determined by the time/temperature profile from the point of harvest to the point of consumption.

Given suitable temperatures (≥15°C, possibly lower), *V. vulnificus* are able to grow in BMS, with the extent of growth depending on the time at suitable temperatures. *V. vulnificus* growth also appears to vary from one lot of oysters to the next (DaSilva *et al.*, 2012). Suitable growth conditions may occur during the holding period between harvest and transport/processing.

Once refrigeration is achieved, growth of *V. vulnificus* will cease. New Zealand data on refrigeration conditions from the point of harvest to the point of sale (including any retail or food service steps) are not readily available, but there is a regulatory requirement that BMS must be cooled to 7ºC after harvest (Section 5.1.1). The concentration of *V. vulnificus* decreases in BMS held at 7ºC. Refrigerated storage time from harvest to consumption for New Zealand has been reported as 1-5 days with a most likely time of two days (FAO/WHO, 2005). These time periods would be expected to achieve only modest (<1 $\log_{10}$ CFU/g) reductions in concentrations if refrigeration is maintained. There is potential for *V. vulnificus* to grow after the point-of-sale if consumers do not maintain the cool chain. A survey of 127 domestic refrigerators in New Zealand homes identified some that were operating above 15°C (Gilbert *et al.*, 2007).

2.6 DATA ON *V. VULNIFICUS* IN BMS FROM OTHER COUNTRIES

KEY FINDINGS

V. vulnificus have been isolated from oysters, mussels and clams sampled directly from growing waters in multiple countries. There are few published surveys of Pacific oysters; data from Brazil indicates a prevalence of approximately 6% over four years and concentrations up to approximately 100 MPN/g (values that are both lower than measured in New Zealand Pacific oysters). Most published data for other oyster species are for samples from USA waters. Prevalence of up to 97% and concentrations up to 10^6 MPN/g have been reported for the USA. Published data on mussels and clams tend to be from surveys of European waters. The reported prevalence varied (8-90%) and some of these data come from surveys with small sample sizes.

Data from overseas surveys of BMS for *V. vulnificus* are presented in Appendix A.3.

3. EVALUATION OF ADVERSE HEALTH EFFECTS

3.1 DISEASE CHARACTERISTICS

KEY FINDINGS

Foodborne exposure to *V. vulnificus* can lead to primary septicaemia in people with underlying health conditions. Primary septicaemia is a serious condition and approximately half of infected patients die. Gastrointestinal infections without septicaemia have been documented but this appears to be rare and is usually self-limiting. Antibiotic resistance appears to be widespread and inherent amongst *V. vulnificus*.

V. vulnificus causes three distinct syndromes: Wound infections, primary septicaemia and gastrointestinal infections (Daniels, 2011). Wound infections, which can lead to systemic infection and are potentially fatal (even in otherwise healthy people), are not caused by foodborne transmission. This section focusses on foodborne (oral) exposure.

A recent review of *V. vulnificus* described this organism as the "single most fatal foodborne pathogen in the United States, and possibly in the world" (Oliver, 2015). Approximately half the number of people with *V. vulnificus* primary septicaemia die (the mortality rate for people with *V. vulnificus* wound infections is lower, approximately 15%) (Daniels, 2011). This is, in part, due to people with underlying health conditions being more susceptible to infection via oral exposure. The incubation period is short, averaging 26 hours (Oliver, 2015).

V. vulnificus cells that survive the acidity of the upper gastrointestinal tract can penetrate the intestinal wall (most likely in the ileum) to enter the bloodstream and manifest as primary septicaemia (Horseman and Surani, 2011). Symptoms of primary septicaemia include fever (94%), chills (86%), nausea (60%), abdominal pain (44%), hypotension (43%), and the development of secondary skin lesions (69%), which typically develop on the extremities (Oliver, 2015). Diarrhoea may also be experienced by some patients (Bachman, 1983). Almost one third of patients are in septic shock at hospital admission and the characteristic skin lesions appear within 24 hours of symptom onset (Bross *et al.*, 2007). The median time until death is two days after symptom onset. Cases of primary septicaemia require intensive care and rapid antibiotic treatment (Daniels, 2011). Necrotic skin lesions may require debridement (removal of dead tissue) or amputation (Stavric and Buchanan, 1997).

V. vulnificus septicaemia is most common in patients with suppressed immune systems, especially alcohol-induced liver disease or chronic Hepatitis B or C (both of which can also cause liver disease) (Bross *et al.*, 2007). Data from the USA found that 97% of patients with primary septicaemia had some chronic disease, including liver disease (80%), alcoholism (65%), diabetes (35%), malignancy (17%) and renal disease (7%). Liver disease makes people particularly susceptible because, in addition to immune system dysfunction, they have high concentrations of iron in their serum, and *V. vulnificus* requires iron for survival and growth (Daniels, 2011; Oliver, 2015).

In addition to having underlying medical conditions, the majority of septicaemia cases are males and people over the age of 40 (Oliver, 2015). There are a number of possible reasons for this, e.g. people over the age of 40 are more likely to have developed underlying health conditions, males are more likely to work in the seafood industry, and oestrogen has a protective role against *V. vulnificus* endotoxin.

The gastroenteritis syndrome is characterised by abdominal pain or cramps, nausea, vomiting, diarrhoea, fever, and chills (Horseman and Surani, 2011). Skin lesions do not occur. Gastrointestinal illness may lead to hospitalisation, but is usually self-limiting and very few deaths are reported (Daniels, 2011). Affected patients do not necessarily have predisposing health conditions (Stavric and Buchanan, 1997).

There is likely to be some underreporting of the gastrointestinal syndrome, since infected people rarely require medical attention. However, further information suggests that cases of *V. vulnificus* infection presenting with gastroenteritis without septicaemia appear to be infrequent (perhaps ≤5%) (Daniels and Shafaie, 2000; European Commission, 2001; Evans et al., 1999; FAO/WHO, 2005). Foodborne outbreaks of *V. vulnificus* gastroenteritis associated with BMS consumption have not been reported (Section 3.4). An analysis of 141 cases of *V. vulnificus* infection found 16 (11%) were described as gastroenteritis (Hlady and Klontz, 1996). Further details about most of these cases are not available but one was reported to be simultaneously infected with *V. parahaemolyticus* and one had underlying health conditions. Other documented cases have been reported as having predisposing medical conditions or co-infection by other enteric pathogens (Johnstone et al., 1986; Klontz et al., 1988; USFDA, 1994).

A review of antibiotic resistance across 12 countries found reports of antibiotic resistance amongst *V. vulnificus* isolates and some multi-drug resistance (Elmahdi *et al.*, 2016). Both environmental and clinical isolates showed similar antibiotic resistance profiles. The most frequently observed antibiotic resistance profiles involved ampicillin, penicillin and tetracycline, regardless of the country. One of the studies included in the review found that 17% of 151 *V. vulnificus* isolates were resistant to eight or more antibiotics (Baker-Austin *et al.*, 2009). These isolates were from USA coasts and 10 patients with primary septicaemia, yet the researchers did not identify any difference in antibiotic resistance between virulent and non-virulent strains, nor between environmental isolates from pristine and anthropogenically-impacted waters, which suggested antibiotic resistance was widespread and inherent.

3.2 DOSE RESPONSE

KEY FINDINGS

The dose of *V. vulnificus* required to cause gastroenteritis or septicaemia is not known. Estimates of 10^3 and 10^4 cells have been made for people with pre-existing health conditions that make them more susceptible to infection, but the actual dose may be lower or higher.

The infective dose of *V. vulnificus* is not known. There are no human volunteer studies investigating the dose-response relationship for *V. vulnificus*. Some experiments have been done on mice, rats, hamsters, rabbits or guinea pigs. These animal experiments suggested that all *V. vulnificus* strains might be equally virulent (DePaola *et al.*, 2003). However, this assumption has been questioned as others have indicated that only a few strains of the diverse *V. vulnificus* are linked to human disease (Section 2.1.1).

The infectious dose might be as low as 1,000 cells (European Commission, 2001; Jackson *et al.*, 1997). This is equivalent to 3.3 *V. vulnificus*/g of tissue for a 300 g seafood serving, or approximately 100 *V. vulnificus*/g in a single oyster.[21] Others have proposed that the dose may be higher (Daniels, 2011) or lower (Oliver, 2015; USFDA, 2012). Estimating the infectious dose is complicated by the presence and relative numbers of the C- and E-genotype in BMS (and the uncertainty over their virulence), and the influence of underlying illness in patient

[21] If the meatweight of a dredge oyster is 12.75 g (Table 1, King & Lake 2013), the concentration is 78 *V. vulnificus*/g. Equivalent calculations for Pacific and rock oysters produce the values 110 and 165 *V. vulnificus*/g, respectively.

susceptibility to infection. In addition, oysters growing adjacent to one-another have been shown to carry very different numbers of *V. vulnificus* so it is not possible to know how many *V. vulnificus* cells were ingested by an infected person. A single oyster may contain enough *V. vulnificus* cells of the C-genotype to cause infection and death (Oliver, 2015).

A dose-response model was developed for *V. vulnificus* in raw oysters as part of a quantitative risk assessment, where the response measured was development of septicaemia (FAO/WHO, 2005). The model predicts that a dose of $1x10^4$ *V. vulnificus*/serving can cause illness. The approach combined estimates of oyster consumption within the susceptible population with data on the number of oyster-associated cases reported in the USA. However, the model assumed that all strains were equally virulent and there were no seasonal/regional changes in virulence.[22]

Experiments with simulated stomach and intestinal conditions have demonstrated that antacids increase the survival of *V. vulnificus*, which would elevate the risk of infection for people consuming such medications (Koo *et al.*, 2000a, 2000b; Koo *et al.*, 2001).

3.3 NEW ZEALAND HUMAN HEALTH SURVEILLANCE

KEY FINDINGS

V. vulnificus infection is not notifiable in New Zealand unless an outbreak is detected or the sick person has an occupation that puts others at risk of infection. Gastrointestinal disease as a result of *V. vulnificus* infection will be underreported.

There have been no reported cases or outbreaks of *V. vulnificus* infection in New Zealand where the illness was linked to consumption of BMS. Five cases of *V. vulnificus* infection have been reported in New Zealand. Two had wound infections. There is no information on the transmission route for the other three cases.

A considerable proportion of the New Zealand population is susceptible to severe *V. vulnificus* infection but public health surveillance data suggest that actual infections are very uncommon in New Zealand.

V. vulnificus infection is not a notifiable disease in New Zealand so cases are not routinely reported to New Zealand's notifiable disease database, EpiSurv (Ministry of Health, 2013).[23] However, cases of *V. vulnificus* may be reported to EpiSurv as "acute gastroenteritis" if there is a suspected common source (i.e. an outbreak) or if the sick person is in a "high risk" category (e.g. a food handler, an early childhood service worker).

Cases developing primary septicaemia and who are hospitalised and/or die from the infection will be reported in the Ministry of Health's databases on hospital discharges and/or mortality.

Testing of faecal clinical specimens for *Vibrio* spp. is performed routinely by only a small number (2/13) of laboratories who responded to a New Zealand public health laboratory survey, suggesting that gastrointestinal cases are unlikely to be diagnosed (Lake *et al.*, 2009). For an estimated 80% of faecal samples submitted by acute gastrointestinal cases in New Zealand, no pathogen is identified by routine laboratory testing (Lake *et al.*, 2009). However,

[22] The model is not directly applicable to New Zealand because it is based on data specific to the USA population (e.g. oyster consumption patterns, percentage susceptible population) and data on oysters harvested from the Gulf Coast of the USA (e.g. temperature/salinity parameters, post-harvest practices).

[23] ESR operates the national notifiable disease surveillance database, EpiSurv, on behalf of the New Zealand Ministry of Health (https://surv.esr.cri.nz/episurv/index.php, accessed 11 July 2016).

diagnostic tests may be specifically requested should symptoms (e.g. sepsis) or other information warrant it.

Most (but not all) *Vibrio* spp. isolates from cases are referred to the ESR Enteric Reference Laboratory for species confirmation.

3.3.1 BMS consumption as a risk factor for *V. vulnificus* infection in New Zealand

There have been no reported cases or outbreaks of *V. vulnificus* infection in New Zealand where the illness was linked to consumption of BMS.

3.3.2 *V. vulnificus* infection in New Zealand

No sporadic cases of *V. vulnificus* infection were reported to EpiSurv during the period January 1998 to July 2016. In New Zealand there have been no known cases of foodborne septicaemia caused by *V. vulnificus* infection (Cruz *et al.*, 2016).

Five cases of *V. vulnificus* infection have been reported elsewhere. Two of these cases presented at a Whakatane hospital in 1990 with wound infections (Wright, 1991). Another case was recorded by Rotorua hospital in 1989. The patient in this case died from septicaemia, but further details are not available (McCoubrey, 1996). The Ministry of Health collects data on hospital discharges, and in 2013 the code 'B96.82 *Vibrio vulnificus* as the cause of disease classified to other chapters' was introduced to the reporting system (E. Lewis, Ministry of Health, pers. comm.). Only two cases have been recorded against this code since 2013. Both were males aged 76 and 54, and were discharged during 2015 from hospitals in the Waikato and Bay of Plenty regions. Further details are not available.

No outbreaks of *V. vulnificus* infection have been reported in New Zealand.

3.3.3 The susceptible population in New Zealand

People with underlying medical conditions are more susceptible to primary septicaemia as a result of *V. vulnificus* infection. It is not possible to predict what proportion of the population is considered to be immunocompromised or of above-normal sensitivity to foodborne illness, but some information on specific health conditions are available (Cressey, 2013). This section gathers some preliminary information, for indicative purposes only.

Information gathered in the 2014/15 New Zealand Health survey of 13,497 adults (Ministry of Health, 2015) indicated that:[24]

- 17.7% of the adult population were classified as "hazardous drinkers", an estimated 646,000 adults;[25] and
- 6.1% of the adult population were diagnosed with diabetes, or an estimated 222,000 adults.

Hospital discharge data shows that 205 people were discharged from hospitals during the 2012/13 reporting period with the diagnosis of chronic viral hepatitis (ICD-10 code B18).[26] Over the same period, 1,834 people were discharged for diseases of the liver (ICD-10 codes K70-K77), including 404 people with alcoholic liver disease (ICD-10 code K70) and 458 people with fibrosis and cirrhosis of the liver (ICD-10 code K74). Considerably more people were

[24] Additional data are available at https://www.health.govt.nz/publication/annual-update-key-results-2014-15-new-zealand-health-survey (accessed 11 July 2016).
[25] "Hazardous drinking" refers to an established drinking pattern that carries a risk of harming the drinker's physical or mental health, or having harmful social effects on the drinker or others (Ministry of Health, 2015).
[26] Data are for discharges from public and privately-funded hospitals for the period 1 July 2012 to 30 June 2013, as reported to the Ministry for Health. Data obtained from https://www.health.govt.nz/nz-health-statistics/health-statistics-and-data-sets/hospital-event-data-and-stats (accessed 11 July 2016).

discharged with various cancers. Note that some patients may have been admitted to hospital more than once during the reporting period.

These data suggest that a considerable proportion of the New Zealand population may be susceptible to severe *V. vulnificus* infection. However, public health surveillance data suggest that actual infections are very uncommon in New Zealand. This could be due to underreporting of *V. vulnificus* cases,[27] lower exposures for susceptible people compared with those without the underlying medical conditions that increase susceptibility, low concentrations of *V. vulnificus* in the environment, or a combination of these. The discrepancy is explored further in Section 4.2.2.

3.4 *V. VULNIFICUS* INFECTION OVERSEAS

KEY FINDINGS

In contrast with New Zealand data, approximately 60 cases of *V. vulnificus* infection are reported yearly in the USA, where the disease is notifiable. This includes cases with wound infections, but consumption of oysters (particularly raw oysters) has been confirmed as the most important foodborne transmission route for *V. vulnificus* infections in the USA. Most foodborne cases are associated with oysters harvested from the warm waters of the Gulf of Mexico.

Vibrio spp. infection is notifiable in two Australian states but it appears that cases are infrequently reported in these regions.

There are no reported outbreaks of *V. vulnificus* infection linked to consumption of BMS.

As for New Zealand, gastroenteritis as a result of *V. vulnificus* infection is probably underreported in other countries. However, overseas data supports the view that primary septicaemia as a result of *V. vulnificus* infection is rare. A likely reason is that a combination of factors are required for infection to occur, in particular ingestion of a virulent strain by a person with underlying chronic disease (Oliver, 2015; USFDA, 2012).

Data on *V. vulnificus* infection in other countries are presented in Appendix B.1.

[27] The severity of infection means that septicaemia cases will be reported, but the *vulnificus*-specific ICD code was only introduced to hospital systems in 2013, plus this does not distinguish between septicaemia and wound infections. Gastroenteritis cases rarely require medical attention, so are potentially underreported.

4. EVALUATION OF RISK

4.1 EXISTING RISK ASSESSMENTS

KEY FINDINGS

In 1996, an assessment of four North Island commercial oyster farms concluded that the salinity levels were too high for *V. vulnificus* to become endemic (McCoubrey, 1996). This assessment was based on the limited data available at the time, and surveys conducted more recently have found a prevalence of up to 43% in oysters from the upper North Island (Section 2.5.1). No other risk assessments have been published for New Zealand.

A quantitative risk assessment for *V. vulnificus* in raw oysters was published in 2005, which considered oysters harvested from the Gulf coast of the USA and consumed in that country, measuring primary septicaemia as the health outcome (FAO/WHO, 2005). The exposure model found the highest risk was associated with oysters harvested in the spring and summer months, and that most of the *V. vulnificus* growth occurred during post-harvest storage.

See Appendix B.2 for further information on relevant overseas risk assessments. Guidelines for risk assessment of *V. vulnificus* in BMS have recently been published in an effort to standardise international approaches (FAO/WHO, 2016).

4.2 EVALUATION OF RISK FOR NEW ZEALAND

KEY FINDINGS

For the purpose of this assessment of risk it is assumed that cases of *V. vulnificus* gastrointestinal disease in people without predisposing medical conditions are rare in New Zealand. Lack of scientific certainty means three additional assumptions are necessary to make an assessment of risk:

1. Although some strains of *V. vulnificus* appear to be more likely to cause disease, all strains are considered equally virulent and potentially able to cause disease (gastroenteritis or primary septicaemia).

2. All members of the susceptible New Zealand population (those with underlying health conditions, particularly liver disease and immunosuppression) are equally susceptible to primary septicaemia and gastroenteritis. Some members of the general New Zealand population may be susceptible to gastroenteritis.

3. The presence of *V. vulnificus* in BMS at any concentration has the potential to cause illness.

These assumptions mean that the following assessment overestimates risk.

RMQ1: What is the risk to human health from *V. vulnificus* in BMS consumed in New Zealand?

Based on the available information, and the assumptions above, those in the susceptible population are at risk of foodborne *V. vulnificus* infection (gastroenteritis or primary septicaemia) from BMS harvested from New Zealand waters and consumed raw. The risk

is greatest when the BMS consumed raw are those harvested during the summer months from waters in the northern half of the North Island.

This assessment of risk suggests that there should be cases of *V. vulnificus* infection reported regularly in New Zealand, but there is currently no evidence of this. *V. vulnificus* gastrointestinal infection is likely to be underreported in New Zealand as testing of faecal samples for *Vibrio* spp. is uncommon. Specific reporting of *V. vulnificus* as a cause of hospitalisation was introduced in 2013, but only two cases have been reported up to 2016.

Other factors may be contributing to this apparent discrepancy. The concentration of *V. vulnificus* measured in most positive samples of Pacific oysters harvested from New Zealand waters was ≤10 MPN/g and an analysis of 30 isolates found the majority to be those less likely to cause human disease (data on other BMS species, and on BMS from other geographical regions are scarce). Population exposure is low; BMS are consumed by only a small proportion of New Zealanders on a daily basis and the shellfish are cooked in approximately two-thirds of servings. Of the oysters consumed raw, we predict from available data that the majority are likely to be dredge oysters sourced from southern Foveaux Strait waters rather than Pacific oysters. Furthermore, the size of the susceptible population and their BMS consumption patterns have not been properly estimated for New Zealand.

There are insufficient data to determine the risk to New Zealand consumers of *V. vulnificus* infection from imported BMS.

RMQ2: Does the commercial harvest of Pacific oysters in New Zealand during the summer months pose a public health risk for consumers of this food with respect to *V. vulnificus*?

The commercial harvest of Pacific oysters in New Zealand during the summer months poses a public health risk for consumers of this food with respect to *V. vulnificus*, particularly BMS consumers in the susceptible population. Pacific oysters harvested from New Zealand waters during the summer months are more likely to contain *V. vulnificus* than oysters harvested at other times of the year. The available data suggest that Pacific oysters harvested from farms located in the northern half of the North Island are more likely to contain *V. vulnificus* compared with those harvested from the Marlborough region.

RMQ3: Is there currently scientific justification for additional risk management controls over commercial harvests of Pacific oysters in New Zealand during the summer months, to protect consumers of this food from *V. vulnificus* infection?

The primary purpose of the current BMS monitoring and testing regimes is to prevent illness from contamination by faecal pathogens or biotoxins. There are no requirements for monitoring or controlling *V. vulnificus* in BMS or BMS growing areas unless specific monitoring is included in a Risk Management Programme or a case or outbreak of *V. vulnificus* infection is linked to BMS. Currently the concentration of *V. vulnificus* is being indirectly controlled through post-harvest cooling requirements, which reduces the opportunity for *V. vulnificus* to multiply.

While there is evidence that consumers of Pacific oysters harvested during the summer months in New Zealand could be exposed to *V. vulnificus*, at this time additional risk management controls would be difficult to justify from a scientific perspective. The current uncertainties over dose response and pathogenicity markers make it difficult to quantify the risk of *V. vulnificus* infection should this pathogen be detected in oysters. In addition, BMS consumption has not yet been linked to any cases of *V. vulnificus* infection in New Zealand so there is little evidence to support this food/hazard combination as an important contributor to the overall burden of foodborne disease in this country at this time.

4.2.1 Assumptions

There is no evidence to support *V. vulnificus* as an important cause of gastrointestinal disease in New Zealand, although only a small number of laboratories reported that they routinely test faecal specimens for this species. In New Zealand, where *Vibrio* spp. infection is not a notifiable disease, outbreaks and sporadic cases of *V. parahaemolyticus* gastrointestinal infection have been reported (King *et al.*, 2016), but there are no such reports for *V. vulnificus* infection. This is similar to observations in other countries, where reports of the gastrointestinal syndrome in otherwise healthy people (with *V. vulnificus* as the only pathogen isolated from stool samples), are uncommon. The available information from hospitalisations caused by *V. vulnificus* in New Zealand suggest that this organism is a rare cause of hospitalisation.

For the purpose of this assessment of risk it will be assumed that cases of *V. vulnificus* gastrointestinal disease in people without predisposing medical conditions are rare in New Zealand. It is acknowledged that people with underlying medical conditions may also present with gastroenteritis without septicaemia.

In their 2005 quantitative risk assessment of primary septicaemia as a result of *V. vulnificus* in raw oysters, the FAO and WHO based their risk calculations on three important assumptions (FAO/WHO, 2005):

1. All strains of *V. vulnificus* were equally virulent.

2. All members of the susceptible population were equally susceptible to illness.

3. A dose of $1x10^4$ *V. vulnificus*/serving can cause illness.

The science presented in preceding chapters of this document show that these assumptions continue to be necessary for risk assessment, but are not accurate. Our qualitative evaluation of the risk to New Zealand consumers from BMS contaminated with *V. vulnificus* is underpinned by the following assumptions:

1. Although some strains of *V. vulnificus* appear to be more likely to cause disease, all strains are considered equally virulent and potentially able to cause disease (gastroenteritis or primary septicaemia). It is acknowledged that the pathogenic potential appears to differ between *V. vulnificus* strains but there is currently no single nor suite of markers that can reliably separate the strains that will cause illness from those that will not.

2. All members of the susceptible New Zealand population (those with underlying health conditions, particularly liver disease and immunosuppression) are equally susceptible to primary septicaemia or gastroenteritis. Some members of the general New Zealand population may be susceptible to gastroenteritis but overseas epidemiological data suggests that such cases are uncommon (≤5%).

3. The presence of *V. vulnificus* in BMS at any concentration has the potential to cause illness. It is acknowledged that doses of 10^3 or 10^4 cells may be necessary to cause primary septicaemia but the dose of *V. vulnificus* required to cause gastroenteritis or septicaemia is not known. In addition, the actual number of *V. vulnificus* cells ingested by a consumer of raw BMS cannot be easily predicted, since a small population of *V. vulnificus* at harvest may multiply between harvest and consumption and/or a consumer may eat multiple shellfish contaminated at low concentrations at a single sitting. Moreover, calculations presented in Section 2.5.1 illustrate how measurement of a low concentration of *V. vulnificus* in a pooled sample of BMS (3.57 MPN/g) can, at its extreme, be the result of one shellfish in that sample being contaminated at levels thought to be necessary to cause human illness ($1.4x10^3$ cells).

Clearly the above assumptions lead to an overestimation of risk, since together they imply that the presence of any strain of *V. vulnificus* in BMS, at any concentration, will cause illness in the susceptible population and may cause illness in the general population. Current scientific evidence suggests otherwise, but further investigations are needed before an alternative position can be taken with confidence, especially considering the serious nature of the primary septicaemia syndrome.

4.2.2 Risk associated with BMS harvested from New Zealand waters: What is the risk to human health from *V. vulnificus* in BMS consumed in New Zealand?

This section responds to the risk management question:

What is the risk to human health from *V. vulnificus* in BMS consumed in New Zealand?

The risk is first discussed for BMS harvested from New Zealand waters and consumed raw.

Based on the available information, and the assumptions above, those in the susceptible population are at risk of foodborne *V. vulnificus* infection (gastroenteritis or primary septicaemia) from BMS harvested from New Zealand waters and consumed raw. The risk is greatest when the BMS consumed raw are those harvested during the summer months from waters in the northern half of the North Island.

This assessment of risk is supported by the following:

- *V. vulnificus* are present in the New Zealand coastal marine environment, as indicated by BMS surveys: Their presence is not related to faecal contamination, so routine tests for microbiological markers of faecal contamination are not relevant to informing the risk of *V. vulnificus* contamination.
- Water salinity and water temperature are not barriers to the occurrence of *V. vulnificus* during the summer months in New Zealand: New Zealand strains of *V. vulnificus* appear to be well adapted to high salinities. Waters in northern areas of New Zealand provide favourable temperatures for *V. vulnificus* over the summer periods. Spring and autumn periods may also support the presence of *V. vulnificus* in these waters. The La Niña phase of the southern oscillation brings warmer temperatures to New Zealand. It is notable that the highest concentration of *V. vulnificus* measured in BMS sampled from New Zealand (9,300 MPN/g) was during 2011, when the La Niña phase was particularly prolonged.
- Based on New Zealand surveys of Pacific oysters (and the assumption that all BMS bioaccumulate *V. vulnificus* similarly to oysters), BMS harvested from northern waters during summer are most likely to be contaminated with *V. vulnificus*: *V. vulnificus* have been detected in Pacific oysters sampled from harbours located in the Northland, Auckland and Coromandel regions, but only in one sample from the Marlborough region. The prevalence and concentrations were higher during summer months when sea surface temperatures were ≥20ºC. *V. vulnificus* were detected in Pacific oyster samples growing in intertidal and subtidal locations.

It should be noted that there are few data to inform the risk of *V. vulnificus* infection from consumption of BMS other than Pacific oysters commercially harvested from northern New Zealand waters. It is possible that there are other regions of New Zealand where the risk of BMS becoming contaminated with *V. vulnificus* is similar to that observed in Pacific oysters from northern waters. Several non-commercially harvested species occupy intertidal niches in warmer regions of New Zealand (e.g. cockles, pipi and toheroa). *V. vulnificus* were isolated from pipi and cockles non-commercially harvested from the Bay of Plenty during the 1995/96 summer.

New Zealand nutrition surveys have found that mussels, oysters and scallops were consumed more often than other types of BMS. Half of the oyster servings were raw, 40% of the mussel servings were raw or marinated (marinating is not a reliable control for *V. vulnificus*). The

prevalence of *V. vulnificus* amongst populations of mussels has not been well established for New Zealand, so the risk posed by mussels is unclear. *V. vulnificus* have been detected in mussels harvested from European waters.

This assessment of risk suggests that there should be cases of *V. vulnificus* infection reported regularly in New Zealand, but epidemiological evidence for New Zealand shows that reports of *V. vulnificus* infection are rare. Four reasons help to explain this apparent discrepancy:

- The assumptions (Section 4.2.1) overestimate risk. In New Zealand surveys of BMS, the concentration of *V. vulnificus* in most positive samples of Pacific oysters was low (≤10 MPN/g) and an analysis of 30 isolates found the majority to be those less likely to cause human disease.
- Section 3.3.3 suggests that the susceptible population may be considerable but this population subset has not been properly estimated for New Zealand. In addition there are no data comparing BMS consumption patterns between people with underlying health conditions that make them more susceptible to *V. vulnificus* infection and those without. Available data suggest that the risk of infection is lower for those without such health conditions.
- *V. vulnificus* infection is underreported in New Zealand. *V. vulnificus* infection is non-notifiable unless there is an outbreak (no confirmed foodborne outbreaks of *V. vulnificus* infection have been reported by any country). Cases of primary septicaemia will come to the attention of medical authorities, but specific reporting of *V. vulnificus* as a cause of disease was only introduced in 2013. The self-limiting and comparatively mild form of the gastroenteritis syndrome means that patients are less likely to seek medical attention. Moreover, human vibrio infections are unlikely to be detected because most laboratories do not routinely test clinical samples for *Vibrio* spp. (no pathogen is identified by routine laboratory testing for an estimated 80% of faecal samples submitted by acute gastrointestinal cases in New Zealand).
- Population-level exposure is low. Based on New Zealand nutrition surveys, BMS are consumed by only a small proportion of New Zealanders on a daily basis (estimates of 1.5% of adults in 2009 and 0.5% of children in 2002; Section 2.5.4), and the shellfish are consumed cooked in approximately two-thirds of these servings (*V. vulnificus* are rapidly killed with heat; Section 2.4.1, 5.2.2). In addition, New Zealand nutrition surveys clearly show that oysters are commonly consumed raw, but do not identify the species of oyster consumed by respondents. A proportion of these will be dredge oysters; probably the majority proportion. Data from 2011 shows that dredge and Pacific oysters are harvested in approximately the same quantities (by weight), but the majority of Pacific oysters are exported and are not available to New Zealand consumers (King and Lake, 2013). The relationship between water temperature and *V. vulnificus* suggests that dredge oysters present a much lower risk of *V. vulnificus* infection compared to Pacific oysters because of their more southern and subtidal habitat (the majority of commercially harvested dredge oysters are from Foveaux Strait).

This assessment of risk does not take into account post-harvest conditions for live or raw BMS since these may increase risk by supporting *V. vulnificus* population growth (e.g. non-refrigeration) or decrease risk by causing population decline (e.g. cooling, freezing). There are no New Zealand surveys for *Vibrio* spp. in BMS at point-of-sale (or point-of-departure, for exports), nor time/temperature profiles for BMS from harvest to point-of-sale. Such data would improve this risk assessment.

There are insufficient data to determine the risk to New Zealand consumers of *V. vulnificus* infection from imported BMS. *Vibrio* spp. are not monitored as part of the microbiological clearance limits for imported shellfish (Section 5.1.2) and there are no microbiological surveys of imported BMS. The majority of BMS imported into New Zealand (by weight) are frozen.

Frozen storage reduces the concentration of *Vibrio* spp. that may have contaminated the product, but is not a reliable control. New Zealand nutrition surveys do not distinguish imported BMS from other sources and it is not known how much imported BMS are consumed raw.

4.2.3 Risk associated with Pacific oysters

This section responds to two risk management questions.

1. **Does the commercial harvest of Pacific oysters in New Zealand during the summer months pose a public health risk for consumers of this food with respect to *V. vulnificus*?**

Yes, based on the assessment of risk explained in sections 4.2.1 and 4.2.2. Pacific oysters harvested from New Zealand waters during the summer months are more likely to contain *V. vulnificus* than oysters harvested at other times of the year. The available data suggest that Pacific oysters harvested from farms located in the northern half of the North Island are more likely to contain *V. vulnificus* compared with those harvested from the Marlborough region. The presence of *V. vulnificus* poses a health risk to the susceptible population.

The risk for live or raw oysters will be attenuated by cooling and cold-chain requirements up until the point of sale, and by any freezing (sections 2.4 and 5). These controls will reduce the concentration of *V. vulnificus* but are not reliable methods for ensuring complete elimination.

While this Risk Profile considers the risk to New Zealand consumers, it is acknowledged that the majority of Pacific oysters harvested in New Zealand (by weight) are exported chilled or frozen. The risk to consumers in destination countries will depend on the time of year the oysters were harvested.

2. **Is there currently scientific justification for additional risk management controls over commercial harvests of Pacific oysters in New Zealand during the summer months, to protect consumers of this food from *V. vulnificus* infection?**

The primary purpose of the current BMS monitoring and testing regimes is to prevent illness from contamination by faecal pathogens or biotoxins. There are no specific requirements for monitoring or controlling *V. vulnificus* in BMS or BMS growing areas in New Zealand unless a case or outbreak of *V. vulnificus* infection is linked to BMS (Section 5). A further exception is where businesses that process harvested BMS have included specific monitoring for *Vibrio* spp. in their Risk Management Programme, which includes microbiological limits and processes in place to divert failed BMS into vibriocidal treatments such as low temperature pasteurisation. Currently the concentration of *V. vulnificus* in Pacific oysters is being indirectly controlled through post-harvest cooling requirements (which reduces the opportunity for *V. vulnificus* to multiply if present in the oysters) and, possibly, through harvest closures during high rainfall events (although the relationship between *V. vulnificus* concentration and water salinity in New Zealand's Pacific oyster growing areas requires study).

As explained above, consumers of Pacific oysters commercially harvested from New Zealand waters during the summer months could be exposed to *V. vulnificus*. However, important gaps in scientific knowledge currently make it difficult to quantify the risk of *V. vulnificus* infection should this pathogen be detected in oysters. Key data gaps include dose response and the uncertainty over pathogenicity markers; these and other data gaps are listed in Section 4.4. In addition, BMS consumption has not yet been linked to any cases of *V. vulnificus* infection in New Zealand so there is little evidence to support this food/hazard combination as an important contributor to the overall burden of foodborne disease in this country at this time.

In summary, while there is evidence that consumers of Pacific oysters harvested during the summer months in New Zealand could be exposed to *V. vulnificus*, at this time additional risk management controls would be difficult to justify from a scientific perspective.

4.2.4 Risks associated with other foods

Because *V. vulnificus* are natural inhabitants of estuarine and marine environments they are also found in other seafoods, and consumption of non-BMS seafoods (e.g. shrimp, crab, fish) has caused *V. vulnificus* infection in other countries (Inoue *et al.*, 2004; Weis *et al.*, 2011). Other foods may be cross-contaminated (e.g. through liquids spreading from contaminated seafood) although this appears to be uncommon (Desmarchelier, 2003). Garnish in contact with raw fish has been identified as a cause of *V. vulnificus* infection (Yokochi *et al.*, 2013). It is not known how important other seafoods are as vehicles of *V. vulnificus* infection in New Zealand.

4.3 THE BURDEN OF *V. VULNIFICUS* INFECTION IN NEW ZEALAND

KEY FINDINGS

There are no estimates of the burden of *V. vulnificus* infection for New Zealand.

4.3.1 Burden of disease from BMS contaminated with *V. vulnificus*

There are no estimates for the burden of disease from BMS contaminated with *V. vulnificus*.

4.3.2 Burden of disease from all *V. vulnificus* infections

No assessment of economic or health costs associated with *V. vulnificus* infection has been carried out for New Zealand. *V. vulnificus* was not considered in previous enteric pathogen burden of foodborne disease reports for New Zealand (Cressey, 2012; Cressey and Lake, 2007; Cressey and Lake, 2008, 2009; Gadiel, 2010).

4.4 DATA GAPS

KEY FINDINGS

Aside from internationally-recognised data gaps around pathogenicity and dose-response, the assessment of risk for New Zealand would be improved with additional data on *V. vulnificus* in BMS harvested from New Zealand waters other than Pacific oysters (including at the point-of-sale), and the incidence of acute gastroenteritis in New Zealand as a result of *V. vulnificus* infection.

The important data gaps identified in this document that impact on the assessment of risk are:

- The ability to identify strains of *V. vulnificus* that will cause infection from those that will not, considering both the septicaemia and gastroenteritis syndromes;
- Population susceptibility to the *V. vulnificus* gastroenteritis syndrome, and a dose-response relationship for this syndrome;
- A dose-response relationship for the *V. vulnificus* primary septicaemia syndrome;
- The prevalence and concentration of *V. vulnificus* in BMS (commercial and non-commercial) other than Pacific oysters, from the upper half of the North Island and from other regions of New Zealand (including measuring for pathogenicity markers);
- The prevalence and concentration of *V. vulnificus* in live or raw BMS at the point-of-sale; and
- The incidence of *V. vulnificus* acute gastroenteritis in New Zealand.

Other data gaps identified in this document that impact on the assessment of risk are:

- Environmental surveys for New Zealand to better evaluate the relationship (if any) between environmental variables (e.g. water temperature, salinity), site characteristics (e.g. nearby landuse, BMS farming methods) and *V. vulnificus* concentrations in water, sediment and BMS;
- The interaction (if any) between Ostreid herpesvirus infection of BMS and the presence/concentration of *Vibrio* spp.;
- The interaction (if any) between the E- and C-genotypes within individual BMS;
- Post-harvest time/temperature profiles for BMS harvested in New Zealand intended for sale as live or raw product; and
- Clarity over the risk posed by *V. vulnificus* in the VBNC state: Proof that *V. vulnificus* can move into, and out of, the VBNC state under normal environmental conditions, that VBNC *V. vulnificus* can be found inside BMS, and that VBNC cells lose their virulence over time.

A recent international guideline lists the data necessary to produce a quantitative risk assessment or risk models (FAO/WHO, 2016).

5. AVAILABILITY OF CONTROL MEASURES

5.1 CURRENT NEW ZEALAND CONTROL MEASURES

KEY FINDINGS

There are no regulatory controls specific to *V. vulnificus* in BMS. Temperature requirements will help minimise growth of this bacterium in BMS after harvesting. In addition, public health protection measures are put in place if there is sufficient epidemiological evidence to link infection by *V. vulnificus* with consumption of BMS. However, such measures rely on knowing what levels of *V. vulnificus* are of concern for public health, which is currently unclear. A BMS processor may choose to include monitoring for *Vibrio* spp. as part of their Risk Management Programme. There are no microbiological standards for *V. vulnificus* in BMS.

Current seafood safety advice for New Zealand consumers advises them to cook seafood thoroughly, which will reduce the risk of *Vibrio* spp. infection. Data from New Zealand and overseas shows that people at high-risk of primary septicaemia from ingestion of *V. vulnificus* are often not aware of the risk posed by raw BMS.

5.1.1 Regulatory controls over the New Zealand BMS industry

Businesses that grow, harvest, process, store or transport BMS for human consumption are subject to the *Animal Products Act 1999* and associated regulations and notices.

The food safety requirements for BMS growers, harvesters and "operators"[28] are set out in the Animal Products (Regulated Control Scheme – Bivalve Molluscan Shellfish) Regulations 2006 and the Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006 (Cartwright, 2006; Knox, 2006).[29] These can be referred to as the BMSRCS Regulations and BMSRCS Notice. Both apply to BMS harvested from aquaculture schemes (land-based or marine) and wild stocks.

Classification of BMS harvesting areas is subject to microbiological monitoring as part of a wider sanitary survey and annual review process, including an evaluation of all actual or potential pollution sources in the growing area catchment. All BMS commercially harvested in New Zealand for human consumption must come from a shellfish growing area that is registered with MPI and classified for harvest for human consumption, and such areas are monitored for faecal coliforms (water) and generic *E. coli* (shellfish).[30] The microbiological monitoring requirements do not include standards for *Vibrio* spp.

Each area has an individually-formulated sampling programme and criteria for when the area shall be closed to harvesting. Because most of the human pathogens of concern are carried into BMS growing areas with stormwater, threshold values from salinity metres, river gauges

[28] The BMSRCS Regulation defines an "operator" as a harvest operator, transport operator, sorting shed operator, BMS depot operator, or relay operator. Activities such as wet storage and depuration are also covered in the BMSRCS Regulation, but only where these are not covered by a Risk Management Programme.
[29] http://www.foodsafety.govt.nz/industry/sectors/seafood/bms/index.htm (accessed 18 July 2016).
[30] A list is maintained by MPI. Version as at 1 July 2016 available at: http://www.foodsafety.govt.nz/elibrary/industry/bms-shellfish-growing-areas.pdf (accessed 18 July 2016).

or rainfall gauges often form part of the criteria. The closure time may also depend on the conditions, for example, after 25mm of rain an area closes for 24 hours, after 75 mm the area closes for five days. The rate of change of salinity during tidal cycles may also be used for determining closing and opening of harvest areas (FAO/WHO, 2011).

While testing for *Vibrio* spp. is not a requirement under the BMSRCS Notice, the Notice sets out temperature control requirements that would help to minimise growth of *Vibrio* spp. should the bacteria be present in the shellfish. Operators are required to keep BMS cool through various measures (shading, water sprays, and ice), and the transport environment must be maintained at 7ºC or cooler. In addition, Schedule 4 mandates maximum periods between harvest and the point where the temperature must be maintained at 7ºC or less. The maximum time from harvest to temperature control depends on the average maximum daily air temperature for the month:

- 36 hours where average maximum is ≤18ºC;
- 24 hours where average maximum is 19-27ºC; and
- 20 hours where average maximum is ≥27ºC.

Schedule 4 includes air temperature data for the major shellfish harvesting regions of New Zealand.

In addition to the controls above, the BMSRCS notice sets out a series of actions to be taken if BMS are implicated in an outbreak involving two or more people who are not from the same household where there is sufficient epidemiological evidence to link the cases with BMS (Part 13). The actions depend on whether the contamination occurred in the growing area or post-harvest. These requirements apply to all human microbial pathogens, including *Vibrio* spp. It should be noted that outbreaks of *V. vulnificus* infection have not been reported by any country, but the notice also provides for actions if one person has become ill ("in the case of marine biotoxin poisoning or as the regional shellfish specialist determines relevant"). The severity of most foodborne *V. vulnificus* infections suggests that this would apply. Section 76 (7) states "where a naturally occurring pathogen is the problem, the officer must keep the area closed until it has been determined that levels of naturally occurring pathogens are not a public health concern." Section 79 describes decision steps for dealing with pathogens in shellfish, using regulatory tolerance levels to make decisions. When there is no regulatory level set (as for *V. vulnificus*), then a public health risk assessment is necessary to make management decisions.

Part 13 of the notice also sets out actions to be taken if human pathogens are detected in BMS, which primarily involves checking the classification of the growing area.

Businesses that process BMS, including depuration and land-based wet storage, must operate under a registered Risk Management Programme (RMP).[31] Generic RMPs for half-shell mussels and oysters are available and these list *Vibrio* spp. among the possible microbiological hazards to be considered.[32] A BMS processor may choose to include monitoring for *Vibrio* spp. as part of their RMP. BMS processors must also comply with the Animal Products (Specifications for Products Intended for Human Consumption) Notice, and the most recent version of this notice came into effect on 1 April 2016.[33] Sections 14.12 to

[31] http://www.foodsafety.govt.nz/industry/sectors/seafood/bms/processors.htm (accessed 18 July 2016).
[32] http://www.foodsafety.govt.nz/elibrary/industry/code-practice-seafood/generic-rmp-model.pdf (accessed 18 July 2016).
[33] http://www.foodsafety.govt.nz/elibrary/industry/animal-products-specifications-asd/index.htm (accessed 18 July 2016).

14.34 set out specific requirements; none are specific to *Vibrio* spp. BMS must be alive when they arrive at the processor.

A revised Australia New Zealand Food Standards Code came into effect on 1 March 2016.[34] Schedule 27 of Standard 1.6.1 (microbiological limits in food) specifies a microbiological standard for *E. coli* in BMS (excluding scallops). There is no standard for *Vibrio* spp. in BMS.

5.1.2 Regulatory controls over imported BMS

BMS imported into New Zealand must be cooked, dried or frozen, and also shelled (unless imported from the EU with a permit) (MAF Biosecurity, 2004, 2008).

Regardless of country of origin, BMS and products containing BMS are classified as a food of "High Regulatory Interest (HRI)" because they are known to present an increased risk to human health (MPI, 2016d). BMS always require food safety clearance before being imported into New Zealand. *Vibrio* spp. are not included amongst the microbiological clearance limits (MPI, 2016a).

From 1 March 2016, seafood importers are required to be registered with MPI or import using a registered agent (MPI, 2016c). The registered importer must be a New Zealand resident. There is a transition period for food importers to become registered that expires on 30th June 2017.[35]

5.1.3 Voluntary industry controls

Some voluntary monitoring for *V. vulnificus* and *V. parahaemolyticus* occurs (C. Johnston, Aquaculture New Zealand, pers. comm.). Testing in-shell oysters for *V. parahaemolyticus* is required to maintain access to the Canadian market during the Canadian summer.

5.1.4 Consumer and food handler communications

In June 2013, MPI updated resources that promote food safety for seafood gatherers.[36] MPI advise only to collect "shellfish from areas where the seawater is not contaminated in any way", which will reduce the risk from many of the viruses and bacteria that can cause gastrointestinal infection, but not from *Vibrio* spp. However, advice to store shellfish under cool conditions, consume within two days and cook thoroughly will reduce the risk of *Vibrio* spp. infection.

Some products imported into New Zealand considered 'high risk' (by the producers) were labelled with phrases such as "cook before consumption". However, such labelling was not effective at preventing illness as shown by an outbreak of norovirus infection in New Zealand (Simmons *et al.*, 2007). These instructions can be easily ignored or the interpretation of the extent of cooking required unclear.

Overseas surveys have revealed that the majority of high-risk people (people with underlying medical conditions) are unaware of the risks associated with raw shellfish consumption (and swimming in warm seas) (Daniels, 2011). A small study in New Zealand found that of 20 "at-risk" individuals, eight were "not concerned" or "slightly concerned" about the risk of disease from eating raw oysters (McCoubrey, 1996). An attempt at using warning signs at food service outlets serving raw oysters in the State of California, USA, was not effective at reducing the number of reported *V. vulnificus* cases linked to raw oyster consumption (Vugia *et al.*, 2013). An educational campaign targeted at people with underlying illnesses was successful in the State of Florida, USA (Weis *et al.*, 2011).

[34] http://www.foodstandards.gov.au/code/Pages/default.aspx (accessed 18 July 2016).
[35] The steps required for the importation of seafood can be found at: http://www.mpi.govt.nz/importing/food/seafood/steps-to-importing/ (accessed 19 July 2016).
[36] http://www.mpi.govt.nz/food-safety/community-food/wild-foods/food-safety-when-fishing-or-gathering-seafood/, http://www.mpi.govt.nz/document-vault/1058 (accessed 19 July 2016).

5.2 ADDITIONAL CONTROLS

KEY FINDINGS

Low temperature pasteurisation, freezing, high hydrostatic pressure and irradiation are effective vibriocidal treatments for BMS. Other treatments that have demonstrated antimicrobial activity towards *V. vulnificus* in oysters include electrolysed oxidising water, ultrasound, ozone, organic acids and biological controls (e.g. predatory bacteria, bacteriophages).

There is a large body of scientific literature concerning the effectiveness of a variety of treatments for reducing *V. vulnificus* in BMS. The purpose of this section is to provide an overview and some examples from recent or relevant studies. Fully evaluating the effectiveness of each control option and its relevance to the New Zealand BMS industry is beyond the scope of this Risk Profile. A review (Drake *et al.*, 2007) summarises information from many older studies.

The USFDA now recognises irradiation, hydrostatic pressure, and individual quick freezing (IQF) with extended frozen storage as processes that are designed to retain raw product characteristics and that can be used to reduce *V. vulnificus* and *V. parahaemolyticus* to non-detectable (<30 MPN/g) levels (USFDA, 2011). Predictive modelling using water quality parameters is also being investigated as a way to predict the presence, abundance and potential virulence of *V. vulnificus* (Froelich *et al.*, 2013b; Froelich and Noble, 2016; Jacobs *et al.*, 2014; Urquhart *et al.*, 2015), but such models need to be site specific and well validated. IQF and frozen storage are currently used in New Zealand but irradiation and hydrostatic pressure are not.

There are strain-dependant differences in resistance to control methods (e.g. Kural and Chen, 2008; Staley *et al.*, 2011) and the level of resistance may also change depending on other stressors the cells were exposed to prior to a control intervention.

5.2.1 Management techniques

Depuration (short term storage of shellfish in seawater tanks) can reduce the concentration of *V. vulnificus* inside BMS but is not a reliable method for eliminating these bacteria from BMS, particularly because vibrios reside within various oyster tissues. *V. vulnificus* can also spread from contaminated oysters to those that are not (Ramos *et al.*, 2012a). After 48 hours of depuration in seawater at temperatures up to 27ºC, the concentration of naturally bioaccumulated *V. vulnificus* either increased approximately 100-fold then stabilised, or changed very little (Ramos *et al.*, 2012a; Tamplin and Capers, 1992). One study found that using UV light and chlorine to control microbes in the circulating seawater increased *V. vulnificus* depuration from oysters (Ramos *et al.*, 2012a), but another study did not measure improvement using UV light and/or 0.2 µm pore-size filtration (Tamplin and Capers, 1992). The concentration of *V. vulnificus* in the oysters was much higher in the latter study and the authors suggested that growth of the organism in the shellfish and water exceeded the bactericidal activities of the UV light. The temperature of depuration can also affect its effectiveness (Chae *et al.*, 2009). Depuration is not common practice in New Zealand.

The sensitivity of *V. vulnificus* to high salinities has prompted investigations to measure the effectiveness of relaying contaminated oysters to high salinity waters. Experiments in Chesapeake Bay found that the concentration of naturally-present *V. vulnificus* in oysters relayed from low (14-15‰) or medium (22-25‰) salinity sites to high salinity sites (≥30‰) decreased 2-3 $\log_{10}$ MPN/g after 14 days (Audemard *et al.*, 2011). The mortality rate for the oysters was low (4%), even for oysters relayed from the low salinity site. An earlier experiment also found that the concentration of *V. vulnificus* in naturally-contaminated oysters reduced by 3-4 $\log_{10}$ MPN/g within two weeks of relaying to high salinity water (32-34‰), with an oyster

mortality of <6% (Motes and DePaola, 1996). Further trials demonstrated that high salinity (35‰) depuration of naturally contaminated oysters is effective but the rate and extent of depuration is inconsistent (Larsen *et al.*, 2013; Larsen *et al.*, 2015).

New Zealand data show that oysters are harvested from waters already at high salinities (>30‰) so the capacity for high-salinity relaying in New Zealand, without purpose-built tanks, appears limited. Relaying is a strictly controlled activity and requires a permit in New Zealand. BMS may be relayed to make them fit for human consumption if they have been exposed to faecal pollution.

Commercial harvesting of BMS may be stopped when rain or water salinity gauges reach pre-determined thresholds that indicate freshwater influx as a result of rainfall (Section 5.1.1). The purpose is to prevent harvested BMS from containing unacceptable concentrations of faecal microorganisms. Data from other countries suggest that lower salinities can favour higher concentrations of *V. vulnificus* in the water. This monitoring may be useful during summer months for indicating when there is a risk of Pacific oysters being contaminated with high concentrations of *V. vulnificus*.

It may be possible to selectively breed BMS that are more resistant to *V. vulnificus* contamination. A number of studies have been undertaken to understand more about the immune defence mechanisms of oysters (e.g. Allen and Burnett, 2008; Brousseau *et al.*, 2014; Faisal *et al.*, 1998; Gagnaire *et al.*, 2007; Genthner *et al.*, 1999; Harris-Young *et al.*, 1995) but scientists still do not know how to prevent bivalves naturally taking up *V. vulnificus* from the marine environment when they are naturally present in the water column. Selective breeding is being investigated for resistance to *Vibrio* spp. pathogenic to shellfish (Azéma *et al.*, 2015).

5.2.2 Temperature controls

Exposure to mild heat treatments above 45°C causes death of *V. vulnificus*. Low temperature pasteurisation (50ºC, 10 minutes) of oysters naturally contaminated with *V. vulnificus* (10^5-10^7 MPN/g) reduced the concentration of this pathogen to non-detectable levels, and this treatment was also effective in artificially contaminated oysters (Andrews *et al.*, 2000; Cook and Ruple, 1992; Ye *et al.*, 2012). Treatment at 50ºC for only 5 minutes (or treatment at 45ºC for 20 minutes) only achieved a reduction of 1.8 $\log_{10}$ MPN/g (Ye *et al.*, 2012). A heat-shock process (1-4 minutes, internal temperature >50ºC) was also effective against *V. vulnificus* (Hesselman *et al.*, 1999).

Ice slurries were effective for rapidly cooling oysters (24ºC to 10ºC within 12 minutes), but repeated dipping of oysters caused the ice to become contaminated with faecal coliforms, *Clostridium perfringens*, *V. vulnificus* and *V. parahaemolyticus* (Lydon *et al.*, 2015). However, the concentrations of *Vibrio* spp. were unchanged in the flesh of the oysters after 15 minutes submersion in the contaminated ice slurry. Another study, where oysters were immersed in ice for three hours before refrigeration, concluded that the advantages of icing in controlling *V. vulnificus* were outweighed by the disadvantages of increased faecal coliforms and total bacterial contamination (Quevedo *et al.*, 2005).

As demonstrated by data in Section 2.4, *V. vulnificus* are susceptible to freezing, but freezing cannot be relied upon to eliminate this pathogen without process validation. Cryogenic individual quick freezing with extended frozen storage is an USFDA-approved control for *Vibrio* spp. The combination of vacuum packaging and freezing (-20ºC) was more effective at reducing the concentration of *V. vulnificus* in oysters than freezing inside normal-sealed packaging (Parker *et al.*, 1994).

5.2.3 High (hydrostatic) pressure processing (HPP)

It has been found that HPP inactivates *V. parahaemolyticus* by damaging the cell membrane, cell wall and degrading cellular proteins (Wang *et al.*, 2013). The treatment probably impacts *V. vulnificus* cells in a similar manner.

A >5 $\log_{10}$ reduction in the concentration of naturally occurring *V. vulnificus* in oysters was observed after a pressure treatment of 250 MPa for two minutes (Cook, 2003), and the same was observed when *V. vulnificus* were artificially bioaccumulated in oysters (Ye *et al.*, 2012). Alternative effective pressure/time regimes have been reported in other studies (Koo *et al.*, 2006; Kural and Chen, 2008). These differences are not unexpected since the methods, *V. vulnificus* strains, and the BMS species and form (e.g. shucked, whole, homogenised) differ between experiments. Combining HPP with low temperature pasteurisation has a synergistic effect on killing *V. vulnificus* (Ye *et al.*, 2012).

A Monte Carlo simulation predicted that the mildest HPP treatment investigated in the model (250 MPa, 1ºC, 2 minutes) would achieve the USA recommendation of a 3.52 $\log_{10}$ reduction in *V. vulnificus* counts with an endpoint of non-detectable (<30 CFU/g), even during warmer periods (Serment-Moreno *et al.*, 2015).

A recent study has found that the performance of the HPP process was not affected by the conditions oysters were stored under prior to treatment (Ye *et al.*, 2013). Oysters were artificially-contaminated with a pressure-resistant strain of *V. vulnificus*, stored under various conditions (air, seawater, frozen), then shucked, and the meat subjected to nine different HPP regimes (225-275 MPa, 2 minutes at 4, 21 or 35ºC). HPP at 275 MPa was more effective at reducing the number of *V. vulnificus*, but in general, neither the pre-HPP storage conditions nor the temperature of the HPP significantly affected the performance of the HPP process, as measured by the number of *V. vulnificus* survivors. However, frozen storage was the most effective pre-HPP storage condition for reducing the concentration of *V. vulnificus*, demonstrating that a combination of frozen storage and HPP was an effective multi-hurdle control.

5.2.4 Irradiation

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.*, 2003; Drake *et al.*, 2007; 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 *V. vulnificus* artificially bioaccumulated in whole shell oysters from 10^7 MPN/g to non-detectable levels, and had the same effect on naturally present *V. vulnificus* (10^3 MPN/g) (Andrews *et al.*, 2003). A 5-log reduction in the concentration of artificially bioaccumulated *V. vulnificus* was achieved by an X-ray treatment of 0.75 kGy in half shell oysters, and 2 kGy in whole shell oysters (Mahmoud, 2009). The oysters were able to survive a treatment of 3 kGy followed by storage at (5ºC) for up to seven days.

5.2.5 Other treatments

In order for oysters to be treated with a compound that is added to tank water, the oyster must not be able to detect it, otherwise they close their shells and cease pumping water. This is the case with diacetyl, found in butter, which reduced *V. vulnificus* in shucked oysters but was not so effective in shell-stock (Birkenhauer and Oliver, 2003). Other treatments that have demonstrated antimicrobial activity towards *V. vulnificus* in oysters include electrolysed oxidising water, ultrasound, ozone and organic acids (Borazjani *et al.*, 2003; Mahmoud, 2014; Ren and Su, 2006).

Biological controls offer alternative treatments for *V. vulnificus*. Predatory bacteria are naturally present in seawaters and laboratory experiments have demonstrated how these bacteria can reduce the concentration of *V. vulnificus* in seawater and oysters (Richards *et al.*, 2012). An antibacterial-producing bacteria *Phaeobacter inhibens* eradicated *V. vulnificus* in oyster juice but was unable to prevent *V. vulnificus* from contaminating live oysters (Porsby

and Gram, 2016). Bacteriophages are also being investigated (Lee *et al.*, 2012; Pelon *et al.*, 2005), as are extracts from marine algae (Genovese *et al.*, 2012).

5.3 CONTROL MEASURES IN OTHER COUNTRIES

KEY FINDINGS

Monitoring *V. vulnificus* in BMS harvesting areas as part of a control programme is uncommon (FAO/WHO, 2016). Control of temperature between harvest and sale is seen as a major element in controlling risk. General food hygiene measures, including cooling and controlling cross-contamination, are internationally recognised as being important for controlling growth of *Vibrio* spp. in BMS.

Codex recommended water temperature and salinity levels are established for a harvesting area to indicate increased risk of *Vibrio* spp. contamination, and that environmental monitoring of harvesting areas is put in place (including monitoring human illness, predictive modelling and prevalence studies). There are no microbiological standards set for EU member states considering *Vibrio* spp. in BMS, but a real-time mapping programme is in place to predict the presence of *Vibrio* spp. in European coastal waters.

The USA has put in place monitoring and control plans for *V. vulnificus* and *V. parahaemolyticus* in BMS. Controls (e.g. area closure, post-harvest processing) are implemented when illnesses are linked to a BMS harvesting area or when elevated water temperatures are measured. Since 2003, raw oysters harvested from the Gulf of Mexico from April to October have not been permitted for sale in the State of California unless they have been processed to reduce *V. vulnificus* to non-detectable levels (<30 MPN/g). The USA have guideline levels for *V. vulnificus* of “not detected” (<30 MPN/g) in cooked, ready-to-eat fishery products or BMS carrying the label “processed to reduce *Vibrio vulnificus* to non-detectable levels”.

Appendix C contains further details on controls measures for *V. vulnificus* in BMS that have been recommended by international organisations or put in place by other countries.

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APPENDIX A: HAZARD AND FOOD

A.1 *V. vulnificus* growth and survival

V. vulnificus is a motile, Gram-negative, curved rod-shaped bacteria with a single polar sheathed flagella tail and two circular chromosomes (Baumann *et al.*, 1984; Drake *et al.*, 2007; Farmer III and Hickman-Brenner, 2006). It does not form spores. *V. vulnificus* are halophilic (i.e. they require sodium chloride (NaCl) for growth) and are usually restricted to estuarine and coastal marine waters where they occur naturally. They can be free living (planktonic) but are frequently attached to suspended matter or sediments, or form biofilms on marine biotic surfaces (e.g. on BMS shells). They have been isolated from aquatic vegetation (Chase *et al.*, 2015). *V. vulnificus* in the water or attached to suspended sediments can be taken up by marine animals including mammals, fish, shellfish, crustaceans and plankton. Their presence is not due to faecal pollution.

Vibrio vulnificus was first recognized, identified and described as a human pathogen in 1976 by the USA Communicable Disease Center Enteric Disease Laboratory, when it was referred to as the lactose positive or the halophilic *Vibrio* species (USFDA, 1994). At that time the characteristics of 38 isolates of halophilic bacterium isolated from blood cultures, cerebrospinal fluid and wound infections were described (Hollis *et al.*, 1976). This same bacterium, along with three other species, had earlier been isolated by scientists from the Pacific Ocean near the Hawaiian Islands and they named the genus *Beneckea* (Baumann *et al.*, 1971). Therefore, literature published between 1971 and the 1980s often refers to *Vibrio parahaemolyticus* and *Vibrio vulnificus* as *Beneckea parahaemolyticus.* Subsequent taxonomic revisions have placed this organism in the Vibrio genus. In 1979 the pathogen was officially named by Farmer as *Vibrio vulnificus* (Daniels, 2011).

The literature highlights the global prevalence of *V. vulnificus*, with identifications occurring in the marine environment and seafood samples from Australia (Maxwell *et al.*, 1991; Wise and Newton, 1992), Brazil (Rodrigues *et al.*, 1992), France (Cantet *et al.*, 2013) Korea (Chong *et al.*, 1982; Park *et al.*, 1991), India (Thampuran and Surendran, 1998), Japan (Osaka *et al.*, 2004), Malaysia (Paydar and Thong, 2013), New Zealand (McCoubrey, 1996), North Sea (Veenstra *et al.*, 1994), Thailand (Thamlikitkul, 1990; Wongpaitoon *et al.*, 1985), Taiwan (Chuang *et al.*, 1992), Russia (Nair *et al.*, 2007), Saudi Arabia (Chagla *et al.*, 1988), Scandinavia (Andersen, 1991; Bauer *et al.*, 2006; Melhus *et al.*, 1995) and all coastal regions of the USA including the Hawaiian Islands (Nip-Sakamoto and Pien, 1989; USFDA, 1994).

General information on *V. vulnificus* can be found in a hazard datasheet prepared for the New Zealand Ministry of Health (ESR, 2001) available from:

http://www.foodsafety.govt.nz/science-risk/hazard-data-sheets/pathogen-data-sheets.htm,

and from the United States Food and Drug Administration (USFDA) Bad Bug Book (USFDA, 2012).

This appendix includes additional details and any recent information relevant to this Risk Profile.

Temperature

The growth temperature range for *V. vulnificus* is often cited as 13-43ºC, but two studies have measured growth of *V. vulnificus* in laboratory broth at 10 and 11ºC (Burnham *et al.*, 2009;

Kim *et al.*, 2012).[37] At non-freezing temperatures <10ºC the concentration of culturable cells decreases. Cells may die or move into a VBNC state.

V. vulnificus are capable of surviving for long periods at freezer temperatures. Lower temperatures increase survival, possibly due to decreased ice crystal formation (Seminario *et al.*, 2011). When *V. vulnificus* were suspended in a laboratory buffer solution and frozen at -10, -35 and -80ºC for 7-9 days, the concentration reduced by 4.6, 1.1 and <0.1 $\log_{10}$ CFU/ml, respectively, as measured against the concentration immediately after freezing (Seminario *et al.*, 2011). Initial freezing reduced the concentration of *V. vulnificus* by 1.5-1.8 $\log_{10}$ CFU/ml but there was no significant difference between the three temperatures. Thus the temperature of frozen storage had more effect on *V. vulnificus* survival than the initial freezing rate.

Exposure to mild heat treatments above 45°C causes death of *V. vulnificus* in laboratory broth (Cook and Ruple, 1992). The $D_{47°C}$ time for 52 strains averaged 78 seconds. The $D_{47°C}$ for 18 of the most heat-resistant strains averaged 114 seconds and $D_{50°C}$ was 40 seconds (mean z value = 7.1°C). The *V. vulnificus* morphotype influences thermal death times. D values measured at 45, 47, 49 and 51ºC were almost always higher when *V. vulnificus* strains were in the opaque (encapsulated) form compared to in the translucent form, although the difference was statistically significant only at 45 and 49ºC (Kim *et al.*, 1997). Higher z values were also calculated for the opaque form (2.4-2.5ºC compared with 1.7-2.1ºC for the translucent form).

Salinity

Regression between temperature and salinity indicates 17‰ is optimal (FAO/WHO, 2005).

A study of the growth of *V. vulnificus* over six days in sterilised seawater of varied salinities at 14ºC found that the concentration of *V. vulnificus* reduced at salinities of 30, 35 and 38‰ (Kaspar and Tamplin, 1993). These results contrasted with studies of New Zealand isolates (Cruz *et al*. (2016), see Section 2.3.1), which measured growth at 40‰. However, Kaspar and Tamplin (1993) only measured the behaviour of one *V. vulnificus* isolate and the seawater media contained less nutrients than the media used by Cruz *et al.* (2016).

pH

Low pH is quite lethal to *V. vulnificus* (Koo *et al.*, 2001). The numbers of *V. vulnificus* were close to limit of detection after 100 seconds at pH 2 and 24 minutes at pH 3 (Koo *et al.*, 2000b). Pre-exposure to acidic conditions has been shown in increase resistance of *V. vulnificus* to subsequent stressors (freeze-thaw, cold storage, acid), although resistance appears to be strain-specific and varies dependant on the acid used (Bang and Drake, 2005; Drake *et al.*, 2007).

Temperature, salinity and pH

Laboratory experiments using combinations of temperature, salinity and pH indicate that biotype 1 *V. vulnificus* isolates may be more adapted to a range of marine habitats (e.g. are more tolerant of low and high salinities) and grow faster than isolates of biotypes 2 and 3. The survival of *V. vulnificus* isolates of biotypes 1, 2 and 3 were measured in laboratory media at combinations of salinity (5-40‰) and pH (7.0 or 8.0) at temperatures supportive of growth (25, 30 and 37°C) (Chase and Harwood, 2011). Growth at all salinity-pH combinations was observed at all temperatures, supporting the results of Cruz *et al*. (2016). However, biotype 1 grew on average 1.7 and 1.9 times faster than biotypes 2 and 3, respectively, and biotype 2 grew on average 1.2 times faster than biotype 3. All biotypes grew best at 37°C, pH 7.0, and within the salinity range of 15 to 30‰. Under these optimal conditions the fastest average

[37] The 2001 *V. vulnificus* hazard datasheet lists the minimum growth temperature as 8ºC, as cited in ICMSF (1996). ICMSF (1996) does not provide a supporting reference and studies published since do not support growth at 8ºC.

generation time for biotype 1 was 15 minutes. The factor that most affected the growth rate of biotype 1 was temperature. The growth rates of biotypes 2 and 3 were most affected by high or low salinities.

A.2 *V. vulnificus* testing and typing

Testing and typing methods for *V. vulnificus* have been recently reviewed and evaluated (FAO/WHO, 2016). General guidance is now available for selection of methods fit-for-purpose, with the intention that internationally comparable datasets are generated (FAO/WHO, 2016).

The *vvh*A gene, which encodes a haemolysin, is unique to *V. vulnificus* and can be used to identify an isolate to species level in addition to (or instead of) biochemical tests. A variety of molecular approaches can be used to separate isolates into the C- or E-genotypes (Oliver, 2015).

Efforts have been directed towards developing better molecular-based detection to improve sensitivity, shorten testing time and indicate *V. vulnificus* concentration (e.g. (Garrido-Maestu *et al.*, 2014; Jones *et al.*, 2012; Wang *et al.*, 2016). Culture-based techniques are still important for obtaining a bacterial isolate, thus there are also efforts to improve culturing techniques (e.g. (Cruz *et al.*, 2013; Froelich *et al.*, 2014; Griffitt and Grimes, 2013; Jones *et al.*, 2013; Nigro and Steward, 2015; Williams *et al.*, 2013). Multiplex PCRs have been developed to simultaneously detect a suite of virulence markers in a *V. vulnificus* isolate (Bier *et al.*, 2015).

In New Zealand, when foods suspected of causing illness are tested for *Vibrio* spp., it is routine to identify any *Vibrio* spp. to species level. Isolates of *V. vulnificus* from food are not routinely tested for virulence indicators.

A.3 *V. vulnificus* in BMS overseas

Data from surveys of *V. vulnificus* in BMS have been summarised in TABLE 5. The data presented in this table are only from surveys of shellfish freshly harvested from their growing areas, usually as part of wider environmental microbiology studies. There are many surveys measuring *Vibrio* spp. in shellfish at retail (e.g. (Cook *et al.*, 2002; Fukushima and Seki, 2004; Normanno *et al.*, 2006; Robert-Pillot *et al.*, 2014)) but these are not informative for the New Zealand situation since the concentration of *Vibrio* spp. can change between harvest and retail sale (plus there are currently no data on *Vibrio* spp. in BMS at retail in New Zealand for comparison). Studies of *V. vulnificus* in freshly harvested BMS, in combination with data on water temperature and salinity, are more informative.

It should be noted that the prevalence data are not directly comparable between studies and are only indicative because:

- Different methods are used to detect *V. vulnificus*; and
- Some studies are temporal (same site tested repeatedly over time), some are spatial (multiple sites tested once) and some are both.

The prevalence data summarised in the table are for the study as a whole. None of these studies measured the ratio of C- or E-genotypes in the BMS.

TABLE 5: Prevalence and concentration of *V. vulnificus* measured in raw BMS sampled in other countries (surveys published in the scientific literature)

COUNTRY	DATE OF SURVEY	WATER TEMPERATURE (range, °C)[1]	WATER SALINITY (range, ‰/ppt)[1]	PREVALENCE OF *V. VULNIFICUS* (%)[2]	CONCENTRATION OF *V. VULNIFICUS* IN POSITIVE SAMPLES (MPN/g or CFU/g)	COMMENTS ON *V. VULNIFICUS* RESULTS	REFERENCE
Pacific oysters (*C. gigas*)							
Hong Kong	1986	NR	NR	3/50 (6)	NR		(Chan *et al.*, 1989)
Brazil (6 sites)	2006-2007	18-29	NR	9/180 (5)	4-7		(Ramos *et al.*, 2012b)
Brazil (6 sites)	2008-2009	20-29	NR	6/60 (10)	Mean 6 (max. $1.3x10^2$)	Concentration positively correlated with temperature, no correlation with salinity.	(Ramos *et al.*, 2014)
Oysters (*C. virginica* or species not identified)							
Mexico (12 sites, Pueblo Viejo Lagoon)	2002-2003	NR	NR	39/143 (27)	NR	Most detected when salinity >18‰, temperature > 24ºC.	(Quinones-Ramirez *et al.*, 2010)
USA (estuary, New Hampshire/Maine)	1989-1990	0-25 (monthly mean)	0-24 (monthly mean)	25/66 (38)	Geometric mean $1.2x10^2$ (max. $4.6x10^3$)	Concentrations increased with increasing temperature and salinity (detected when water >10ºC and ≥5‰ salinity).	(O'Neill *et al.*, 1992)
USA (2 sites, Chesapeake Bay)	1991-1992	6-26	8-19	12/20 (60)	$1x10^3$-$4.7x10^4$	Detected in oysters from waters at 7.6ºC.	(Wright *et al.*, 1996)
USA (North Carolina waters)	2004	26	31	137/155 (88)	Mean 10^4 (max. 10^6)		(Sokolova *et al.*, 2005)
USA (2 sites, east coast)	2005-2006	11-31	0-27	Individual oysters (not pooled): 85/100 (85)	NR	Highest concentration in waters preceded highest concentration in oysters. Concentration in oysters positively correlated to water temperature, negatively correlated to salinity.	(Warner and Oliver, 2008)
USA (Chesapeake Bay)	2011	24-26	8-11	NR (n=24)	Average $4x10^5$ (max. $1.1x10^6$)		(Shaw *et al.*, 2014)
USA (Long Island Sound)	2012	NR	NR	66/68 (97)	Median 0.97 (max. 3.3 $\log_{10}$)	No correlation with temperature or salinity.	(Jones *et al.*, 2014)

COUNTRY	DATE OF SURVEY	WATER TEMPERATURE (range, °C)[1]	WATER SALINITY (range, ‰/ppt)[1]	PREVALENCE OF *V. VULNIFICUS* (%)[2]	CONCENTRATION OF *V. VULNIFICUS* IN POSITIVE SAMPLES (MPN/g or CFU/g)	COMMENTS ON *V. VULNIFICUS* RESULTS	REFERENCE
Mussels (*M. edulis*, *M. galloprovincialis*)							
Denmark (1 site)	1996	3-14	NR	7/17 (41)	NR	Detected in mussels and water at low temperatures (8ºC).	(Hoi *et al.*, 1998)
Denmark, Sweden (2 sites, Baltic Sea)	2006	16-24	9-17	12/19 (63)[3]	NR		(Collin and Rehnstam-Holm, 2011)
France (6 sites)	1999	18-25.7	29-40	3/12 (25)	NR		(Hervio-Heath *et al.*, 2002)
France (Mediterranean coastal lagoons)	2006-2007	20-24	20-40	1/6 (17)	0.04[3]		(Cantet *et al.*, 2013)
Italy (lagoon)	NR (7 months)	10.5-30.0	22-29	2/24 (8)	NR	Isolated when water >22ºC and 2.3-2.6% salinity.	(Beneduce *et al.*, 2010)
Clams (*Chaemelea gallina*, *Donax* spp., *Ruditapes* spp., *Mercenaria mercenaria*)							
France (Mediterranean)	2006-2007	20-24	20-40	2/3 (67)	0.04-15 (*vvhA* PCR)[3]		(Cantet *et al.*, 2013)
Italy (Emilia Romagna)	2011-2014	NR	NR	8/79 (10)	NR		(Passalacqua *et al.*, 2016)
Spain (Mediterranean coast)	1995-1997	12.6-27.1	31-38	*C. gallina*: 2/12 (17) *Donax* spp: 3/10 (30)	Data not reliable	Not detected in seawater.	(Arias *et al.*, 1999)
USA (Long Island Sound)	2012	NR	NR	27/30 (90)	Median -0.08 $\log_{10}$ (max. 1.6 $\log_{10}$)	No correlation with temperature or salinity.	(Jones *et al.*, 2014)

ND, not detected; NR, not reported.

[1] Estimated from graph if data not specified.

[2] Unless indicated, each sample (the denominator) was formed from a pooled number of shellfish (the number of shellfish pooled and the size of the homogenate tested varied between studies).

[3] As indicated by PCR, targeting the *vvh* gene and/or *viuB* gene.

A.4 Additional information on BMS in New Zealand

A.4.1 BMS species

TABLE 6: BMS species in New Zealand[1]

COMMON NAMES[2]	SCIENTIFIC NAME	COMMON TIDAL HABITAT	NEW ZEALAND DISTRIBUTION
Cockle	*Austrovenus stutchburyi*	Intertidal region	Widespread
Deepwater clam	*Panopea zelandica*	5-25 m below low tide	Widespread
Dosinia, fine (silky)	*Dosinia subrosea*	Subtidal surf zone	Widespread (more common in northern NZ)
Dosinia, ringed	*Dosinia anus*	5-10 m below low tide	Widespread (more common in northern NZ)
Frilled venus shell	*Bassinia yatei*	6-9 m below low tide	Widespread
Mussel, blue	*Mytilus* spp.	Below low water to 60 m	More common around South Island
Mussel, green-lipped	*Perna canaliculus*	Below low tide to 60 m	Widespread (most common in central and northern NZ), aquaculture
Mussel, horse	*Atrina zelandica*	Below low tide to 50 m	Widespread
Mussel, little black	*Xenostrobus pulex*	Midtide	Widespread
Mussel, ribbed	*Aulacomya atra maoriana*	At or below low tide	South Island
Oyster, dredge/Bluff/flat	*Ostrea chilensis*	Intertidal and below low tide to 50m	Widespread
Oyster, Pacific	*Crassostrea gigas*	Intertidal and below low tide	Widespread
Oyster, rock	*Saccostrea glomerata*	Intertidal and below low tide	More common around North Island
Pipi	*Paphies australis*	Midtide to 7 m below low tide	Widespread
Scallop	*Pecten novaezelandiae*	Low tide to 60 m	Widespread
Scallop, queen	*Zygochlamys delicatula*	Subtidal, from 110 m	East coast, South Island
Toheroa	*Paphies ventricosum*	Intertidal	Widespread (most common on west coast of northern NZ)
Triangle shell	*Spisula aequilatera*	3-8 m below low tide	Central and southern NZ
Trough shell	*Mactra discors*	Subtidal surf zone	Widespread (more common around southern NZ)

COMMON NAMES[2]	SCIENTIFIC NAME	COMMON TIDAL HABITAT	NEW ZEALAND DISTRIBUTION
Trough shell, large	*Mactra murchisoni*	Subtidal surf zone	Widespread (more common around southern NZ)
Tuatua	*Paphies subtriangulata*	Low intertidal to 4 m below low tide	Widespread (more common around North Island)
Tuatua, southern/ deepwater	*Paphies donacina*	Subtidal surf zone	Widespread (more common around central NZ)

[1] Information from MPI's 2016 fishery assessment plenary reports (available from http://fs.fish.govt.nz/Page.aspx?pk=61&tk=212, accessed 28 July 2016), Turner *et al.* (2005) and Manaaki Taha Moana Research Team (2012).

[2] A summary of alternative common names, scientific names and Māori names is available from http://www.foodsafety.govt.nz/elibrary/industry/specification-scientific-names-human-consumption/nz-fishnames-list.pdf (accessed 28 July 2016).

A.4.2 Data on imported Pacific oysters, New Zealand

Data obtained from Statistics New Zealand show that between 0.5 and 5.3 million frozen, shucked Pacific oysters were imported annually since 2006 (FIGURE 2).[38] FIGURE 2 has been compiled from three codes relating to Pacific oysters (frozen, meat):

- Code 0307100048 "Molluscs; rock or Pacific oysters, frozen, meat": Used prior to 2010. No values have been entered against this code after 2010, and up until this date imports of Pacific oysters were not reported separately from imports of rock oysters.
- Code 0307100036 "Molluscs; Pacific (*Crassostrea gigas*), frozen, meat": Values were only entered against this code in 2011.
- Code 0307190035 "Molluscs; Pacific oysters (*Crassostrea gigas*), frozen, meat": Values were only entered against this code from 2012 onwards. The data presented in FIGURE 2 differ from official published statistics (see footnote).

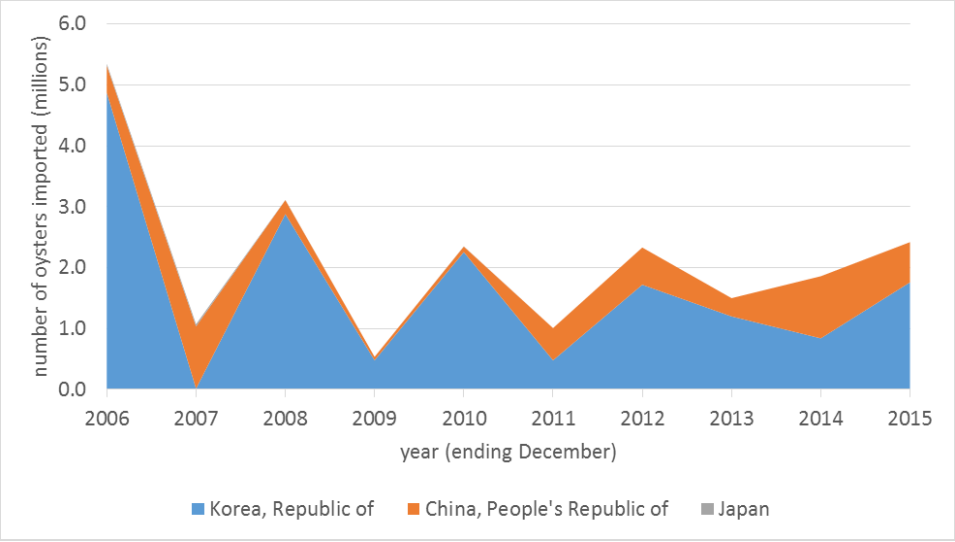

FIGURE 2: Number of frozen and shucked Pacific oysters imported into New Zealand for the years 2006-2015 and their countries of origin

[38] Data retrieved from Statistics New Zealand Infoshare, http://www.stats.govt.nz/infoshare/ (accessed 13 April 2016 and 1 June 2016) and updated data for 2012-2015 directly provided by Statistics New Zealand (September 2016). Updated data differs from published official statistics.

APPENDIX B: EVALUATION OF ADVERSE HEALTH EFFECTS

B.1 *V. vulnificus* infection in other countries

V. vulnificus infection is a notifiable disease in some countries. "Vibriosis" became a nationally notifiable disease in the USA in January 2007; infection from any species of the family Vibrionaceae, other than toxigenic *Vibrio cholerae* O1 or O139, must be reported.[39] USA notification data are collected in the Cholera and Other Vibrio Illness Surveillance System (COVIS).

Vibrio spp. infection is not a nationally notifiable disease in Australia, but "*Vibrio* food poisoning" and "*Vibrio* disease (invasive)" is notifiable in the Northern Territory and "*Vibrio* infection" in Tasmania.[40]

The European Centre for Disease Prevention and Control (ECDC) does not collect data on *Vibrio* spp. infection from European Union Member States. *Vibrio* spp. infection is not notifiable in Canada, but may be kept under surveillance in some States (e.g. in Alberta).[41]

A recent review noted that increases in *Vibrio* spp. infections have been reported in northwest Spain and the Baltic Sea, Israel and New Caledonia (Oliver, 2015). This has been correlated with global warming. Oliver (2015) commented that foodborne *Vibrio* spp. infections were also increasing in the USA but the increase did not appear to be due to global climate change, and increased virulence amongst *Vibrio* spp. must also be considered. However, it is difficult to know for sure whether reported cases of *Vibrio* spp. infection are, in fact, increasing in the USA, or whether this observation is an artefact of the disease only being notifiable since 2007.

B.1.1 Incidence of *V. vulnificus* infection

USA

TABLE 7 summarises annual data from COVIS for the most recent five-year period for which data are available (2010-2014). Note that these data are for all *V. vulnificus* cases, including wound infections. Based on the USA's annual estimated resident population for 2014 (319 million), the incidence for 2014 was 0.004 per 100,000.[42] Notable trends were observed for vibriosis cases each year (data on these trends was not specific to *V. vulnificus*):

- The majority of vibriosis cases were reported from coastal states on the east and west coasts – the Atlantic, Gulf and Pacific states;
- Numbers of confirmed and total foodborne cases peaked in summer months; and
- From cases with domestically-acquired foodborne vibriosis who reported eating a single seafood item in the week before onset of illness, oysters (particularly raw oysters) was the seafood item most often reported.

[39] https://wwwn.cdc.gov/nndss/conditions/vibriosis/ (accessed 5 July 2016).
[40] http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-casedefs-statedis.htm (accessed 6 July 2015).
[41] https://www.nml-lnm.gc.ca/NESP-PNSME/index-eng.htm and http://www.health.alberta.ca/documents/Notifiable-Disease-List-2015.pdf (accessed 6 July 2016).
[42] United States Census Bureau population data, http://www.census.gov/popest/data/national/totals/2015/index.html (accessed 6 July 2016).

TABLE 7: Reported cases of *V. vulnificus* infection in the USA (COVIS)[1]

YEAR	NUMBER OF CASES	MEDIAN AGE (RANGE)	% MALE	HOSPITALISATIONS[2]	DEATHS[2]	% OF DOMESTICALLY ACQUIRED CASES CONFIRMED AS FOODBORNE[3]
2014	124	59.5 (10-93)	81	97/123 (79)	21/117 (18)	12
2013	137	65 (7-90)	91	120-127 (94)	32/115 (28)	11
2012	119	61 (7-93)	87	101/116 (32)	34/106 (32)	5
2011	113	60 (8-91)	78	89/113 (87)	34/108 (31)	23
2010	133	57 (2-87)	85	93/124 (75)	36/116 (31)	21

[1] COVIS annual reports available at http://www.cdc.gov/nationalsurveillance/cholera-vibrio-surveillance.html (accessed 6 July 2016). Data in table are only for patients from which *V. vulnificus* were exclusively isolated. Additional cases were reported with *V. vulnificus* in combination with another *Vibrio* spp.

[2] Data only for cases where hospitalisation or mortality status was reported.

[3] Estimated from graph.

V. vulnificus are responsible for more than 95% of seafood-related deaths in the USA (Elmahdi *et al.*, 2016). Some analyses have confirmed that oysters are a common vehicle of infection in that country:

- Of *V. vulnificus* infections reported in the USA for the period 1988-1996, the majority (96%) of cases diagnosed with primary septicaemia had reported consumption of raw oysters (Shapiro *et al.*, 1998). Of the cases with traceback information available, 89% had consumed oysters harvested from the Gulf of Mexico when the water temperature was >20ºC. Underlying liver disease was associated with fatal outcome.
- Of 276 *V. vulnificus* infections reported in Florida during the period 1998-2007, 91 cases were associated with oyster consumption (Weis *et al.*, 2011). There were 76 deaths and 56% of these were associated with raw oyster consumption. An earlier study of *V. vulnificus* infections in Florida (1981-1992) found raw oyster consumption was reported by 81% (58/72) of primary septicaemia patients and 78% (14/18) of gastrointestinal patients (Hlady *et al.*, 1993).

Clams have also been reported as vehicles of *V. vulnificus* infection in the USA (Slayton *et al.*, 2014). Other sporadic cases of *V. vulnificus* infection have also been reported where patients with underlying medical conditions became ill after consuming shellfish (Ulusarac and Carter, 2004).

Australia

For the period 2011-2015 there were 10 cases of *Vibrio* food poisoning and 3 cases of *Vibrio* disease (invasive) reported in the Northern Territory.[43] Seven of the *Vibrio* food poisoning cases were reported during 2011; 5/7 were *V. parahaemolyticus* infections, 2/7 were *V. cholerae* infections and 6/7 were reported as overseas acquired (Harlock, 2012). Additional information was available for the 2015 year, which reported that the one case of *Vibrio* food poisoning was *V. parahaemolyticus* infection, and the case reported eating oysters during the incubation period (Draper, 2016). The *Vibrio* spp. was not reported for the remaining foodborne cases and no further information was located on the cases of *Vibrio* disease (invasive).

[43] Northern Territory Disease Control Bulletin, volumes 23(1), 22(1), 20(1) and 19(1). Available at http://health.nt.gov.au/Centre_for_Disease_Control/Publications/NT_Disease_Control_Bulletin/index.aspx (accessed 27 October 2016).

Available information for Tasmania shows one case of *Vibrio* spp. infection reported in during 2015 (*Vibrio* spp. not identified) and one case reported during 2013 (*V. cholerae*). Data for 2014 were not accessible.[44]

Other countries/regions

Three deaths have been reported in New Caledonia as a result of eating oysters (Cazorla *et al.*, 2011). Other sporadic cases of *V. vulnificus* infection have linked to consumption of raw BMS in Japan (Matsuoka *et al.*, 2013; Tsuzuki *et al.*, 1998).

B.1.2 Outbreaks of *V. vulnificus* infection associated with BMS

Only one reported outbreak of *V. vulnificus* infection possibly linked to BMS consumption was identified from the scientific literature. This outbreak involved two cases and was linked to "molluscs". The outbreak was identified from an analysis of 188 seafood-associated outbreaks reported in the USA's Foodborne Disease Outbreak Surveillance System, for the period 1973-2006 (Iwamoto *et al.*, 2010). No further information is available.

B.1.3 Case control studies

No case control studies investigating consumption of BMS as a risk factor for *V. vulnificus* infection were located, possibly because the link between this hazard and food is so well established.

B.1.4 Attribution studies

A Canadian expert elicitation process during 2014 estimated that the median proportion of *V. vulnificus* enteric infections attributed to foodborne transmission was 92.8% (90% Credible Interval (CI) 77.8-99.1%) (Butler *et al.*, 2015). Smaller proportions were assigned to the other transmission routes of waterborne (3.8%, drinking contaminated water only), animal contact (1.1%, presumably contact with contaminated marine animals) and person-to-person (2.3%). An earlier Canadian expert elicitation study had attributed the majority of *Vibrio* spp. infections to the food category "seafood" (Davidson *et al.*, 2011).

B.2 Risk assessments and risk-related activities overseas

In 2001, the European Commission adopted the opinion of the Scientific Committee on Veterinary Measures relating to Public Health, who had assessed the risk to health of *V. vulnificus* and *V. parahaemolyticus* in raw and undercooked seafood and examined the appropriateness of setting standards for these pathogens (SCVMRPH, 2001). The Committee concluded that the risk of infections caused by these pathogens seems to be low, but cautioned that there were insufficient data on the incidence of infections caused by these pathogens in Europe, and the risk from shellfish imported from other (warmer) countries was not well understood.

In 2005, the FAO and the WHO jointly published a quantitative risk assessment for *V. vulnificus* in raw oysters harvested and consumed in the USA (FAO/WHO, 2005). The assessment focussed on the risk of primary septicaemia from consumption of raw oysters from the Gulf Coast (USA) because data were more readily available for this scenario.

The exposure model in this risk assessment predicted that oysters harvested in the winter months would contain *V. vulnificus* at a mean concentration of 80 organisms/g at the time of consumption, and 57,000 organisms/g in the summer. An average serve of 196g of oyster meat corresponded to average ingested doses of 1.6×10^4 organisms during winter and 1.1×10^7 organisms during summer. Most of the predicted growth was during post-harvest storage. The model predicted that a delay of five hours between oyster harvest and the time until first

[44] Communicable Disease Reports (4th quarter 2015, 2013 annual report). Available at http://www.dhhs.tas.gov.au/publichealth/communicable_diseases_prevention_unit (accessed 27 October 2016).

refrigeration almost doubled the predicted number of cases, irrespective of the season the oysters were harvested.

The quantitative risk assessment assumed that all strains of *V. vulnificus* were equally virulent, and that all members of the susceptible population were equally susceptible to illness, irrespective of the type and progression of the underlying condition that precluded them belonging to the group. The model predicted that oysters harvested from the Gulf Coast would cause a mean of 11.7 (90% uncertainty interval (UI) 9.8-14.0) cases of septicaemia during spring, and 12.2 (90% UI 10.5-14.1) during summer. The predicted mean numbers of septicaemia cases during autumn and winter were 8.0 (90%UI 1.5-11.9) and 0.5 (90%UI 0.1-2.8), respectively.

APPENDIX C: CONTROL MEASURES IN OTHER COUNTRIES

C.1 International controls

C.1.1 Codex Alimentarius

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 Alimentarius, 2010). The guidelines recognise that general food hygiene controls (e.g. cooling, measures to minimise cross-contamination) will also control *Vibrio* spp., but also recommend water temperature and salinity levels are established for a harvesting area to indicate increased risk of *Vibrio* spp. contamination.

The Annex sets out specific control measures for *V. vulnificus* and *V. parahaemolyticus* in bivalve molluscs intended for consumption in a live, raw or partially treated state.[45] 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. Good Hygienic Practices (GHP) and HACCP are recommended for post-harvest operations, along with validating the effectiveness of any treatments (e.g. freezing, high pressure) and monitoring such treatments.

C.1.2 European Union

There are no microbiological criteria for *V. vulnificus* in fishery products placed on the market in the EU (Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs).[46] In 2001, the EU's Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) concluded that the available scientific data do not support setting specific criteria for pathogenic *V. vulnificus* and *V. parahaemolyticus* in seafood. The SCVPH recommended that codes of practice be established to ensure that good hygiene practice has been applied.

The most recent amendment to Commission Regulation (EC) No 2073/2005, signed into law 8 December 2015, requires BMS production areas to be classified into one of three categories according to the level of faecal contamination (as measured by *E. coli* concentration in shellfish). This amendment, Commission Regulation (EU) 2015/2285, did not consider *V. parahaemolyticus* or *V. vulnificus*, the presence of which is not related to faecal contamination.[47]

Individual member states can set additional regulations for their country in addition to these EU-wide requirements.

The European Food Safety Authority (EFSA) hosts "Vibrio viewer". This is a real-time map that incorporates daily remote sensing data (e.g. water temperature, salinity) into a model to predict the environmental suitability for *Vibrio* spp. in coastal waters.[48] The model driving the mapping software has been calibrated to the Baltic Region in Northern Europe.

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[45] "Partially treated" is where a bacteriocidal treatment has been applied with the intention to reduce *V. parahaemolyticus* and/or *V. vulnificus*, but not eliminate these bacteria.
[46] (EC) No 2073/2005 is available at http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R2073 (accessed 19 July 2016).
[47] (EU) 2015/2285 is available at http://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1468917512617&uri=CELEX:32015R2285 (accessed 19 July 2016).
[48] https://e3geoportal.ecdc.europa.eu/SitePages/Vibrio%20Map%20Viewer.aspx (accessed 19 July 2016).

C.2 Country-specific controls

Monitoring *V. vulnificus* in BMS harvesting areas as part of a control programme is uncommon (FAO/WHO, 2016). Control of temperature between harvest and sale is seen as a major element in controlling risk.

C.2.3 Australia

Standard 1.6.1 from the Australia New Zealand Food Standards Code (see Section 5.1.1) also applies in Australia but there are no microbiological limits set for *Vibrio* spp. in Schedule 27 associated with this standard.

Standard 4.2.1 (primary production and processing standard for seafood) applies only in Australia, and applies from pre-harvesting production up to, but not including, manufacturing operations.[49] Under this Standard, "A seafood business must systematically examine all of its primary production and processing operations to identify potential seafood safety hazards and implement controls that are commensurate with the food safety risk". There are no requirements specific to managing the risk from *Vibrio* spp.

C.2.4 USA

The sanitary control of shellfish produced and sold for human consumption in the USA is overseen by the National Shellfish Sanitation Program (NSSP), with the purposes of improving sanitation of shellfish moved interstate and promoting uniformity of State shellfish programmes (National Shellfish Sanitation Program, 2011). A code has been published (National Shellfish Sanitation Program, 2011) and State or local shellfish control authorities are responsible for the enforcement of this Code. This includes monitoring *V. vulnificus* and *V. parahaemolyticus* illnesses, conducting annual risk evaluations, and (if necessary) implementing Control Plans for *V. parahaemolyticus* and *V. vulnificus*. A Control Plan is implemented if there has been two or more etiologically confirmed or epidemiologically linked *V. vulnificus* septicaemia illnesses from the consumption of commercially harvested raw or undercooked oysters that originated from the growing waters of that state within the previous ten years. The Control Plan includes identifying triggers that indicate when control measures are needed and specifying the controls to be implemented. The triggers include illnesses linked to the harvesting area and elevated water temperatures.

From April 2003, raw oysters harvested from the Gulf of Mexico from April to October were not permitted for sale in the California State, unless they were processed to reduce *V. vulnificus* to non-detectable levels (Vugia *et al.*, 2013). After the control was put in place, there was a reduction in the number of reported *V. vulnificus* infections in California where the only exposure was raw oysters. This reduction was not observed in States not imposing this control. Food consumption surveys suggest that the reduction in California was not due to less people eating raw oysters. After the success in California, in 2009 the USFDA announced that all Gulf Coast oysters harvested during the summer months would be subject to post-harvest processing, but implementation of this regulation has been postponed due to multiple issues, including the potential for economic losses (Froelich and Noble, 2016; Muth *et al.*, 2013).[50]

The *Food Safety Modernization Act* was signed into law in January 2011, and since then the USFDA has published Final Rule on Preventative Controls for Human Food.[51] Under this rule, covered facilities must establish and implement a food safety system that includes an analysis of hazards and risk-based preventive controls. "Farms", which includes operations that "raise seafood" are not subject to this rule, but processors of fish and fisheries products are. Guidance is available, which considers *Vibrio* spp. to be an important hazard and describes

[49] https://www.legislation.gov.au/Series/F2012L00291 (accessed 19 July 2016).
[50] http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2009/ucm190513.htm (accessed 19 July 2016).
[51] http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334115.htm (accessed 19 July 2016).

controls such as cool storage to prevent growth, plus the kill-steps of cooking, pasteurisation, quick freezing with extended storage, mild heat, high hydrostatic pressure and irradiation (USFDA, 2011). The guidance recommends that these kill-steps reduce the presence of *V. vulnificus* and *V. parahaemolyticus* to non-detectable levels (defined as <30 MPN/g).

The guidance specifically describes a circumstance where it is reasonably likely that shellfish will be contaminated with *V. vulnificus* at an unsafe level (so kill-steps should be implemented): Oysters harvested from areas that have been confirmed as the original source of oysters associated with two or more *V. vulnificus* illnesses (e.g. states bordering the Gulf of Mexico).

Microbiological guidelines have been published for *V. vulnificus* (USFDA, 2011):

- Cooked ready-to-eat fishery products (minimal cooking by consumer): Not detected.
- Post-harvest processed BMS that make a label claim of "processed to reduce *Vibrio vulnificus* to non-detectable levels": <30 MPN/g.

A 2004 survey of post-harvest processed oysters carrying the label claim detected *V. vulnificus* in 10/61 samples, but the concentration of *V. vulnificus* only exceeded 30 MPN/g in two samples (DePaola *et al.*, 2009). These samples were treated by freezing, and were taken before the 49-day freezing period validated for the company's process had been completed.

C.2.5 Canada

During the summer months, oysters harvested from Canadian waters and intended for sale in the shell should only be harvested from sites where the concentration of *V. parahaemolyticus* is ≤100 MPN/g, unless a validated post-harvest processing step is applied that will reduce *V. parahaemolyticus* to this level (FAO/WHO, 2016). This will also reduce the risk of oysters being contaminated with *V. vulnificus*.

Registered BMS processors must implement a Quality Management Program and this should consider controls for *V. parahaemolyticus*, including ensuring BMS suppliers and transporters have adequate cooling procedures, and ensuring that the time/temperature controls along the BMS processing line are being followed and are effective.[52] Such controls will also be effective for *V. vulnificus*. There is no microbiological guideline or standard for *V. vulnificus*.

[52] http://www.inspection.gc.ca/food/fish-and-seafood/communiques/archive/2013-07-23/eng/1371488770625/1371488872212 (accessed 25 July 2016).

INSTITUTE OF ENVIRONMENTAL SCIENCE AND RESEARCH LIMITED

Kenepuru Science Centre
34 Kenepuru Drive, Kenepuru, Porirua 5022
PO Box 50348, Porirua 5240
New Zealand
T: +64 4 914 0700 **F:** +64 4 914 0770

Mt Albert Science Centre
120 Mt Albert Road, Sandringham, Auckland 1025
Private Bag 92021, Auckland 1142
New Zealand
T: +64 9 815 3670 **F:** +64 9 849 6046

NCBID – Wallaceville
66 Ward Street, Wallaceville, Upper Hutt 5018
PO Box 40158, Upper Hutt 5140
New Zealand
T: +64 4 529 0600 **F:** +64 4 529 0601

Christchurch Science Centre
27 Creyke Road, Ilam, Christchurch 8041
PO Box 29181, Christchurch 8540
New Zealand
T: +64 3 351 6019 **F:** +64 3 351 0010

www.esr.cri.nz