New Zealand Food Safety
Haumaru Kai Aotearoa

Risk Profile:

Vibrio Parahaemolyticus in Bivalve Molluscan Shellfish

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Prepared for the Ministry for Primary Industries
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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 parahaemolyticus in bivalve molluscan shellfish

ESR Report 16033

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

While 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. parahaemolyticus foodborne infection, the 2003 Risk Profile for V. parahaemolyticus in BMS was unable to estimate the risk due to insufficient data. Previous V. parahaemolyticus infections in New Zealand were mainly associated with the consumption of seafood harvested from Pacific Islands and privately imported to New Zealand.

In the intervening period 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, the current Risk Profile again concludes that there are insufficient data to accurately estimate the risk to New Zealand consumers of V. parahaemolyticus 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. parahaemolyticus infection is poorly understood, as are the determinants of pathogenicity of V. parahaemolyticus and its dose-response.

MPI concludes that the current risk to New Zealanders of V. parahaemolyticus 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. parahaemolyticus 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. parahaemolyticus.

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. parahaemolyticus from BMS should these environmental determinants change. The effect of any changes on the risk of Vibrio vulnificus and ciguatoxins, will also be particularly considered in any further project on climate change.

RISK PROFILE:

VIBRIO PARAHAEMOLYTICUS IN BIVALVE MOLLUSCAN SHELLFISH

October 2016

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DISCLAIMER

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SUMMARY

This Risk Profile considers *Vibrio parahaemolyticus* in bivalve molluscan shellfish (BMS). A Risk Profile considering *V. parahaemolyticus* in seafood was published in 2003 (Lake *et al.*, 2003). The 2003 Risk Profile identified that *V. parahaemolyticus* infection in New Zealand was strongly linked to the personal importation and consumption of seafood from the Pacific Islands.

This current document only considers the risk of *V. parahaemolyticus* infection from BMS harvested in New Zealand from wild stocks (commercial or non-commercial harvesting) and from aquaculture (commercial production), and consumed raw. Discussion of the risk from imported BMS and the impact of controls (e.g. cooking) has been included.

The purpose of this Risk Profile is to critically review available information to answer the following Risk Management Question (RMQ): Has the risk to human health from *V. parahaemolyticus* changed since 2003?

V. parahaemolyticus 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. Foodborne exposure to *V. parahaemolyticus* can lead to gastroenteritis. The disease is usually self-limiting but hospitalisation may be necessary. Septicaemia can develop in people with immunocompromising health conditions, which results in death in an estimated 20% of cases.

Not all *V. parahaemolyticus* strains are pathogenic to humans. A number of virulence factors have been identified including possession of genes encoding haemolysins (*tdh, trh*), Type III secretion systems and urease. An isolate containing one or more of these is likely to be pathogenic, but their absence is not necessarily an indication that a strain will not be pathogenic. Science has not yet identified a reliable method for determining *V. parahaemolyticus* pathogenicity.

The most important environmental parameter influencing the presence and concentration of *V. parahaemolyticus* in the marine environment and in BMS is water temperature. Numbers are highest during summer months and lowest during winter months. The available information indicates that *V. parahaemolyticus* are rarely isolated from seawaters below 10ºC and are released from sediments into waters at temperatures above 14ºC. Growth is particularly favoured at temperatures above 20ºC. The influence of water temperature over the prevalence and concentration of pathogenic (*tdh+/trh+*) strains is not well established, but there is some evidence to suggest a positive correlation. Other environmental factors, including salinity and turbidity, have also been linked to *V. parahaemolyticus* environmental prevalence but the correlations are inconsistent. Any relationships are likely to be specific to a region or site. The global distribution of *V. parahaemolyticus* is widening as ocean temperatures rise in response to climate change.

V. parahaemolyticus can multiply in BMS after harvesting if the temperature is suitable. Laboratory studies show that *V. parahaemolyticus* will multiply in BMS stored at 15ºC or above, and modelling studies predict that *V. parahaemolyticus* may grow in BMS stored at 12ºC. At ≥20ºC, multiplication to stationary phase occurs within 1-2 days. *V. parahaemolyticus* will not grow in BMS stored at 10ºC or lower. In unfrozen BMS, the concentration of *V. parahaemolyticus* remains stable or decreases at these cool temperatures, but survival for up to three weeks has been reported. *V. parahaemolyticus* dies in frozen BMS but can survive for up to six months.

There are data on *V. parahaemolyticus* in BMS harvested from New Zealand waters. Most data comes from Pacific oyster samples. These New Zealand surveys show that the

prevalence and concentration of *V. parahaemolyticus* are higher in commercial BMS harvested from harbours in the north half of the North Island compared with the Marlborough Sounds. Up to 100% of Pacific oyster samples from harbours located in the upper half of the North Island yielded *V. parahaemolyticus*, at concentrations as high as 4.8×10^4 MPN/g. Potentially pathogenic *V. parahaemolyticus* were also detected in these samples but at lower prevalences (up to 27%) and concentrations (maximum 933 MPN *trh*+ *V. parahaemolyticus* cells per gram). *V. parahaemolyticus* were also detected in 42% (16/38) of samples of green-lipped mussels from northern North Island harbours in a survey from 2009-2012. From three surveys of Pacific oysters from the Marlborough Sounds, the highest *V. parahaemolyticus* prevalence measured was 30% and the highest concentration was 7.4 MPN/g. *V. parahaemolyticus* were also detected in samples of dredge oysters (1/21, 0.36 MPN/g) and green-lipped mussels (2/17) from the Marlborough Sounds. The *tdh* and *trh* genes were not detected in any samples from the Marlborough Sounds.

Overall, the New Zealand surveys detected *V. parahaemolyticus* in Pacific oysters more often and at higher concentrations during summer months compared with other seasons, when sea surface temperatures were ≥19ºC. There was no significant correlation with water salinity (*V. parahaemolyticus* were isolated from Pacific oysters at salinities >35‰).

V. parahaemolyticus 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. parahaemolyticus* infection will be underreported.

From January 1998 to July 2016 there were 53 sporadic cases of *V. parahaemolyticus* infection reported in New Zealand. In eight of these sporadic cases, BMS were specifically implicated as the vehicle of infection and the available information suggested that these shellfish were harvested in New Zealand. The implicated BMS were oysters or mussels, commercially or non-commercially harvested. Seafood imported from a Pacific island was the implicated vehicle of illness in a further eight cases.

From January 1998 to July 2016 there were eight reported outbreaks of *V. parahaemolyticus* infection, involving a total of 44 cases. The most likely vehicle of infection for seven of these outbreaks was seafood imported from Pacific Islands. BMS harvested from New Zealand waters were not implicated in any of these reported outbreaks.

Has the risk to human health from *V. parahaemolyticus* changed since 2003?

The 2003 Risk Profile found there were insufficient data to make an assessment on the risk associated with BMS harvested from New Zealand waters, although it was noted that the salinities of New Zealand coastal waters were greater than the optimum for *V. parahaemolyticus*, and the water temperatures in regions south of Auckland were less than optimal. Given the lack of a comparative baseline to measure any change in risk from BMS harvested from New Zealand waters, this Risk Profile instead discusses the risk from these BMS (when consumed raw), and provides commentary on whether the risk may have changed.

Based on the available information, New Zealand consumers of raw BMS harvested from New Zealand waters are at risk of foodborne *V. parahaemolyticus* infection. *V. parahaemolyticus* are present in the New Zealand coastal marine environment where water temperature and salinity are not barriers to its survival. The seawaters of New Zealand provide favourable temperatures for *V. parahaemolyticus*, particularly during summer months. New Zealand surveys indicate that BMS harvested from northern waters during summer are most likely to be contaminated with *V. parahaemolyticus*, and the concentrations measured in some samples from upper North Island harbours could be high enough to cause illness. These surveys have also detected potentially pathogenic strains of *V. parahaemolyticus* in BMS. Thus, the human health 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.

The science demonstrates potential for BMS harvested from New Zealand waters to cause *V. parahaemolyticus* infection, but there is a lack of reported cases in New Zealand's public health surveillance data. This is not unexpected, primarily because *V. parahaemolyticus* infection will be underreported in this country. However, during the period January 1998 to July 2016, eight outbreaks of *V. parahaemolyticus* infection were reported in New Zealand (seven most likely caused by seafood privately imported from Pacific Islands). This suggests that the existing public health surveillance systems would detect at least some outbreaks of foodborne *V. parahaemolyticus* infection from consumption of BMS harvested from New Zealand waters, if these were happening frequently.

The risk of infection may be attenuated by a number of factors which, together, mean that New Zealanders consuming raw BMS harvested from New Zealand waters are not often exposed to pathogenic strains of *V. parahaemolyticus* in high enough concentrations to cause illness. These factors include the possibly low prevalence and concentration of pathogenic *V. parahaemolyticus*, consuming BMS harvested during cooler seasons or from southern locations, and cool-chain requirements for industry.

There are few data to inform the risk of *V. parahaemolyticus* infection from consumption of BMS other than Pacific oysters harvested from northern New Zealand waters. The available data indicate mussels might present a similar risk since these are also consumed raw or marinated. It is possible that there are other regions of New Zealand where the risk of BMS becoming contaminated with *V. parahaemolyticus* 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).

There were very little data available prior to 2003 to make an assessment on the risk associated with BMS harvested from New Zealand waters. The data in this current document suggest that the risk of *V. parahaemolyticus* infection from consumption of raw BMS harvested from New Zealand waters has not changed since 2003. Seafoods privately imported from the Pacific Islands are still an important cause of sporadic cases and outbreaks of *V. parahaemolyticus* infection in New Zealand. There are insufficient data to determine the risk to New Zealand consumers of *V. parahaemolyticus* infection from commercially imported BMS.

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

A Risk Profile considering *V. parahaemolyticus* in seafood was published in 2003 (Lake *et al.*, 2003). The 2003 Risk Profile identified that *V. parahaemolyticus* infection in New Zealand was strongly linked to the personal importation and consumption of seafood from the Pacific Islands. This current document only considers information presented in the 2003 Risk Profile relevant to BMS harvested from New Zealand waters.

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

- Has the risk to human health from *V. parahaemolyticus* changed since 2003?

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. vulnificus* in BMS has been prepared alongside this current document (King *et al.*, 2016). Both *V. parahaemolyticus* and *V. vulnificus* may also infect wounds when existing wounds are exposed to seawater 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 PARAHAEMOLYTICUS*

KEY FINDINGS

V. parahaemolyticus are ubiquitous in tropical and temperate marine, coastal, and estuarine environments throughout the world. As a result, *V. parahaemolyticus* are commonly found in seafood, including BMS.

Not all *V. parahaemolyticus* strains are pathogenic to humans. A number of virulence factors have been identified including possession of genes encoding haemolysins (*tdh*, *trh*), Type III secretion systems and urease. An isolate containing one or more of these is likely to be pathogenic, but their absence is not necessarily an indication that a strain will not be pathogenic. Science has not yet identified a reliable method for determining *V. parahaemolyticus* pathogenicity.

2.1.1 The microorganism

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

V. parahaemolyticus are halophilic (salt-loving), motile bacteria that occur naturally in estuarine and coastal marine environments. Their presence is not due to faecal pollution or human contamination (e.g. domestic or industrial discharges to water). *V. parahaemolyticus* was first isolated in 1950 in Osaka, Japan when there was an outbreak of illness associated with boiled and semi-boiled sardines (Hara-Kudo and Kumagai, 2014). Since this first isolation, *V. parahaemolyticus* have been found in tropical and temperate estuarine and coastal marine environments throughout the world (European Commission, 2001; FAO/WHO, 2011).

V. parahaemolyticus can be free living but are frequently attached to suspended matter (abiotic or living, e.g. plankton) or sediments, or form biofilms on marine biotic surfaces (e.g. on BMS shells or zooplankton). They are able to break down chitin so can embed themselves in the shells of marine animals (Daniels, 2011). *Vibrio* spp., including *V. parahaemolyticus*, can be transported around the world's marine environments by ship ballast water, migratory bird and fish species, tidal currents and imported and exported seafood (DePaola *et al.*, 1994; Martinez-Urtaza *et al.*, 2013). Changes in the distribution of plankton species may also affect *Vibrio* spp. distribution (Vezzulli *et al.*, 2016). The dynamics of the El Niño waters have been identified as a driving force for introducing and propagating *V. parahaemolyticus* in South American waters (Martinez-Urtaza *et al.*, 2008a).

2.1.2 Pathogenicity and virulence markers

The most common clinical manifestation of *V. parahaemolyticus* infection is gastroenteritis (FAO/WHO, 2011). Septicaemia may develop in people with underlying medical conditions.

Not all strains of *V. parahaemolyticus* cause illness. The 2003 Risk Profile reported that the pathogenicity of *V. parahaemolyticus* was not clearly understood but recognised that strains with certain pathogenicity markers were associated with human infections. The association between clinical cases and *V. parahaemolyticus* strains with certain virulence markers is still recognised, but science has not yet identified a reliable method for determining *V. parahaemolyticus* pathogenicity. *V. parahaemolyticus* research over the last decade has identified that the pathogenicity factors are not as well understood or as not as predictable as earlier thought (Bechlars *et al.*, 2015; Jones *et al.*, 2012; Nydam *et al.*, 2014; Saito *et al.*, 2015).

As not all strains of *V. parahaemolyticus* are pathogenic, scientific research has been directed towards identifying genotypic and phenotypic traits that can be relied on as indicators for pathogenicity. A number of virulence factors have been identified and an isolate containing one or more of these is likely to be pathogenic.

Appendix A.3 contains details of recognised virulence factors for *V. parahaemolyticus*. Testing for virulence factors is now usually done using molecular methods. The most commonly measured virulence factors are:

- Thermostable Direct Haemolysin (TDH), with the ability to produce this haemolysin depending on possession of the *tdh* gene (*tdh*+ isolates are also Kanagawa positive isolates, see Appendix A.3); and
- TDH-Related Haemolysin (TRH), linked to possession of the *trh* gene.

Other genes less commonly targeted include those within the Type III secretion systems (T3SS), particularly T3SS2α and T3SS2β, as well as genes associated with the production of urease.

Urease production was once considered a useful indicator for pathogenicity because it usually correlated with possession of *trh*. However, isolates that possess *trh* but do not produce urease have also been identified (Jones *et al.*, 2012). Such isolates may still harbour the genes for urease production.

While possession of the *tdh* and/or *trh* genes indicates that a *V. parahaemolyticus* isolate is likely to cause illness in humans, an increasing proportion of clinical isolates possess neither gene and these isolates have been associated with severe cases requiring hospitalisation (FAO/WHO, 2011). In one study of clinical isolates, 27% (21/77) were *tdh*-/*trh*- (Jones *et al.*, 2012). Details from other studies are outlined in Appendix A.3. The absence of the *tdh* and *trh* genes in some clinical isolates demonstrates that these genes cannot be relied on as markers of pathogenicity with absolute certainty. It may also be possible that the methods used may not have detected the *trh* gene (Nilsson and Turner, 2016).

The search for reliable pathogenicity markers is further complicated by the finding that there are both evolutionary and ecological forces acting on *V. parahaemolyticus* populations (Loyola *et al.*, 2015; Paranjpye *et al.*, 2012; Raghunath, 2011). The presence of pathogenicity islands (physical groupings of virulence-related genes) in *V. parahaemolyticus* may foster rapid micro-evolution, promote growth and survival, and result in transmission of factors (including virulence) between strains by horizontal gene transfer (Frischer *et al.*, 1990; Ichige *et al.*, 1989; Iida *et al.*, 1998). Bacteriophages may also genetically alter vibrios (Baross *et al.*, 1978; Hurley *et al.*, 2006; Wang *et al.*, 2006). As a result, it is unlikely that there is one simple set of pathogenicity factors, and these factors will vary regionally.

In summary, it is clear that not all strains of *V. parahaemolyticus* will cause illness. While there is still uncertainty over the characteristics that enable a strain to infect a human host, an isolate containing the *tdh* and/or *trh* gene is more likely to be able to cause human disease. It could be that there are other as-yet unknown virulence factors possessed by *V. parahaemolyticus*.

V. parahaemolyticus can be differentiated by serotyping (based on the O and K antigens, see Appendix A.2). Serotyping is useful for indicating the presence of some recognised pathogenic strains, the so-called “pandemic clones”. The 2003 Risk Profile introduced the first known pandemic clone of *V. parahaemolyticus*, of the serotype O3:K6. The clone was first recognised in India in 1996 and by 2007 had been isolated throughout Asia, America, Africa and Europe (Nair *et al.*, 2007). While the O3:K6 clone exhibited increased adherence and cytotoxicity in tissue culture, and was isolated from waters previously thought to be too cold to support growth, the specific traits that contributed to the clone’s dominance in the environment and human cases were not determined (Boyd *et al.*, 2008; Nair *et al.*, 2007; Yeung *et al.*, 2002). Other serotypes have also emerged, with genotyping showing that they are related to

the O3:K6 pandemic strain. These serotypes are called serovariants and share the defining characteristics of the O3:K6 clone. Twenty serovariants have been reported (Letchumanan *et al.*, 2014). See Appendix A.4 for further information.

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 show that cockles and Foveaux Strait dredge oysters were harvested in the highest amounts by weight (approximately 1,000 tonnes each).

New Zealand green-lipped mussels and Pacific oysters are commercially farmed in New Zealand. During 2011, approximately 100,000 tonnes of green-lipped mussels and 1,800 tonnes of Pacific oysters were harvested. The majority of Pacific oysters were exported.

Recreational harvesters collect more scallops and mussels than other types of shellfish.

Pacific oysters and scallops make up the majority of imported BMS products, by weight.

For the year 2011, an estimated 13,000 tonnes (meatweight) 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. parahaemolyticus* are not usually 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.5. FIGURE 1 explains the sources of BMS available to New Zealand consumers.

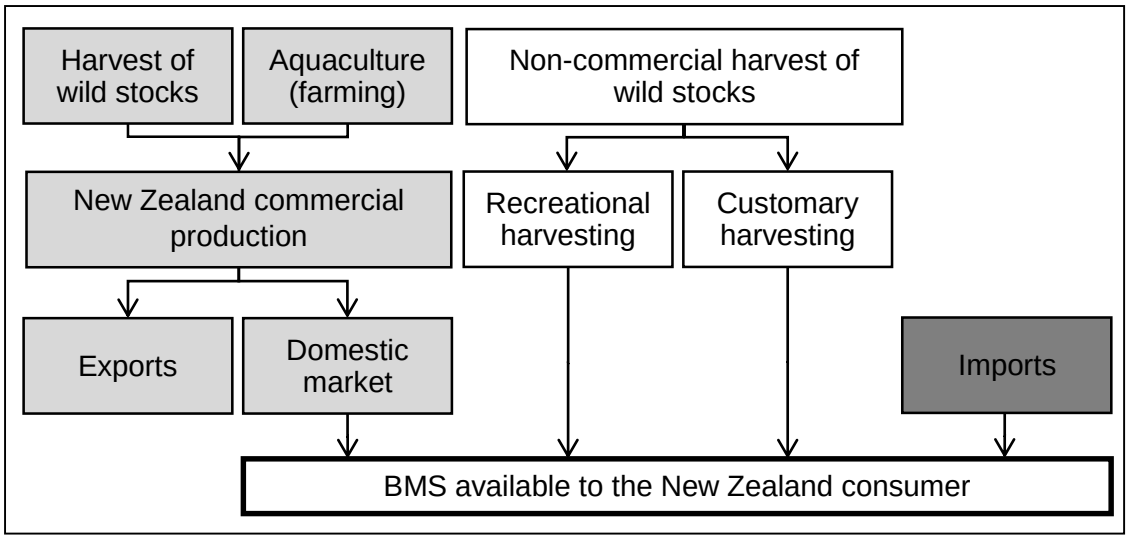

FIGURE 1: Sources of BMS available to New Zealand consumers (reproduced from King and Lake 2013)

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.[3] The amounts listed represent a summation of data for specific areas (Quota Management Areas) around New Zealand.[4] 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.[5]

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 oyster = 102 g (MPI, 2016d) 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. Green-lipped mussels are grown on ropes permanently submerged in subtidal waters. During 2015, 80,000 tonnes of New Zealand

[3] Quota are the same for the 2015/16 fishing year but full data on reported landings are not available until October 2016.

[4] Not all quota management areas for a single species are managed under the QMA so additional harvesting may have occurred that was not reported.

[5] http://www.mpi.govt.nz/travel-and-recreation/fishing/fishing-rules/ (accessed 31 May 2016).

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 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). 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). Oysters are grown on racks, or in baskets, mesh trays or bags attached to racks in the intertidal zone, or sometimes on subtidal long-lines (Castinel *et al.*, 2015). The oysters grown in the subtidal zone are usually transferred to the intertidal zone for some time before harvest to harden the shells. They 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.[6]

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 International trade

New Zealand imports some BMS and BMS meat.[7] 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 (96%) of these imported oysters were from the Republic of Korea.

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

[6] http://www.cawthron.org.nz/aquaculture-park/pacific-oyster-spat-sales/ (accessed 9 August 2016).
[7] Import data obtained from Statistics New Zealand Infoshare, http://www.stats.govt.nz/infoshare/ (accessed 13 April 2016 and 1 June 2016). Updated data on Pacific oysters for 2012-2015 directly provided by Statistics New Zealand (September 2016). Updated data differs from published official statistics.
[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.

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]

2.2.3 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).

2.3 CONTAMINATION OF BMS BY *V. PARAHAEMOLYTICUS*

KEY FINDINGS

V. parahaemolyticus are common in temperate waters, including those found in New Zealand, and concentrations are primarily influenced by seawater temperature. Numbers are highest during summer months and lowest during winter months. The available information indicates that *V. parahaemolyticus* are rarely isolated from seawaters below 10ºC and are released from sediments into waters at temperatures above 14ºC. Conditions become more favourable for growth as temperatures increase, and growth is particularly favoured at temperatures above 20ºC. The influence of water temperature over the prevalence and concentration of pathogenic (*tdh*+/*trh*+) strains is not well established but there is some evidence to suggest a positive correlation.

Other environmental factors, including salinity and turbidity, have also been linked to *V. parahaemolyticus* environmental prevalence but the correlations are inconsistent. Any relationships are likely to be specific to a region or site.

The global distribution of *V. parahaemolyticus* is widening as ocean temperatures rise in response to climate change.

Laboratory studies show that *V. parahaemolyticus* can enter a viable but non-culturable state under cold, nutrient poor conditions. It has not yet been established whether *V. parahaemolyticus* in this state are present in BMS growing under normal environmental conditions, and whether they remain able to cause illness in humans.

BMS will naturally bioaccumulate *V. parahaemolyticus* to concentrations higher than the surrounding waters. The environmental conditions and natural processes such as shellfish

[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.

immunity, predatory bacteria and bacteriophages, affect the presence and concentration of *V. parahaemolyticus* in BMS. *V. parahaemolyticus* will multiply in BMS located in intertidal regions when they are out of the water, if the temperature is suitable. *V. parahaemolyticus* are depurated but the rate of depuration from BMS living in their growing waters is probably variable. Enforced depuration after harvest does not reliably eliminate all *V. parahaemolyticus* from BMS.

The 2003 Risk Profile reported that the presence of *V. parahaemolyticus* in coastal marine waters or BMS is influenced by season, with highest prevalences and concentrations occurring in the warmer months. Information collated in the 2003 Risk Profile also suggested that the majority of *V. parahaemolyticus* isolates from seawater were negative for the Kanagawa phenomenon (KP-, equivalent to *tdh*-, see Appendix A.3), and that human illness from BMS contaminated with *V. parahaemolyticus* increased with warmer water temperature. Research conducted since does not alter these findings, but has provided greater understanding.

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

V. parahaemolyticus are ubiquitous in tropical and temperate marine, coastal, and estuarine (brackish) environments, but not in the open sea (Codex Alimentarius, 2010; Colwell, 1984; European Commission, 2001). Scientists have isolated *V*. *parahaemolyticus* from marine sediments, sea water and marine species such as plankton, arthropods, crab larvae and other marine organisms (Tamplin, 1994). A recent molecular analysis of environmental *V. parahaemolyticus* isolates did not find any relationship between habitat (water, oyster, plankton, sediment) and phylogenetic characteristics, showing that *V. parahaemolyticus* are able to survive in a variety of habitats (Turner *et al.*, 2013). Sediments serve as a reservoir for *Vibrio* spp. (Huehn *et al.*, 2014).

V. parahaemolyticus have been isolated from the intestinal contents of many fish species (Nair *et al.*, 1980). A recent study measured higher concentrations of *Vibrio* spp. in the intestines of fish compared with sediment, water and oysters from the same environment (Givens *et al.*, 2014). There is also evidence to show that *V. parahaemolyticus* can multiply in the digestive tract of shellfish (FAO/WHO, 2011; Greenberg *et al.*, 1982). These findings suggest fish and shellfish have important roles in the environmental cycling of *V. parahaemolyticus*, and that these bacteria might be considered marine enteric bacteria (Baumann and Baumann, 1977; European Commission, 2001; Givens *et al.*, 2014).

The 2003 Risk Profile reported a correlation between water temperature and the concentrations of *V. parahaemolyticus* in shellfish or seawater, reporting that this pathogen was seldom isolated from shellfish grown in water at <15ºC. The document also reported that levels of *V. parahaemolyticus* were also related to water salinity, with an optimal salinity of 19‰, but noted that the salinity of coastal seawaters changes with influxes of freshwater. The environmental factors influencing *Vibrio* spp. in marine ecosystems have been well studied since 2003. Water temperature, and to a lesser extent water salinity, are still the most important factors affecting *V. parahaemolyticus* concentrations in the environment (USFDA, 2005b). However, studies since 2003 suggest that the relationships of both total *V. parahaemolyticus* and pathogenic strains of *V. parahaemolyticus* with salinity, water temperature and other environmental variables are complex. These relationships are also likely to be regional and even site specific (Urquhart *et al.*, 2016; Young *et al.*, 2015; Zimmerman *et al.*, 2007).

<u>Temperature</u>

V. parahaemolyticus are detected throughout the year in tropical waters (Natarajan *et al.*, 1980). In other geographical areas where *V. parahaemolyticus* have been detected, the prevalence and concentration of these bacteria follow a distinct seasonal cycle, with highest

counts recorded in the summer and autumn and lowest counts in the winter. In temperate waters, like those of New Zealand, *V. parahaemolyticus* have an annual cycle, surviving in the sediment during the winter and being released when the water temperature rises above 14ºC (Codex Alimentarius, 2010; European Commission, 2001; Kaneko and Colwell, 1973).

V. parahaemolyticus are rarely found in waters below 10ºC, however they have been isolated from BMS growing in water at 0.6ºC and from sediments at these cooler temperatures (Baross and Liston, 1970; Bauer *et al.*, 2006; Desmarchelier, 2003; Kaneko and Colwell, 1973; Strom and Paranjpye, 2000). The emergence of cold-tolerant strains has been reported (Vasconcelos *et al.*, 1975; Xu *et al.*, 2015). The ability of some strains to tolerate cooler water temperatures has been supported by a study of New Hampshire waters in the United States of America (USA), which found that the genetic diversity of *V. parahaemolyticus* strains isolated from colder waters (1-11ºC) was less than the overall collection of isolates (Ellis *et al.*, 2012). The diversity increased with temperature.

Seawater temperatures above 20ºC favour *V. parahaemolyticus* multiplication (Cantet *et al.*, 2013). The concentration of *V. parahaemolyticus* can reach 100 cells/ml when seawater temperatures increase to 25ºC (DePaola *et al.*, 1990; Kaneko and Colwell, 1973).

Multiple environmental and epidemiological studies verify the temperature linkage. A two-year study of seawater and molluscs from the Adriatic Sea in Italy found that *V. parahaemolyticus* were most prevalent during the summer months (Croci *et al.*, 2001). A study of Norwegian waters only detected *V. parahaemolyticus* during the summer months of July and August (Gjerde and Boe, 1981). An intensive three-year study measured a significant association between surface seawater temperature and *V. parahaemolyticus* in water, oysters and sediments collected from four coastal locations in the USA (Johnson *et al.*, 2012). An increasing incidence of *V. parahaemolyticus* infection linked to the Atlantic Northwest, USA, was attributed to warmer than usual ocean temperatures and the introduction of a lineage of ST36 strains that were adapted to the cooler waters of the Pacific (Xu *et al.*, 2015). The 2004/05 Chilean outbreak of *V. parahaemolyticus* infection, causing over 10,000 cases, was associated with the reversal of the Humboldt Current during the El Niño Southern Oscillation, bringing warm waters to the Chilean coast (Fuenzalida *et al.*, 2007; Martinez-Urtaza *et al.*, 2010). Most of the environmental surveys summarised in Appendix A.6 measured increased prevalence and/or concentration of *V. parahaemolyticus* in BMS with increasing water temperature, and often the correlation was statistically significant.

While the relationship between total *V. parahaemolyticus* and water temperature is fairly well-established, there are few data to assess the importance of water temperature on potentially pathogenic *V. parahaemolyticus*, as indicated by possession of the *tdh* and/or *trh* genes. The main issue is that there are often insufficient numbers (prevalence, concentration) detected to make statistically significant comparisons with environmental variables. Recent studies show mixed evidence that temperature is linked to the prevalence of potentially pathogenic strains.

One study found a statistically significant association between the concentration of these potentially pathogenic strains and suspended particulate matter, and to a lesser extent chlorophyll *a*, but not water temperature (Johnson *et al.*, 2012). In contrast, another study found a statistically significant and positive correlation between *tdh*+ or *trh*+ isolates and temperature (Jones *et al.*, 2014). Similarly, in the USA, wild oysters collected in June, July and September 2001 were tested for *V. parahaemolyticus*, and while the total *V. parahaemolyticus* population, water temperature and salinity were similar between all three months, *trh* and *tdh* were detected only in samples collected in the summer months of June and July (Kaufman *et al.*, 2003). Potentially pathogenic *V. parahaemolyticus* isolates have been recovered from BMS harvested from waters at 16ºC (Passalacqua *et al.*, 2016).

To summarise, a recent review concluded that “most studies that attempt to correlate *tdh*- and/or *trh*-positive *V. parahaemolyticus* strains in oysters to environmental parameters have had little success” (Froelich and Noble, 2016).

Nevertheless, public health surveillance data clearly show increased reports of *V. parahaemolyticus* infections during summer months (Section 3), which suggests that pathogenic strains do respond to seawater temperatures in some way. Epidemiological data and environmental surveys suggest the largest risk to human health comes when BMS are harvested from waters ≥20ºC. Studies in the USA have found a more than ten-fold difference in the concentration of *V. parahaemolyticus* in BMS and seawater at 10ºC compared with those at 25°C (DePaola *et al.*, 1990). A study in Taiwan identified a relationship between outbreaks of *V. parahaemolyticus* illness and the increasing average temperature and salinity of the ocean over the previous six months (Hsiao *et al.*, 2016). The number of reported cases of foodborne *V. parahaemolyticus* infection peaks during the summer months in the USA (Appendix B.2.1).

However, outbreaks of *V. parahaemolyticus* infection have occurred from BMS harvested from waters at lower temperatures. For example, in 2004 an outbreak on a cruise ship was associated with oysters harvested from an Alaskan farm, where the lowest water temperature associated with this farm during the harvest period of July-August 2004 was 15.3ºC (McLaughlin *et al.*, 2005).

Salinity

The relationship between salinity and *V. parahaemolyticus* appears to be variable and complex (Johnson *et al.*, 2012). Salinity appears to be a major factor in the prevalence of V. *parahaemolyticus* in tropical waters (FAO/WHO, 2011; Lopez-Hernandez *et al.*, 2015). In temperate regions it seems that the salinity relationship may vary with different regions and sites. It has been reported that adsorption of *V. parahaemolyticus* onto plankton or chitin-containing materials occurs with higher efficiency under conditions of lower estuarine salinity (<17‰) (Kaneko and Colwell, 1975). This may lead to greater uptake by BMS feeding on the plankton.

Studies summarised in Appendix A.6 demonstrate the variable effect of water salinity on *V. parahaemolyticus* prevalence and/or concentration in BMS. Some studies found no correlation, and others a negative or positive correlation. One study in Spain found the concentration of *V. parahaemolyticus* was positively correlated to water salinity, but the concentration of potentially pathogenic *V. parahaemolyticus* was negatively correlated (Lopez-Joven *et al.*, 2015). An earlier study in Spain found salinity to be the primary factor governing the temporal and spatial distribution of *V. parahaemolyticus*, whereby lower salinities favoured this bacterial species (Martinez-Urtaza *et al.*, 2008b). This study also found that seawater temperature only modulated *V. parahaemolyticus* abundance during periods and in areas of reduced salinities, despite the wide range measured (11.7-20.8ºC).

The relationship between water salinity and *V. parahaemolyticus* has even been found to vary within a region. For example, the concentration of *V. parahaemolyticus* in oysters collected at two sites in the Gulf of Mexico showed that in one site salinity was positively correlated, but not at the other (Zimmerman *et al.*, 2007). These authors estimated that a 10‰ change in salinity corresponded to a 1.2 $\log_{10}$ change in *V. parahaemolyticus* concentration in oysters, although the salinities measured at this site were low (4-13‰).

A study in southern France found that increased freshwater inputs into coastal lagoons as a result of heavy rainstorms and flash flooding coincided with increased *Vibrio* spp. in the lagoon waters (Esteves *et al.*, 2015). The fresh water inputs reduced the water salinity between 2.2 and 16.4‰ within 15 days, depending on the site. The highest concentrations of *V. parahaemolyticus* were measured at water salinities between 10 and 20‰.

Other environmental parameters

The relationship between *V. parahaemolyticus* and other environmental parameters such as suspended particulate matter, chlorophyll *a* and dissolved organic carbon is not well established. As with salinity, any relationship is probably specific to an area.

It was hypothesised that conditions that increased SPM (or turbidity) might also increase *V. parahaemolyticus* concentration in the water through releasing sediment-bound *V. parahaemolyticus*, or by increasing the nutrients available for *V. parahaemolyticus* growth (Zimmerman *et al.*, 2007). These conditions may also cause oysters to shut down filter feeding so depuration of any accumulated *V. parahaemolyticus* ceases. Several studies have found that the amount of suspended particulate matter (SPM) in the water was positively correlated with *V. parahaemolyticus* concentrations in oysters, water and sediment collected from the Gulf Coast, USA (Johnson *et al.*, 2012; Johnson *et al.*, 2010; Zimmerman *et al.*, 2007). For example, a 21-month study of multiple sites in the USA found SPM was an important predictor for total and potentially pathogenic *V. parahaemolyticus*, although the sea surface temperature, levels of dissolved organic carbon and salinity were relatively more important (Johnson *et al.*, 2012). Another study has found that storm events that increased turbidity were not associated with any significant increase in *V. parahaemolyticus* in oysters (Shaw *et al.*, 2014).

Other factors that might influence *V. parahaemolyticus* in BMS growing waters include the amount of zooplankton in the shellfish growing area, the rate of tidal flushing and levels of dissolved oxygen in the water (USFDA, 2005b). Given the ability of *V. parahaemolyticus* to attach to living and non-living surfaces, the type of habitat (e.g. rocky, sandy, coral) may also be influential, although no studies were located investigating this hypothesis.

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. parahaemolyticus*. Climate change will also affect the salinity of coastal and estuarine systems due to changes in precipitation and stream flow patterns (Marques *et al.*, 2010). Warmer temperatures appear to be the cause of *V. parahaemolyticus* extending its geographical range into areas such as Alaska, Europe and Chile (Gonzalez-Escalona *et al.*, 2005; Ma and Su, 2011; Martinez-Urtaza *et al.*, 2013; McLaughlin *et al.*, 2005). Rising water temperatures in shellfish growing areas have been associated with the increasing incidence of *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* spp. infections may be linked to rising ocean temperatures (Baker-Austin *et al.*, 2013; Gonzalez-Escalona *et al.*, 2005; McLaughlin *et al.*, 2005; Paz *et al.*, 2007; Sims *et al.*, 2011).

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. prevalence and concentration.

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. 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.

During the last 15 years, there has been a significant shift from sporadic (single) cases of *V. parahaemolyticus* infection towards large outbreaks attributed to the consumption of oysters harvested from northern waters, which in turn has been linked to higher mean water temperatures (Drake *et al.*, 2007; McLaughlin *et al.*, 2005). Upon investigating an outbreak caused by oysters harvested from Alaskan waters, it was noted that seawater temperature measurements from the implicated farm showed a progressive warming of 0.21°C a year between 1997 and 2004 (McLaughlin *et al.*, 2005).

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). Several laboratory studies have observed *V. parahaemolyticus* entering the VBNC state when subjected to decreased temperature and nutrients (conditions similar to cold seawater), and resuscitating upon temperature upshift (Bates and Oliver, 2004; Mizunoe *et al.*, 2000; Wong *et al.*, 2004b). However, the number of cells recovered decreased with extended low temperature storage, suggesting that the ability of VBNC *V. parahaemolyticus* to resuscitate decreases with time (at least under laboratory conditions).

Cells in the VBNC state are often more resistant to stress. VBNC *V. parahaemolyticus* were found to be more resistant to heat (42 or 47ºC), low salinity and acidic conditions compared with exponentially growing cells (Wong and Wang, 2004).

Thus the available evidence shows that *V. parahaemolyticus* can enter the VBNC state, but investigations are needed to determine whether this phenomenon occurs in marine environments, and whether VBNC *V. parahaemolyticus* are present in BMS growing under normal environmental conditions. It is also important to establish whether *V. parahaemolyticus* in the VBNC state remain virulent. Laboratory studies using mice suggest that VBNC *V. parahaemolyticus* can resuscitate and cause illness *in vivo* (Wong *et al.*, 2004b).

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

Filter feeders such as BMS have the ability to concentrate *Vibrio* spp. from the water and this may protect the pathogens from adverse environmental conditions (Desmarchelier, 2003). During summer months, shellfish often have *V. parahaemolyticus* concentrations two hundred times greater, on average, than those found in the corresponding seawater (DePaola *et al.*, 1990). These concentrations are primarily a result of bioaccumulation, but multiplication within the shellfish can also occur (FAO/WHO, 2011).

Most scientists have focused their attention on understanding the relationship between oysters and *V. parahaemolyticus*, since most foodborne *Vibrio* illnesses are linked to raw oyster consumption (see Section 3). Since *V. parahaemolyticus* have also been isolated from non-oyster BMS species (Appendix A.6) it is assumed that all BMS species have the capacity to accumulate *V. parahaemolyticus* from the aquatic environment. Studies using blue mussels (*Mytilus galloprovincialis*) confirmed that this species actively accumulates *V. parahaemolyticus* from seawater (Croci *et al.*, 2002; Hernroth *et al.*, 2010).

Once ingested by filter feeding, *V. parahaemolyticus* are found in the gills, digestive glands (including stomach, digestive ducts and digestive diverticula), adductor muscle and mantle cilia (Wang *et al.*, 2010a).

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.

Little is known about any differences in uptake of pathogenic *V. parahaemolyticus* strains by BMS compared with environmental strains. *In vivo* laboratory exposure of oysters to a mixture of clinical and environmental *V. parahaemolyticus* isolates showed that both can be incorporated into oysters with some suggestion that the clinical isolate was ingested more readily than the environmental isolate, or that it survived better in oyster tissues (Volety *et al.*, 2001). Surveys of BMS show that the prevalence and concentration of pathogenic *V. parahaemolyticus* (*tdh*+ and/or *trh*+) is usually much lower than total *V. parahaemolyticus* in shellfish growing waters and BMS at the time of harvest (Section 2.6, Appendix A.6).

Microorganisms that persist in oysters must overcome an antimicrobial defence system that consists of cellular and humoral factors that can act internally and externally (Chu, 1988; Feng, 1988). The defensive blood cells (haemocytes) can move from haemolymph sinuses across epithelial barriers into tissues, digestive tract or the pallial cavity (Fisher, 1986). Although little is known of their *in vivo* bactericidal ability, oyster haemocytes are known to kill several species of bacteria, including *V. parahaemolyticus* and *V. vulnificus* (Genthner *et al.*, 1999; Harris-Young *et al.*, 1993). There also appear to be seasonal differences in the oyster cellular defence system, with the bactericidal activity of haemocytes being greater in the summer than in winter (Genthner *et al.*, 1999). One study found that an isolate of *V. parahaemolyticus* survived well in the presence of haemocytes from clams and multiplied in the presence of mussel haemocytes, but this *V. parahaemolyticus* isolate was pathogenic to mussels and other isolates were not tested (Hernroth *et al.*, 2010).

The shellfish species and physiology (e.g. sexual maturity, immune function, metabolic state) can also affect survival and growth of *V. parahaemolyticus* within shellfish (Fisher and DiNuzzo, 1991; Volety *et al.*, 1999). In addition, oysters have been reported to harbour bacteriophages against *V. parahaemolyticus*, and there are vibrio predatory bacteria that have the capacity to control *V. parahaemolyticus* in both seawater and in shellfish (Comeau *et al.*, 2005; Richards *et al.*, 2012).

The number of *V. parahaemolyticus* accumulated in oysters depends on both the general environmental conditions and on the health of the oyster itself. The concentration of *V. parahaemolyticus* 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. parahaemolyticus* populations and the feeding behaviour of oysters (Kaneko and Colwell, 1977; Venkateswaran *et al.*, 1990). Increased concentrations of *V. parahaemolyticus* have also been measured in oysters experiencing one or more causes of stress, e.g. heat (Aagesen and Hase, 2014).

V. parahaemolyticus will grow in oysters when they are out of the water if the temperature is suitable. Summer conditions permit *V. parahaemolyticus* multiplication in BMS exposed by the receding tide as the temperatures of the exposed shellfish can be up to 10ºC above the air temperature. Studies of oysters growing in the intertidal zone found that the concentration of total and pathogenic (*tdh*+, *trh*+) *V. parahaemolyticus* increased when oysters were exposed on the sunny mudflats by a receding tide, then decreased when the tidal waters covered the shellfish and filter-feeding recommenced (Jones *et al.*, 2016). Another study measured *V. parahaemolyticus* concentrations 4-8 times higher at maximum intertidal exposure than at the beginning (Nordstrom *et al.*, 2004).

It is clear that *V. parahaemolyticus* are depurated by BMS, but the length of time any *V. parahaemolyticus* cell remains inside an individual shellfish still residing in its growing area is not well defined, and is probably difficult to predict. *V. parahaemolyticus* have been shown to depurate from mussels (*M. galloprovincialis*) more slowly than *E. coli* (Croci *et al.*, 2002). The pili and flagellar systems of *V. parahaemolyticus* were found to contribute to bacterial persistence in naturally depurating Pacific oysters (*C. gigas*) (Aagesen *et al.*, 2013).

Laboratory experiments found the *V. parahaemolyticus* were retained better in the gills and digestive glands of oysters undergoing depuration, compared with the adductor muscle and mantle cilia (Wang *et al.*, 2010a).

The 2003 Risk Profile cited a New Zealand study that found in 10/11 trials, the concentration of naturally-present *V. parahaemolyticus* decreased by 72% (standard deviation 23%) in oysters depurated for 36 hours (Fletcher, 1985). An increase in *V. parahaemolyticus* in one trial was attributed to the tank water being at 24ºC. Also described was a study in Italy, that found depuration of mussels for 44 hours reduced the number of *V. parahaemolyticus* present by only 85.1%, i.e. <1 $\log_{10}$ reduction (Croci *et al.*, 2002). Another Italian study found depuration effectively removed *V. parahaemolyticus* from mussels but not clams (Barile *et al.*, 2009). The effectiveness of depuration of oysters in chilled (5ºC) water was found to differ depending on the season the oysters were harvested, with *V. parahaemolyticus* being retained longer by summer-harvested oysters (Su *et al.*, 2010). Variable results have been reported elsewhere (Eyles and Davey, 1984; Son and Fleet, 1980). A consistent finding is that depuration does not reliably eliminate *V. parahaemolyticus* from BMS.

2.4 BEHAVIOUR OF *V. PARAHAEMOLYTICUS* IN BMS

KEY FINDINGS

Laboratory studies show that *V. parahaemolyticus* will multiply in BMS stored at 15ºC or above, and modelling studies predict that *V. parahaemolyticus* may grow in BMS stored at 12ºC. At ≥20ºC, growth to stationary phase occurs within 1-2 days.

V. parahaemolyticus will not grow in BMS stored at 10ºC or lower. In unfrozen BMS, the concentration of *V. parahaemolyticus* remains stable or decreases at these cool temperatures, but survival for up to three weeks has been reported. *V. parahaemolyticus* dies under frozen storage but can survive for up to six months.

V. parahaemolyticus in BMS are rapidly killed by cooking.

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

The 2003 Risk Profile provided a small amount of data that indicated *V. parahaemolyticus* in oysters decreased in numbers when the oysters were stored under refrigeration but rapidly grew inside oysters kept in warm conditions. The document concluded that *V. parahaemolyticus* would not grow in seafood stored at refrigeration temperatures and some decline in numbers will occur. The document also cautioned that the time between harvesting and chilling might permit growth, and cited a 1985 report of oysters harvested in New Zealand that were kept at ambient temperatures for 20-21 hours before any refrigeration was attempted (Fletcher, 1985). An increase in *V. parahaemolyticus* was measured between harvests and cooling.

This section provides an updated evaluation of behaviour data.

V. parahaemolyticus have been detected in BMS sampled at retail, demonstrating that these bacteria are retained in harvested shellfish. The presence of these bacteria in shellfish does not alter the appearance, taste or odour of the shellfish.

TABLE 2 summarises studies of *V. parahaemolyticus* survival in harvested BMS under various temperatures. Together, these data show that:

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

- *V. parahaemolyticus* will multiply in BMS at 15ºC, increasing by approximately 2 $\log_{10}$ over two days of storage. No data were located for temperatures in the range 11-14ºC.
- *V. parahaemolyticus* will not grow in BMS stored unfrozen at 10ºC or lower. The concentration has been observed to remain stable or decrease at these cool temperatures. Survival for up to three weeks has been reported.
- *V. parahaemolyticus* dies under frozen storage but can survive for up to six months. The data suggests that death is more rapid at -10 or -18ºC compared with -30ºC. This has been attributed to the formation of larger intracellular ice crystals at the higher temperatures, causing greater cell damage (Shen *et al.*, 2009).

These findings have been supported by two New Zealand studies.[12]

The aim of the first study was to validate an Australian growth model that predicted *V. parahaemolyticus* growth at >15ºC in oysters during storage based on experiments where the oysters were injected with *V. parahaemolyticus* following harvest.[13] Using naturally contaminated Pacific oysters harvested from New Zealand waters, no *V. parahaemolyticus* growth was observed after 12 days at 10 or 15ºC in 2/3 trials (Cruz *et al.*, 2015a).[14] Slow growth rates of 0.0052 and 0.0048 $\log_{10}$ MPN/g were measured at 10 and 15ºC, respectively, in one trial. The concentration of *V. parahaemolyticus* remained fairly static or decreased in oysters held at 5ºC, and growth was measured at 20, 25 and 30ºC. A model produced from the data predicted a minimal temperature for growth of 12.06ºC. The minimal temperature for growth predicted by the Australian model was 13.37°C.

A second study has evaluated the behaviour of *V. parahaemolyticus* in Pacific oysters (naturally contaminated) after flash freezing followed by frozen storage. The aim of this study was to compare inactivation of *V. parahaemolyticus* with that observed in the USA study of Liu *et al*. (2009), using the end-point of a 3.52 $\log_{10}$ MPN/g reduction in 30 samples (see TABLE 2). The results of the New Zealand study were very similar to the USA study.

These studies highlight the importance of temperature control in preventing growth of *V. parahaemolyticus* in shellfish. The time taken for BMS to cool once under refrigeration depends on the efficiency of the cooling system, the quantity of the shellfish to be cooled and their arrangement in the cool room (FAO/WHO, 2011). The available data shows that *V. parahaemolyticus* will continue to grow in oysters after they are placed in refrigeration until the temperature of the oyster flesh falls below 10ºC (European Commission, 2001; FAO/WHO, 2011). Modelling indicates that *V. parahaemolyticus* may grow in BMS at 12ºC. While the concentration of *V. parahaemolyticus* can decrease under cold or frozen storage, these conditions cannot be relied on to eliminate this bacterial species in BMS.

There has been some research examining behavioural differences between total and pathogenic *V. parahaemolyticus* in harvested BMS. A USA study measured an increase in the concentration of naturally present *V. parahaemolyticus* in oysters exposed to ambient air temperatures (28-32ºC) for 5 or 24 hours, but pathogenic (*tdh*+ or *trh*+) *V. parahaemolyticus* concentrations were not significantly different after 5 h storage (Kinsey *et al.*, 2015). However, after 24 h storage, the mean concentration of *tdh*+ *V. parahaemolyticus* increased 2.2 $\log_{10}$ MPN/g (from 0.0 to 2.2 $\log_{10}$ MPN/g) and the mean concentration of *trh*+ *V. parahaemolyticus* increased 2.7 $\log_{10}$ MPN/g.

[12] Work carried out under the Seafood Safety Programme by Plant & Food Research. Preliminary results provided by G. Fletcher, Plant & Food Research, with permission. The data had not been fully analysed and reported.
[13] http://www.explorerisk.com/FSVibrio/Default.aspx (accessed 11 October 2016).
[14] *V. parahaemolyticus* concentrations were measured by qPCR MPN.

TABLE 2: Change in the concentration of *V. parahaemolyticus* 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 ≥20ºC					
Oyster *C. virginica*	University of Delaware, USA	Accumulated in inoculated seawater (initial count 7.8 log_{10} MPN/g)	35ºC or 21ºC, 5 h, air	NC	(Ye *et al.*, 2013)
Oyster *C. virginica*	Alabama waters, USA	Naturally present (~3 log_{10} MPN/g)	Ambient air temperature (28-32ºC), 5 or 24 h	↑ 1.2 (mean at 5 h)[2] ↑ 2.2 (mean at 24 h)[2]	(Kinsey *et al.*, 2015)
Oyster *C. virginica*	Gulf Coast USA	Naturally present	30 or 22ºC, 1 d	↑ 1-2	(Cook and Ruple, 1989)
Blue mussel *M. chilensis*	Inland sea, Chile	Naturally present (up to 3.66 log_{10} MPN/g)	28ºC, 18 h	NC in 2/8 samples ↑ 0.9-2.9 in 6/8 samples[2]	(Aranda *et al.*, 2015)
Oyster *C. virginica*	Gulf Coast waters, USA	Naturally present (sampling means 2.5-2.9 log_{10} CFU/g)	26°C, 24 h	↑ approx. 1	(Kaufman *et al.*, 2003)
Oyster *C. virginica*	Alabama waters, USA	Naturally present (1-3 log_{10} CFU/g)	26ºC, 5 h 26ºC, 10 h 26ºC, 24 h	↑ 0.8 ↑ 1.7 ↑ 2.9	(Gooch *et al.*, 2002)
Oyster *C. virginica*	Maryland and Alabama waters, USA	Naturally present (~2 log_{10} CFU/g)	30ºC, 7 d 25ºC, 7 d 20ºC, 14 d	↑ ~3-4 at all temperatures (most growth during first 2 d)[3]	(Parveen *et al.*, 2013)
Oyster *C. commercialis*	New South Wales waters, Australia	Accumulated in inoculated seawater (initial count 65 CFU/g)	20-25ºC, 4 d	↑ 1.3	(Son and Fleet, 1980)
Oyster *C. virginica*	Maryland waters, USA	Naturally present (3.5 log_{10} CFU/g)	20ºC, 10 d	↑ ~1 after 1 d ↑ ~4 after 10 d	(Mudoh *et al.*, 2014)
Storage 11-19ºC					
Oyster *C. virginica*	Maryland and Alabama waters, USA	Naturally present (~2 log_{10} CFU/g)	15ºC, 14 d	↑ ~2.5 (most growth during first 2 d)[3]	(Parveen *et al.*, 2013)

SHELLFISH TESTED	SHELLFISH SOURCE	INOCULUM	CONDITIONS	CHANGE IN CONCENTRATION (log_{10} MPN/g or log_{10} CFU/g)[1]	REFERENCE
Oyster *C. plicatula*	Zhejiang Province, China	Accumulated in inoculated seawater (4-5 log_{10} MPN/g)	15ºC, 60 h	In-shell: ↑ 2.4 Shucked: ↑ 1.6	(Shen *et al.*, 2009)
Cool storage (≤10ºC, unfrozen)					
Oyster *C. virginica*	Gulf Coast USA	Naturally present	10ºC, 1 d	NC	(Cook and Ruple, 1989)
Oyster *C. virginica*	Maryland waters, USA	Naturally present (3.5 log_{10} CFU/g)	10ºC, 10 d 5ºC, 10 d	NC NC	(Mudoh *et al.*, 2014)
Oyster *C. virginica*	Maryland and Alabama waters, USA	Naturally present (~2 log_{10} CFU/g)	10ºC, 21 d 5ºC, 21 d	↓ to non-detectable levels at both temperatures[3]	(Parveen *et al.*, 2013)
Oyster *C. virginica*	University of Delaware, USA	Accumulated in inoculated seawater (initial count 7.8 log_{10} MPN/g)	10ºC, 1 d, seawater 4ºC, 1-2 d, seawater	↓ 1.6 ↓ 1.5-1.7	(Ye *et al.*, 2013)
Oyster *C. plicatula*	Zhejiang Province, China	Accumulated in inoculated seawater (4-5 log_{10} MPN/g)	5ºC, 96 h 0ºC, 96 h	In-shell: ↓ 1.4 Shucked: ↓ 2.0 In-shell: ↓ 2.1 Shucked: ↓ 2.4	(Shen *et al.*, 2009)
Oyster *C. virginica*	Louisiana waters, USA	Accumulated in inoculated seawater (10^4 MPN/g)	3-5ºC, 21 d	↓ 1.6	(Andrews *et al.*, 2003a)
Oyster *C. virginica*	Alabama waters, USA	Naturally present (1-3 log_{10} CFU/g)	3ºC, 14-17 d	↓ 0.8 log CFU/g	(Gooch *et al.*, 2002)
Oyster *C. virginica*	University of Delaware, USA	Accumulated in inoculated seawater, held 21ºC/5 h (initial count 7.0 or 6.8 log_{10} MPN/g)	Ice slurry, 15 d	In-shell: ↓ 2.6 Shucked: ↓ 3.1	(Ye *et al.*, 2013)

SHELLFISH TESTED	SHELLFISH SOURCE	INOCULUM	CONDITIONS	CHANGE IN CONCENTRATION (log_{10} MPN/g or log_{10} CFU/g)[1]	REFERENCE
Frozen storage					
Oyster *C. gigas*	Washington State waters, USA	Accumulated in inoculated seawater ($3x10^5$ MPN/g)	Ultra flash frozen: -95.5ºC, 12 min then stored at: -10ºC -20ºC -30ºC	 NC (↓ 0.22) 1 month: ↓ 2.45 6 months: ↓ 4.55 1 month: ↓ 1.71 6 months: ↓ 4.13 1 month: ↓ 1.45 6 months: ↓ 2.53	(Liu *et al.*, 2009)
Oyster *C. virginica*	University of Delaware, USA	Accumulated in inoculated seawater, held 21ºC/5 h (initial count 7.0 or 6.8 log_{10} MPN/g)	-18ºC, 15 d	In-shell: ↓ 3.4 Shucked: ↓ 3.3	(Ye *et al.*, 2013)
Oyster *C. plicatula*	Zhejiang Province, China	Accumulated in inoculated seawater (5.5 log_{10} MPN/g)	In-shell: -18ºC, 75 d Shucked: -18ºC, 60 d In-shell: -30ºC, 75 d Shucked: -30ºC, 75 d	↓ 5.1 ↓ ND (<3 MPN/g) ↓ 3.8 MPN/g ↓ 5.1 MPN/g	(Shen *et al.*, 2009)

[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] Using PCR targeting the *tlh* gene as a measure of concentration.

[3] The estimated average growth rates at 15, 20, 25, and 30°C were 0.038, 0.082, 0.228 and 0.219 log_{10} CFU/h, respectively. The average rates at 5 and 10ºC were −0.002 and −0.001 log_{10} CFU/h, respectively.

2.4.1 The effect of shellfish preparation

V. parahaemolyticus are sensitive to low pH (e.g. pH ≤4.2 (Yeung and Boor, 2004)). Studies have shown that acidic marinades or sauces may inhibit *V. vulnificus* survival in or on oysters (Borazjani *et al.*, 2003; Sun and Oliver, 1995). It is not certain whether *V. parahaemolyticus* are similarly affected but there are studies that suggest they are. *V. parahaemolyticus* inoculated into tomato sauce adjusted to pH 4.4 were not detected after incubation for 24 h at 10 or 22°C, or 4 h at 35°C (Beuchat, 1976). However, only a slight reduction in viable cell count was measured in tomato sauce adjusted to pH 5.6-6.5. *V. parahaemolyticus* inoculated on to raw fish was inactivated by lime juice (Mathurand and Schaffner, 2013).

Vibrios are readily destroyed by cooking even when the oysters are highly contaminated (Codex Alimentarius, 2010). The commercial practice of heat shocking oysters in boiling water (three minutes) to facilitate opening also reduced counts of *V. parahaemolyticus* to "undetectable" levels (Hackney *et al.*, 1980). D times of less than one minute are reported for *V. parahaemolyticus* in homogenates of clams or crabs at temperatures above 50°C, and cooking to an internal temperature of 65ºC is claimed to effectively inactivate this organism (ICMSF, 1996). However, such a temperature can affect the quality of oyster meat, so a low temperature pasteurisation of 10 minutes at 50ºC is a more favoured method for eliminating *V. parahaemolyticus* and *V. vulnificus* from shellstock oysters (Andrews *et al.*, 2000).

2.5 EXPOSURE ASSESSMENT

KEY FINDINGS

New Zealand surveys show that the prevalence and concentration of *V. parahaemolyticus* are higher in commercial BMS harvested from harbours in the north half of the North Island compared with the Marlborough Sounds. Most samples were of Pacific oysters, and *V. parahaemolyticus* were detected in Pacific oysters more often and at higher concentrations during summer months compared with other seasons, when sea surface temperatures were ≥19ºC. There was no significant correlation with water salinity (*V. parahaemolyticus* were isolated from Pacific oysters at salinities >35‰).

Up to 100% of Pacific oyster samples from harbours located in the upper half of the North Island yielded *V. parahaemolyticus*, at concentrations as high as 4.8×10^4 MPN/g. Potentially pathogenic *V. parahaemolyticus* were also detected in these samples but at lower prevalences (up to 27%) and concentrations (maximum 933 MPN *trh*+ *V. parahaemolyticus* cells per gram). *V. parahaemolyticus* were also detected in 42% (16/38) of samples of green-lipped mussels from northern North Island harbours in a survey from 2009-2012.

From three surveys of Pacific oysters from the Marlborough Sounds, the highest *V. parahaemolyticus* prevalence measured was 30%. The highest concentration of *V. parahaemolyticus* measured was 7.4 MPN/g. *V. parahaemolyticus* were also detected in samples of dredge oysters (1/21, 0.36 MPN/g) and green-lipped mussels (2/17) from the Marlborough Sounds. The *tdh* and *trh* genes were not detected in any samples from the Marlborough Sounds.

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. parahaemolyticus* 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. parahaemolyticus 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

At the time of the 2003 Risk Profile, there were few data on *V. parahaemolyticus* prevalence and concentration in shellfish harvested from New Zealand waters. The most relevant survey (Fletcher, 1985) detected *V. parahaemolyticus* in 85/149 (57%) Pacific oysters collected from four oyster processors located in the north of the North Island over three summers. The concentration of *V. parahaemolyticus* in oysters generally increased with increasing water temperature. The concentration of *V. parahaemolyticus* was >10 MPN/g in seven samples.

Since the 2003 Risk Profile, there have been four studies of *V. parahaemolyticus* in BMS freshly harvested from New Zealand waters.[15] 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 follows the table. *V. parahaemolyticus* were detected less often and at lower concentrations in Pacific oysters sampled from the Marlborough region (TABLE 3). *V. parahaemolyticus* were also detected in samples of dredge oysters from the South Island (1/21, 0.36 MPN/g) and green-lipped mussels from the North and South Islands (18/55, maximum 95.4 MPN/g). There are no surveys for *Vibrio* spp. in BMS sampled at New Zealand retail or food service outlets.

While it appears that the prevalence of potentially pathogenic *V. parahaemolyticus* has increased over time, the methods used for detecting these differed between the 2008-2012 studies and the more recent studies.

Only two *V. parahaemolyticus* isolates from these surveys were serotyped (Kirs *et al.*, 2010). They were tested against O and K antisera but were not the O3:K6 pandemic clone.

A study spanning three summers (2013-2015) and harbours of the upper North Island and Marlborough Sounds, did not identify a significant relationship between the concentration of *V. parahaemolyticus* in oysters and the water depth (intertidal/subtidal, at different depths) or growing method (except for one harbour, where higher *V. parahaemolyticus* numbers were detected in shellfish growing closer to the sea floor). The sea surface temperature was found to be more important.

[15] The laboratory methods and microbiological media used for these studies were similar but had varied detection limits (see table).

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

SAMPLING PERIOD	LOCATION OF HARBOUR(S) SAMPLED	LIMIT OF DETECTION (MPN/g)	*V. PARAHAEMOLYTICUS* PREVALENCE	MAXIMUM CONCENTRATION OF *V. PARAHAEMOLYTICUS* MEASURED	PREVALENCE OF POTENTIALLY PATHOGENIC *V. PARAHAEMOLYTICUS*
2008-2009	Upper half North Island	3	55/58 (95%)	$1.5x10^3$ MPN/g	2/58 (3%) *tdh*+
2009-2012	Upper half North Island	0.36	174/217 (80%)	$2.4x10^4$ MPN/g	3/174 (2%) *tdh*+
2009-2012	Marlborough Sounds	0.36 or 3	0/18	NA	NA
2013	Upper half North Island	0.31	48/48 (100%)	$2.4x10^3$ MPN/g	11/48 (23%) *trh*+
2014	Upper half North Island	0.31	91/91 (100%)	$2.4x10^4$ MPN/g	24/91 (26%) *trh*+ and/or *tdh*+
2015	Upper half North Island	0.31	21/22 (95%)	$2.4x10^4$ MPN/g	ND
2015	Marlborough Sounds	0.31	6/19 (32%)	7.4 MPN/g	ND
2016	Upper half North Island	0.36	30/30 (100%)	$4.8x10^4$ MPN/g	2/30 (7%) *trh*+ 8/30 (27%) *tdh*+
2016	Marlborough Sounds	0.36	3/10 (30%)	3.57 MPN/g	ND

NA, not applicable; ND, not detected.

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. parahaemolyticus were detected in 55/58 (94.8%) samples (95% Confidence Interval (CI) 85.6-98.9%) and positive samples were distributed relatively evenly across all farms. The arithmetic mean concentration of *V. parahaemolyticus* was 564 MPN/g (range <3-1,500 MPN/g), and five samples had concentrations exceeding 1,000 MPN/g. This is higher than the concentrations reported by Fletcher (1985) but this may be an artefact from different methodology. There was no significant difference between concentrations of *V. parahaemolyticus* in oysters collected while submerged or emerged during the tidal cycle. *V. vulnificus* was also detected during this survey but there was no significant correlation between the concentrations of these two *Vibrio* species.

V. parahaemolyticus containing *tdh* were isolated from two consecutive Pacific oyster samples collected during February at one location (*trh* was not detected in any sample). The proportion of samples containing potentially pathogenic *V. parahaemolyticus* was 3.4% (2/58). The presumptive concentrations of *tdh+ V. parahaemolyticus* in the two samples were ≤15 and 460 MPN/g.

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). While the concentrations of *V. parahaemolyticus* were highest in late February/early March, there was no significant correlation with water temperature, salinity, or any of the other environmental parameters measured (turbidity, precipitation). There was also no significant correlation between concentrations of *V. parahaemolyticus* and growing conditions (production method, distance from the sea floor). The absence of correlation with environmental parameters may have been due to lack of variation in these parameters, or else the fact they were not measured continuously (they were only measured at the time of sample collection).

2009-2012 survey of Pacific oysters and green-lipped mussels (Cruz *et al.*, 2015b)

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.

The overall prevalence of *V. parahaemolyticus* in Pacific oysters was high compared with other shellfish types, but this observation is likely to be an artefact of sampling location since the majority of Pacific oysters were sampled from North Island sites where *V. parahaemolyticus* was more likely to be detected (TABLE 4). The prevalence of *V. parahaemolyticus* measured in this study were higher than earlier studies but the analytical methods differed so the results are not directly comparable.

TABLE 4: Detection of *V. parahaemolyticus* in BMS samples taken from New Zealand aquaculture farms 2009-2012 (data are from Cruz *et al.*, 2015)

SHELLFISH SAMPLED	PREVALENCE OF *V. PARAHAEMOLYTICUS*		CONCENTRATION OF *V. PARAHAEMOLYTICUS* IN POSITIVE SAMPLES (MPN/g)
	SAMPLE LOCATION	PREVALENCE (%)	
Pacific oysters	North Island	174/217 (80)	$0.36x10^4$-$2.4x10^4$
	South Island	0/18	
	Total	*174/235 (74)*	
Dredge oysters	South Island	1/21 (5)	0.36
Green-lipped mussels	North Island	16/38 (42)	0.36-95.4
	South Island	2/17 (12)	
	Total	*18/55 (33)*	
All samples	North Island	190/255 (75)	0.36-$2.4x10^4$
	South Island	3/56 (5)	
	Total	*193/311 (62)*	

The surface seawater temperatures and salinities in the North Island sites were 7.9-25.5ºC (mean 18.7ºC) and 8.5-40‰ (mean 33‰), respectively. In the South Island sites, these measurements were 10.7-20.5ºC (mean 15.0ºC) and 34-37‰ (mean 35‰), respectively. The concentration of *V. parahaemolyticus* in Pacific oysters increased when seawater temperatures were high. The concentration exceeded 1,000 MPN/g only when the seawater temperature exceeded 19ºC. The prevalence and concentration of *V. parahaemolyticus* measured in Pacific oysters sampled during the summer months were significantly higher than in winter months (*V. parahaemolyticus* were detected in 100% of Pacific oysters sampled during summer months). The authors' noted, however, that the concentration of *V. parahaemolyticus* in some of the samples harvested at warm temperatures (20ºC) was low, and the results as a whole indicated that temperature was not the only factor influencing the presence of *V. parahaemolyticus* in shellfish.

The concentration of *V. parahaemolyticus* in other shellfish species was not correlated with seawater temperature but a correlation might have been detected with a larger number of samples (the results for green-lipped mussels suggest a pattern may have become evident with more sampling).

The concentration of *V. parahaemolyticus* was not independently correlated with salinity for any of the shellfish, but increased salinity appeared to contribute to decreased concentration of *V. parahaemolyticus* in Pacific oysters when considered alongside temperature effects. However, samples with $>10^4$ MPN/g *V. parahaemolyticus* were obtained from waters with salinities of 33-35‰.

V. parahaemolyticus isolates from positive samples were tested for presence of the *tdh* and *trh* genes. Only three samples were positive for *tdh* (all were Pacific oysters from North Island sites, one isolated in November 2010 and two in March 2012), which indicates a low prevalence of strains potentially pathogenic to humans. One of these *tdh*+ samples was collected when the water temperature was 17.5ºC. The *trh* gene was not detected.

<u>2013-2015 oyster farming method experiments[16]</u>

During the summer of 2013, the concentration of *V. parahaemolyticus* 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. parahaemolyticus* were detected in 100% of

[16] Research supported through the MBIE-funded Safe New Zealand Seafood Programme.

samples (n=48), and the range of concentrations measured in the positive samples was 0.31-2.4x10^3 MPN/g. Similarly, when the experiment was repeated in a different Northland harbour during the 2014 summer, *V. parahaemolyticus* were detected in 100% of samples (n=91), with concentrations reported in the range of 4.2-2.4x10^4 MPN/g.

During 2013, *trh*+ *V. parahaemolyticus* were isolated from 11/48 of the oyster samples. The *tdh* gene was not detected. This differed from the 2014 survey, where 11/91 samples yielded *trh*+ *V. parahaemolyticus*, 9/91 samples yielded *tdh*+ *V. parahaemolyticus* and 4/91 samples yielded both types. The maximum concentration of potentially pathogenic *V. parahaemolyticus* measured was 933 MPN/g (a *trh*+ sample).

During the 2015 summer, *V. parahaemolyticus* were also measured in samples taken from commercial growing areas in the Coromandel and Marlborough regions, for comparative purposes. *V. parahaemolyticus* were detected in 21/22 (95%) samples from Coromandel (0.36-2.4x10^4 MPN/g) and 6/19 (32%) samples from Marlborough (0.36-7.40 MPN/g). The *trh* and *tdh* genes were not detected in any isolates from these samples.

Overall, *V. parahaemolyticus* were detected in samples from both subtidal and intertidal locations. While there appeared to be some relationship between the concentration of *V. parahaemolyticus* and water depth/growing method, the relationship was only significant in one harbour (higher *V. parahaemolyticus* numbers in shellfish growing closer to the sea floor) and it was acknowledged that further experiments were needed to confirm this finding. The sea surface temperature was more important.

The available data on surface seawater temperature (measured at each sampling) shows temperatures in all four harbours fluctuating between approximately 15 and 25ºC, but the authors report that the temperatures measured during 2015 in the Marlborough site were, on average, lower than those measured at the North Island sites during 2013-2015. There was a significantly positive correlation between *V. parahaemolyticus* concentration and surface sea temperature across all harbours surveyed. Most of the *V. parahaemolyticus*-positive samples and the highest concentrations of *V. parahaemolyticus* were measured when water temperatures were >20ºC, but concentrations >10 MPN/g were measured in samples taken from waters at 17ºC.

<u>2016 survey of Pacific oysters</u>

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. parahaemolyticus* were detected in 33 (83%). All of the seven negative samples were from Marlborough and the concentrations of *V. parahaemolyticus* in the three positive samples from this harbour were low (0.36-3.57 MPN/g) compared to the Northland harbours.

For the 30 Northland samples, the concentration of *V. parahaemolyticus* ranged from 3.57 to 4.8x10^4 MPN/g. The concentration exceeded 1x10^3 MPN/g in nine samples. The prevalence of *trh*+ *V. parahaemolyticus* was 2/30 and the concentrations measured were 42.4 and 73.6 *trh*+ *V. parahaemolyticus*/g. The prevalence of *tdh*+ *V. parahaemolyticus* was 8/30 and the concentrations measured ranged 0.36-4.24 *tdh*+ *V. parahaemolyticus*/g.

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

- *V. parahaemolyticus* were detected from oysters collected from waters of salinities >30‰ in all harbours (the sample containing 4.8x10^4 MPN/g came from waters at 35‰).
- *V. parahaemolyticus* were detected in samples up until the last sampling day, 7 June 2016, although concentrations above 1x10^3 MPN/g were not measured in any samples taken

after mid-April. Water temperature data show a diurnal pattern developing during April, but afternoon/evening temperatures were still ≥20ºC.

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

- For the maximum concentration detected in oysters from a Northland harbour ($4.8x10^4$ MPN/g): The estimated concentration per oyster is $1.5x10^6$ *V. parahaemolyticus*, assuming that the *V. parahaemolyticus* were evenly distributed amongst the 12 pooled oysters. If all the *V. parahaemolyticus* were present in only 1/12 oysters, the estimated concentration in this oyster would be $1.8x10^7$ *V. parahaemolyticus* cells.
- For the maximum concentration detected in oysters from the Marlborough harbour (3.57 MPN/g): The estimated concentration per oyster is 138 *V. parahaemolyticus*, assuming that the *V. parahaemolyticus* were evenly distributed amongst the 12 pooled oysters. If all the *V. parahaemolyticus* were present in only 1/12 oysters, the estimated concentration in this oyster would be $1.7x10^3$ *V. parahaemolyticus* cells.

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

Surface seawater temperature and salinity data gathered during the New Zealand BMS surveys are summarised 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. parahaemolyticus* growth (≥20ºC). These data also show that *V. parahaemolyticus* 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.

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, and 12ºC in southern New Zealand sites.[17] 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).

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.[18] Over the last decade, the La Niña phase has been present during the summers of 2008, 2009 and 2011.[19] The 2011 phase was particularly prolonged, spanning from mid-2010 to mid-2011.

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[17] 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).

[18] 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)

[19] 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.

Based on these data, temperature and salinity do not represent barriers to the occurrence of *V. parahaemolyticus* 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. parahaemolyticus* 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. parahaemolyticus* in BMS, particularly if northern sites are compared with Marlborough, where the prevalence and concentration of *V. parahaemolyticus* in BMS appear lower. However, studies from other countries show that using environmental indicators to predict the presence of *V. parahaemolyticus* 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.

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 5). 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.

TABLE 5: 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.

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.

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 Maori, is an important cultural and dietary component. A survey in the upper North Island found that 11% of households reported collecting seafood more than once a week, 31% collected seafood at least weekly, and 52% reported collecting seafood at least fortnightly (Hay *et al.*, 2000).

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 5), 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 5).

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 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 5). 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. parahaemolyticus* along the food chain

The 2003 Risk Profile concluded that *V. parahaemolyticus* can grow very quickly and reach high numbers in a short period given significant temperature abuse, but under mild temperature abuse numbers in shellfish should decline during storage. This is partially incorrect since data presented in Section 2.4 show that *V. parahaemolyticus* will grow in BMS held at 15ºC, which may be considered a mildly abusive temperature. The concentration of *V. parahaemolyticus* decreases in BMS at 10ºC or below.

Growth of *V. parahaemolyticus* 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 but >10ºC), *V. parahaemolyticus* are able to grow in BMS. The extent of growth depends on the time spent at suitable temperatures. Suitable growth conditions may occur during the holding period between harvest and transport/processing.

Once refrigeration is achieved, growth of *V. parahaemolyticus* will cease. New Zealand data on refrigeration conditions for BMS 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. parahaemolyticus* 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, although data in Section 2.4 suggest that the rate of reduction can vary widely. There is potential for *V. parahaemolyticus* 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. PARAHAEMOLYTICUS* IN BMS FROM OTHER COUNTRIES

KEY FINDINGS

V. parahaemolyticus have been detected in BMS (oysters, mussels and clams) harvested from waters around the world at prevalences ranging from not detected to 100%. The available data suggest that the concentration in BMS can be as high as 10^5 cells/g.

Potentially pathogenic (*tdh*+ and/or *trh*+) *V. parahaemolyticus* have also been detected in BMS harvested from waters around the world, and also at variable prevalences (>50% in some studies). The few available data suggest that the concentration of potentially pathogenic *V. parahaemolyticus* in BMS rarely exceeds 100 cells/g, but a concentration of 10^2 cells/g has been reported. The prevalence or concentration of potentially pathogenic *V. parahaemolyticus* does not appear to be correlated with the prevalence or concentration of total *V. parahaemolyticus* and is usually lower.

The prevalence and/or concentration of *V. parahaemolyticus* in BMS was positively correlated with increasing water temperature in most studies. Correlation with water salinity was inconsistent between studies.

These findings are similar to findings from New Zealand studies.

Appendix A.6 summarises data from surveys of BMS in other countries.

3. EVALUATION OF ADVERSE HEALTH EFFECTS

3.1 DISEASE CHARACTERISTICS

KEY FINDINGS

Foodborne exposure to *V. parahaemolyticus* can lead to gastroenteritis. The disease is usually self-limiting but hospitalisation may be necessary. Septicaemia can develop in people with immunocompromising health conditions, which results in death in an estimated 20% of cases. Antibiotic resistance has been reported.

While the whole population is considered to be susceptible to *V. parahaemolyticus* infection, pre-existing medical conditions may make some people more susceptible. Case control studies have found that *V. parahaemolyticus* infection was six times more likely in people having a pre-existing chronic disease (Odds Ratio (OR) 6.0, 95% Confidence Interval (CI) 1.5-23.7), and eight times more likely in people who had taken antibiotics during the four weeks prior to illness (OR 8.1, 95% CI 1.2-56.4) (Liao *et al.*, 2015; Yan *et al.*, 2015).

The most common clinical syndrome caused by *V. parahaemolyticus* infection via food is gastroenteritis. Common symptoms include vomiting, nausea, abdominal pain, and watery (sometimes bloody) diarrhoea (Odeyemi, 2016). The incubation period is short (4-96 hours).

Infection can lead to hospitalisation and treatment with antibiotics, but the disease is usually self-limiting. The long-term effect of reactive arthritis has been reported (Tamura *et al.*, 1993). Mortality rates for the USA, where *V. parahaemolyticus* infection is a nationally notifiable disease, are 1-2% per year (this includes cases with wound infections).

V. parahaemolyticus infection may lead to septicaemia, a severe, life-threatening disease caused by the multiplication of pathogenic microorganisms and/or the presence of their toxins in circulating blood. Illness is more likely to progress to septicaemia in people with underlying immunocompromising chronic disease, and the probability of this occurring has been estimated as 0.025 (or 25 in every 1,000 people in this subpopulation) (USFDA, 2005b). The probability of illness progressing to septicaemia in the healthy subpopulation has been estimated as 0.00063 (<1/1000 cases). An estimated 20% of people with septicaemia caused by *V. parahaemolyticus* infection die (USFDA, 2005b).

A review of antibiotic resistance across 12 countries found reports of antibiotic resistance amongst *V. parahaemolyticus* isolates (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 countries.

3.2 DOSE-RESPONSE

KEY FINDINGS

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

Appendix B.1 contains further details on *V. parahaemolyticus* dose-response studies.

3.3 NEW ZEALAND HUMAN HEALTH SURVEILLANCE

KEY FINDINGS

V. parahaemolyticus 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. parahaemolyticus* infection will be underreported.

Between January 1998 and July 2016 there were eight sporadic cases of *V. parahaemolyticus* infection reported, where BMS were specifically implicated as the vehicle of infection and where the available information suggested that these shellfish were harvested in New Zealand. The implicated BMS were oysters or mussels, commercially or non-commercially harvested.

BMS harvested from New Zealand waters were not implicated in any reported outbreaks of *V. parahaemolyticus* infection during the same period.

Sporadic cases of *V. parahaemolyticus* infection are reported each year. From January 1998 to July 2016 there were 53 cases, including eight cases where the implicated vehicle of illness was seafood imported from a Pacific island. During the same period there were eight reported outbreaks of *V. parahaemolyticus* infection, involving a total of 44 cases. The most likely vehicle of infection for seven of these outbreaks was seafood imported from Pacific Islands.

V. parahaemolyticus 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).[20] However, cases of *V. parahaemolyticus* 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 requiring hospitalisation, or who 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, 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. Confirmed isolates are routinely tested for the presence of the *tdh* or *trh* genes.

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

From January 1998 to July 2016, there were 46 sporadic, confirmed cases of *V. parahaemolyticus* infection reported to EpiSurv. For 31 of these cases, the likely transmission route was foodborne and the infection was probably domestically acquired (overseas travel during the incubation period was not reported; see Section 3.3.2). Of these 31 cases, there

[20] 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).

were eight cases where BMS were specifically implicated and where the available information suggested that these shellfish were harvested in New Zealand (TABLE 6). The source of infection was not confirmed in any of these cases.

TABLE 6: Eight cases of *V. parahaemolyticus* infection reported in EpiSurv (January 1998-July 2016) where BMS (most likely harvested from New Zealand) was the implicated vehicle of illness

BMS SPECIES IMPLICATED	SOURCE	STATUS AT CONSUMPTION	YEAR REPORTED
Oysters	Commercial (supermarket)	Raw	2015
Oysters (Bluff)	Commercial (restaurant)	Raw	2013
Oysters	Non-commercial (by-product of mussel farming)	Raw	2014
Mussels	Recreational gathering	Raw	2001
Mussels	Recreational gathering	Raw	2006
Mussels	NR* (consumed at a hui)	NR	1998
Mussels	NR	NR	2016
Mussels	NR	NR	2006

* NR, not reported.

BMS harvested from New Zealand have not been implicated in any outbreaks of *V. parahaemolyticus* infection reported to EpiSurv during the period January 1998 to July 2016 (Section 3.3.2). As reported in the 2003 Risk Profile, an outbreak of *V. parahaemolyticus* infection was reported in 1983 where the implicated food was recreationally harvested mussels in Southeast Auckland (Thornton *et al.*, 2002).

3.3.2 *V. parahaemolyticus* infection in New Zealand

The 2003 Risk Profile reported data from EpiSurv for the period 1998 to 2002.

From January 1998 to July 2016, there were 53 cases of infection with *Vibrio* spp. reported to EpiSurv, and all but one of these cases was infected with *V. parahaemolyticus* (one case was infected with *V. mimicus*). There were between zero and four cases per year, except for 1999 (5 cases) and 2000 (16 cases).[21] Of the 52 cases of *V. parahaemolyticus* infection:

- 45 were confirmed cases and 6 were probable cases (1 unknown);
- 28 cases were female and 22 cases were male (2 unknown);
- The case ages ranged from 11 to 82 (mean 44, median 41); and
- 14 were reported as hospitalised, no deaths were reported.[22]

No source of infection was confirmed for any of the 52 cases, either epidemiologically or by laboratory testing. The reason for the higher number of cases reported during 2000 is not clear. Thirteen of these 16 cases resided in the Auckland or Counties Manukau regions, only one reported overseas travel during the incubation period and five reported consumption of seafood imported from a Pacific island. The La Niña phase was strong during the summer of 2000, but also during 1999 and 2001.[23]

[21] The incidence, for years where cases were reported, was 0.07-0.08 cases per 100,000 resident population. No cases were reported in 2004, 2007, 2008 or 2012. Calculated based on population statistics (mean resident year ending December) from Statistics New Zealand Infoshare, accessed 21 September 2016.
[22] Hospitalisation rate was 14/49 (29%).
[23] http://www.bom.gov.au/climate/current/soihtm1.shtml (accessed 31 August 2016).

Ten cases reported overseas travel during the incubation period for *V. parahaemolyticus*. The cause of illness in all ten cases was probably foodborne; nine reported consumption of seafood of whom one specifically reported consumption of BMS (raw oysters, plus crab and sea cucumber).

There were 37 confirmed cases of *V. parahaemolyticus* infection that were not linked to an outbreak and did not report overseas travel during the incubation period. The cause of infection was not confirmed for any of these cases but food was considered the probable cause for 31 cases. None reported recreational contact with seawater. Of these 31 cases, there were eight cases where BMS were specifically implicated and where the available information suggested that these shellfish were harvested in New Zealand (see Section 3.3.1), and there were another eight cases where the implicated vehicle of illness was seafood imported from a Pacific island.

Data from the ESR Enteric Reference Laboratory show that most *V. parahaemolyticus* isolates received by that laboratory contained the *tdh* and/or *trh* genes. Data were available for the period 7 February 2012 to 23 May 2016. Of 25 isolates tested, both genes were detected in 12 isolates, 8 isolates were *tdh*+/*trh*-, 2 isolates were *tdh*-/*trh*+, and neither gene was detected in the remaining 3 isolates.

The Ministry of Health collects data on hospital discharges, and during the period 1998 to 2015 there were 17 cases reported against the code 'A05.3 Foodborne *Vibrio parahaemolyticus* intoxication' (E. Lewis, Ministry of Health, pers. comm.) (TABLE 7). This was slightly higher than the 14 hospitalised cases reported to EpiSurv. Foodborne *Vibrio parahaemolyticus* intoxication was the primary diagnosis for 9/17 cases. These data show that cases of foodborne *V. parahaemolyticus* infection, where symptoms from the infection and any other medical conditions are severe enough to require hospitalisation, occur almost yearly. Hospitalisation has been reported for males and females of a wide age range (12-76 years) but Pacific Island peoples are over-represented. One explanation is consumption of privately-imported seafood by Pacific Island peoples, which was identified in the 2003 Risk Profile as a practice that increased risk of *V. parahaemolyticus* infection in New Zealand, and is supported by the EpiSurv data presented above.

A New Zealand case of fatal necrotising fasciitis due to *V. parahaemolyticus* has been reported in the scientific literature, but this was attributed to exposure of a skin wound to seawater (Pacific Ocean, northern New Zealand) and there was no association with food (Payinda, 2008).

TABLE 7: Patients who were reported to have been treated in a public hospital in New Zealand for foodborne *V. parahaemolyticus* intoxication

YEAR	GENDER	PATIENT AGE AT DISCHARGE	PRIMARY ETHNICITY
1998*	Male	48	Other Pacific peoples
1999*	Female	23	Not stated
1999	Male	39	New Zealand European/Pākehā
1999*	Female	46	Other Pacific peoples
2000*	Female	69	Tongan
2000	Male	38	Tongan
2002	Female	12	New Zealand European/Pākehā
2003	Male	56	New Zealand European/Pākehā
2003*	Male	14	Other Pacific peoples
2005	Male	39	Tongan
2006	Male	35	Cook Island Maori
2007	Female	84	Māori
2009*	Female	38	Tongan
2009*	Female	53	Tongan
2010*	Female	41	Tongan
2012*	Male	65	Tongan
2015	Male	76	New Zealand European/Pākehā

* *V. parahaemolyticus* intoxication was the primary diagnosis.

From January 1998 to July 2016, there were eight outbreaks of *V. parahaemolyticus* infection reported to EpiSurv, involving a total of 44 cases (9 confirmed, 35 probable). As can be seen in TABLE 8, the most likely vehicle of infection for seven of these outbreaks was seafood imported from Pacific Islands. BMS harvested from New Zealand were not implicated in any of these outbreaks. No deaths were reported associated with these outbreaks.

TABLE 8: Outbreaks of *V. parahaemolyticus* infection in New Zealand, as reported in EpiSurv (January 1998-July 2016)

YEAR	EXPOSURE LOCATION	NUMBER OF CASES		IMPLICATED FOOD
		TOTAL	HOSPITALISED	
1999	Auckland	7	2	Imported raw seafood (Tonga)
1999	Hutt Valley, home	8	0	Imported seaworms (Samoa)
2000	Auckland, home	3	1	Imported shellfish (Tonga)
2000	Auckland, home	5	0	Imported raw mussels (Tonga)
2000	Auckland, home	3	0	Imported raw seafood (Tonga)
2007	Overseas, Thailand	11	3	Seafood, salad
2009	Auckland, home	3	0	Imported cooked crab, raw coconut
2009	Auckland	4	0	Imported raw seafood (clams, cockles, fish in coconut juice)

The mean annual number of domestically-acquired foodborne cases of *V. parahaemolyticus* infection has been estimated as 1,049 (90% CI 0-5,979) (Cressey and Lake, 2011).[24] The under-reporting and under-diagnosis factors used to produce this estimate were high (25.5 and 29.3, respectively), and it was estimated that 29% of all cases were travel-related and most (89%) of the domestically-acquired cases were foodborne. The under-reporting and under-diagnosis factors were taken directly from a USA study (Scallan *et al.*, 2011) and their applicability in the New Zealand context is uncertain. The estimate was based on notified cases for the period 2000-2009 (scaled to the 2009 population) so it is possible that it is an overestimate of annual cases since the number of reported cases in 2000 was much higher than usually reported and would have skewed the mean. This is reflected in the wide confidence intervals. The estimated number of domestically-acquired foodborne cases of *V. parahaemolyticus* infections was similar to an estimate calculated for infection by Shiga toxin-producing *Escherichia coli* of serotype O157, and higher than estimates for a number of other potentially foodborne pathogens, including *Listeria monocytogenes* and *Shigella* spp. (Cressey and Lake, 2011).

The annual number of hospitalisations and deaths estimated for domestically-acquired foodborne *V. parahaemolyticus* infection was 10 (90% CI 0-62) and 1 (90% CI 0-4), respectively (Cressey and Lake, 2011). These estimates were produced using data over the time period 2000-2009, and assumed that rates were fairly stable over that period. A repeat analysis using only data from 2009 produced higher estimates, but these were still within the wide 90% confidence intervals of the initial estimations, which reflect uncertainty over data on *V. parahaemolyticus* infection in New Zealand.

3.3.3 The susceptible population in New Zealand

People with underlying immunocompromising chronic disease are more susceptible to septicaemia as a result of *V. parahaemolyticus* infection. The range of health conditions leading to an immunocompromised state makes it difficult to predict the proportion of the population that is considered to be immunocompromised, or of above-normal sensitivity to foodborne illness (Cressey, 2013). In their risk assessment, the USFDA listed liver disease, immunodeficiency, peptic ulcer disease, diabetes, alcoholism, haematological disease, gastric surgery, heart disease, renal disease, cancer or malignancy, treatment with corticosteroids, and transplant recipients as chronic medical conditions for consideration (USFDA, 2005b).

In addition, a case control study in China identified people taking antibiotics as being more susceptible to *V. parahaemolyticus* infection (Yan *et al.*, 2015). The proportion of the New Zealand population undertaking a course of antibiotics at any one time is not known.

3.4 *V. PARAHAEMOLYTICUS* INFECTION OVERSEAS

KEY FINDINGS

Internationally, *V. parahaemolyticus* infection is a leading cause of human gastroenteritis associated with seafood consumption.

Vibriosis has been a notifiable disease in the USA since 2007 so public health data from the USA cannot be meaningfully compared with that from New Zealand. The incidence of *V. parahaemolyticus* infection (all syndromes, including wound infections) for 2014 was an estimated 0.2 per 100,000 (approximately 600 cases reported during the year). The majority of vibriosis cases were reported from coastal states and peaked in summer months. Raw oysters were often implicated as the vehicle of infection.

[24] This converts to an estimate of 24 cases per 100,000, based on the 2009 resident New Zealand population (4,304,900; as extracted from Statistics New Zealand Infoshare, 27 July 2016).

V. parahaemolyticus infection is notifiable in three Australian States. The incidence in Western Australia is higher than reported for the USA; 0.6 per 100,000 each year during the period 2012-2014, and 0.3 per 100,000 in 2015. One case was reported in the Northern Territory during 2014 and this case reported eating oysters during the incubation period.

Outbreaks of *V. parahaemolyticus* infection caused by contaminated BMS have been reported. Of 12 outbreaks reported in the scientific literature (11 occurring in countries of the American Continent and one in Spain), oysters were an implicated vehicle of infection in seven. The arrival of the O3:K6 clone in Chile, combined with higher-than-normal sea temperatures, lead to a very large outbreak involving almost 11,000 people. The most commonly-reported vehicles of infection during this outbreak were clams and mussels. Higher-than-normal sea temperatures were reported as a contributing factor in 4/12 outbreaks.

Data on *V. parahaemolyticus* infection in other countries are presented in Appendix B.2.

4. EVALUATION OF RISK

4.1 EXISTING RISK ASSESSMENTS

KEY FINDINGS

A 2011 quantitative risk assessment has predicted consumption of oysters from New Zealand waters would not cause any *V. parahaemolyticus* illnesses in New Zealand, but the assessment did not incorporate data representative of New Zealand conditions.

A 2005 quantitative risk assessment predicted that consumption of raw oysters would cause a mean of 2,826 *V. parahaemolyticus* illnesses per year in the USA, with the majority arising from consumption of oysters from the Gulf Coast, particularly during summer. The model also predicted seven cases of septicaemia each year from eating raw oysters. The approach was later adapted to predict the annual number cases of *V. parahaemolyticus* illnesses from eating raw oysters in other countries. The model predicted 66 cases per year in Japan, 91 in Australia and 186 in British Columbia, Canada.

Guidelines for risk assessment of *V. parahaemolyticus* in BMS have recently been published in an effort to standardise international approaches (FAO/WHO, 2016).

4.1.1 New Zealand risk assessment and risk-related activities

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) jointly published a Quantitative Risk Assessment (QRA) considering *V. parahaemolyticus* in raw oysters in 2011 (FAO/WHO, 2011). This QRA included a separate assessment of the risk of *V. parahaemolyticus* illness following consumption of oysters, based on data from the Orongo Bay intertidal harvesting area in Northland, New Zealand. The model predicted consumption of oysters would not cause any *V. parahaemolyticus* illnesses in New Zealand.

There are several limitations to this model. Surrogate data from the USA was used for many of the inputs to the model, including the relationship between water temperature and *V. parahaemolyticus* levels in oysters, oyster weights, oyster consumption variables and the under-reporting multiplier. The water activity was set to 0.98 and temperature data from Orongo Bay was considered representative of all oyster production areas in New Zealand.

4.1.2 Risk assessments from other countries

The 2003 Risk Profile signalled that two relevant QRAs were under preparation and both have now been published.

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

The exposure model in this risk assessment predicted that the mean concentration of pathogenic *V. parahaemolyticus* per serving of oysters consumed in the winter months ranged from <1 to 98 organisms. The range during summer months was 460 to 21,000 organisms. Most of the growth occurred during post-harvest storage.

The model predicted both the likelihood and severity of illness following exposure to pathogenic *V. parahaemolyticus* from consumption of raw oysters. The predicted mean annual number of illness for all regions was 2,826, with the majority (73%) of these illnesses arising from consumption of oysters from the Gulf Coast, particularly during summer. The model predicted seven cases of septicaemia each year, of which five would be expected to occur among the immunocompromised population. This was calculated based on a probability of 0.0023 for *V. parahaemolyticus* gastroenteritis progressing to septicaemia.

In 2011, the FAO and WHO jointly published a QRA considering *V. parahaemolyticus* in raw oysters (FAO/WHO, 2011), which was based on the USFDA's QRA but adapted to estimate illness in Australia, Canada, Japan and New Zealand. However, surrogate data from the USA were used for many of the inputs for these countries. The endpoint modelled was gastrointestinal illness from *V. parahaemolyticus* as a result of eating raw oysters. The predicted annual illnesses for countries other than New Zealand were 66 in Japan, 91 in Australia and 186 in British Columbia, Canada.

Two country-specific QRAs have also been published:

- Consumption of raw oysters by people in the São Paulo State, Brazil (Sobrinho *et al.*, 2014): The model estimated that the mean probability of illness per serving of raw oysters was 3.1×10^{-4} in winter and 6.0×10^{-4} in summer. This was based on *V. parahaemolyticus* being present in the oysters at a maximum of 6 $\log_{10}$ MPN/g. Temperature control during oyster transport was identified as an important risk-reduction step.
- Consumption of raw oysters and mussels by people in France (ANSES, 2012): The model predicted there would be 237 cases of gastroenteritis per year caused by eating raw oysters contaminated with *V. parahaemolyticus*, and 963 cases from eating raw mussels. Most of these cases would occur during summer. The authors noted key data gaps, including survey data on the prevalence and concentration of total and pathogenic (*tdh*+/*trh*+) *V. parahaemolyticus* in shellfish.

A QRA for consumption of bloody clams (*Anadara granosa*) by people in southern Thailand identified undercooking as the primary factor that increased the risk of *V. parahaemolyticus* infection (Yamamoto *et al.*, 2008).

4.2 EVALUATION OF RISK FOR NEW ZEALAND

KEY FINDINGS

Based on the available information, New Zealand consumers of raw BMS harvested from New Zealand waters are at risk of foodborne *V. parahaemolyticus* infection. *V. parahaemolyticus* are present in the New Zealand coastal marine environment where water temperature and salinity are not barriers to its survival. The seawaters of New Zealand provide favourable temperatures for *V. parahaemolyticus*, particularly during summer months. New Zealand surveys indicate that BMS harvested from northern waters during summer are most likely to be contaminated with *V. parahaemolyticus*, and the concentrations measured in some samples from upper North Island harbours could be high enough to cause illness. These surveys have also detected potentially pathogenic strains of *V. parahaemolyticus* in BMS. Thus, the human health 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.

The science demonstrates potential for BMS harvested from New Zealand waters to cause *V. parahaemolyticus* infection, but there is a lack of reported cases in New Zealand's public health surveillance data. This is not unexpected, primarily because *V. parahaemolyticus* infection will be underreported in this country. During the period January 1998 to July 2016, BMS harvested from New Zealand waters were not confirmed as the vehicle of infection for

any reported sporadic cases of *V. parahaemolyticus* infection, and were not implicated in any reported outbreaks of *V. parahaemolyticus* infection. Over the same period, seafood privately imported from Pacific Islands were the most likely vehicle of infection for 7/8 outbreaks of *V. parahaemolyticus* infection. This suggests that the existing public health surveillance systems would detect at least some outbreaks of foodborne *V. parahaemolyticus* infection from consumption of BMS harvested from New Zealand waters if these were happening frequently.

The risk of infection may be attenuated by a number of factors which, together, mean that New Zealanders consuming raw BMS harvested from New Zealand waters are not often exposed to pathogenic strains of *V. parahaemolyticus* in high enough concentrations to cause illness. These factors include the possibly low prevalence and concentration of pathogenic *V. parahaemolyticus*, consuming BMS harvested during cooler seasons or from southern locations, and cool-chain requirements for industry.

There are few data to inform the risk of *V. parahaemolyticus* infection from consumption of BMS other than Pacific oysters harvested from northern New Zealand waters. The available data indicate mussels might present a similar risk since these are also consumed raw or marinated. It is possible that there are other regions of New Zealand where the risk of BMS becoming contaminated with *V. parahaemolyticus* 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).

This assessment of risk would be improved by a targeted public health surveillance study of vibriosis in New Zealand, to better understand the incidence of sporadic disease and to identify whether BMS are an important vehicle of illness. Data on *Vibrio* spp. in BMS at the point-of-sale and time/temperature profiles from harvest to that point would also be valuable.

RMQ: Has the risk to human health from *V. parahaemolyticus* changed since 2003?

There were very little data available prior to 2003 to make an assessment on the risk associated with BMS harvested from New Zealand waters. The data in this current document suggest that the risk of *V. parahaemolyticus* infection from consumption of raw BMS harvested from New Zealand waters has not changed since 2003.

Seafoods privately imported from the Pacific Islands are still an important cause of sporadic cases and outbreaks of *V. parahaemolyticus* infection in New Zealand.

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

4.2.1 Risk associated with BMS

This section responds to the risk management question:

Has the risk to human health from *V. parahaemolyticus* changed since 2003?

The 2003 Risk Profile concluded that there was a strong link between personal importation and consumption of seafood from the Pacific Islands and *V. parahaemolyticus* infection. There were very little data at the time to make an assessment on the risk associated with BMS harvested from New Zealand waters, although it was noted that the salinities of New Zealand coastal waters were greater than the optimum for *V. parahaemolyticus*, and the water temperatures in regions south of Auckland were less than optimal.

Given the lack of a comparative baseline to measure any change in risk from BMS harvested from New Zealand waters, this Risk Profile will instead discuss the risk from these BMS (when consumed raw), and provide commentary on whether the risk may have changed.

Based on the available information, New Zealand consumers of raw BMS harvested from New Zealand waters are at risk of foodborne *V. parahaemolyticus* infection. 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. parahaemolyticus* 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. parahaemolyticus* contamination.
- Water salinity and water temperature are not barriers to the occurrence of *V. parahaemolyticus* in New Zealand: New Zealand strains of *V. parahaemolyticus* appear to be well adapted to high salinities. The seawaters of New Zealand provide favourable temperatures for *V. parahaemolyticus*, particularly during summer months. Spring and autumn periods may also support the presence of *V. parahaemolyticus* in these waters. The La Niña phase of the southern oscillation also brings warmer temperatures to New Zealand.
- Based on New Zealand surveys of commercial Pacific oysters and green-lipped mussels (and the assumption that all BMS bioaccumulate *V. parahaemolyticus* similarly to these shellfish), BMS harvested from northern waters during summer are most likely to be contaminated with *V. parahaemolyticus*: The prevalence and concentration of *V. parahaemolyticus* in oysters and mussels sampled from harbours located in the Northland, Auckland and Coromandel regions were higher (up to 100% and 4.8×10^4 MPN/g) than those from the Marlborough region (up to 30% and 7.4 MPN/g). The prevalence and concentrations were higher during summer months when sea surface temperatures were ≥19ºC. *V. parahaemolyticus* were detected in Pacific oyster samples growing in intertidal and subtidal locations.
- New Zealand surveys have detected potentially pathogenic strains of *V. parahaemolyticus* in BMS harvested from northern North Island harbours: The prevalence (up to 27%) and concentration (up to 933 MPN/g) of *tdh*+ and/or *trh*+ *V. parahaemolyticus* were lower compared with total *V. parahaemolyticus*, which is a similar finding to studies in other countries.
- The concentration of *V. parahaemolyticus* in BMS harvested from northern New Zealand waters can be high enough to cause illness: The best estimation for dose-response predicts a 50% probability of illness for a dose between 10^7 and 10^{10} *V. parahaemolyticus* cells. For the maximum concentration detected in pooled oysters from a Northland harbour (4.8×10^4 MPN/g), a single oyster in this sample may contain up to 10^7 *V. parahaemolyticus* cells.

The above evidence demonstrates the potential for BMS harvested from New Zealand waters to cause *V. parahaemolyticus* infection, yet there is a lack of reported cases in New Zealand's public health surveillance data. This is not unexpected, primarily because *V. parahaemolyticus* infection will be underreported due to the disease being non-notifiable (unless an outbreak is detected) and mild enough that most sick people do not seek medical care. Moreover, 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).

For the period January 1998 to July 2016, BMS harvested from New Zealand waters were implicated as the vehicle of infection in 8/52 (15%) reported cases of *V. parahaemolyticus* infection, but the vehicle of infection was not confirmed in any of these cases. However, it is rare for the source of infection to be confirmed for sporadic cases of gastroenteritis. Over the

same period, BMS harvested from New Zealand waters were not implicated in any reported outbreaks of *V. parahaemolyticus* infection. In contrast, seafood privately imported from Pacific Islands were the most likely vehicle of infection for 7/8 outbreaks of *V. parahaemolyticus* infection reported during this period. This suggests that the existing public health surveillance systems would detect at least some outbreaks of foodborne *V. parahaemolyticus* infection from consumption of BMS harvested from New Zealand waters if these were happening frequently.

It may be that the risk of infection is attenuated by several factors, which together mean that New Zealanders consuming raw BMS from New Zealand waters are not often exposed to pathogenic strains of *V. parahaemolyticus* in high enough concentrations to cause illness:

- It is clear that not all strains of *V. parahaemolyticus* will cause illness. While there is still uncertainty over the characteristics that enable a strain to infect a human host, an isolate containing the *tdh* and/or *trh* gene is more likely to be able to cause human disease. New Zealand surveys of BMS show that the prevalence and concentration of *V. parahaemolyticus* containing these genes is much lower than for the total *V. parahaemolyticus* population. The prevalence and concentration of pathogenic strains without these genes may be similarly low. There is currently no evidence that the pandemic clones causing outbreaks in other countries are present in New Zealand waters, but further work is necessary to confirm this.
- The available dose-response data suggest that the number of cells required to cause illness may be very high (consumption of $>10^7$ cells may cause illness in 50% of people, and illness will almost always occur with a dose of 10^9 cells).
- 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. parahaemolyticus* 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 major proportion. Data from 2011 show 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. parahaemolyticus* suggests that dredge oysters present a much lower risk of *V. parahaemolyticus* infection compared to Pacific oysters because of their more southern and subtidal habitat (the majority of commercially harvested dredge oysters are from Foveaux Strait).
- Only a proportion of the total weight of commercially harvested BMS taken from New Zealand waters will be harvested during the summer months, when the risk of *V. parahaemolyticus* contamination is highest.
- Commercial harvesters are required to cool harvested BMS to 7ºC or lower and this temperature control will prevent multiplication of *V. parahaemolyticus*.

It should be noted that there are few data to inform the risk of *V. parahaemolyticus* infection from consumption of BMS other than Pacific oysters commercially raised in northern New Zealand waters. It is possible that there are other regions of New Zealand where the risk of BMS becoming contaminated with *V. parahaemolyticus* 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).

There is support for further research on *V. parahaemolyticus* in mussels harvested from New Zealand waters. New Zealand nutrition surveys have found that mussels, oysters and scallops

were consumed more often than other types of BMS, and 40% of mussel servings were consumed raw or marinated. New Zealand surveys detected *V. parahaemolyticus* in a small sample of green-lipped mussels from northern North Island harbours. Of the eight sporadic cases of *V. parahaemolyticus* infection reported during the period January 1998 to July 2016 where BMS harvested from New Zealand waters was the implicated food, mussels were implicated in five (Section 3.3.1).

There is no evidence to suggest that the risk of *V. parahaemolyticus* infection from consumption of raw BMS harvested from New Zealand waters has changed since 2003. Support for this comes primarily from outbreak data. If the risk had increased, outbreaks are likely to have been reported. The series of microbiological surveys of BMS sampled from 2008 to 2016 do not suggest that *V. parahaemolyticus* contamination of BMS has increased or decreased during this period. The predicted increases in seawater temperature as a result of global warming will increase the risk in the future.

This risk assessment does not take into account post-harvest conditions for live or raw BMS since these may increase risk by supporting *V. parahaemolyticus* 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. This assessment of risk would also be improved by a targeted public health surveillance study of vibriosis in New Zealand, to better understand the incidence of sporadic disease and provide attribution estimates. The basic elements of a clinical surveillance study for *Vibrio* spp. infections have been described (FAO/WHO, 2016).

There are insufficient data to determine the risk to New Zealand consumers of *V. parahaemolyticus* infection from commercially 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.

4.2.2 Risks associated with other foods

As *V. parahaemolyticus* 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, squid, sea urchin) has caused *V. parahaemolyticus* infection in New Zealand (Section 3.3.2) and other countries (Hara-Kudo and Kumagai, 2014; Weis *et al.*, 2011). The 2003 Risk Profile noted that the increasing popularity of raw fish foods such as sushi may make *V. parahaemolyticus* infection more common. There is, as yet, no evidence to suggest that this has happened.

Other foods may be cross-contaminated (e.g. through liquids spreading from contaminated seafood) (Desmarchelier, 2003). This appears to be uncommon but cross-contamination from mussels to fresh produce was the implicated cause of an outbreak in the USA (Davis *et al.*, 2004). *V. parahaemolyticus* infection associated with cooked seafood that was subsequently recontaminated has also been reported (Codex Alimentarius, 2010).

The 2003 Risk Profile identified seafoods privately imported from Pacific Islands as an important cause of *V. parahaemolyticus* infection in New Zealand. Such foods continue to be implicated in sporadic cases and outbreaks of *V. parahaemolyticus* infection in New Zealand (Section 3.3.2).

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

KEY FINDINGS

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

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

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

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

No assessment of economic or health costs associated with *V. parahaemolyticus* infection has been carried out for New Zealand. *V. parahaemolyticus* 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). The identification of *V. parahaemolyticus* infections is likely to be inhibited by the low frequency of testing of clinical specimens. For a high proportion (perhaps 80%) of faecal samples submitted by acute gastrointestinal cases in New Zealand, no pathogen is identified by routine laboratory testing (Lake *et al.*, 2009).

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. parahaemolyticus* in BMS harvested from New Zealand waters other than Pacific oysters (including at the point-of-sale), and the incidence of gastroenteritis in New Zealand as a result of *V. parahaemolyticus* infection.

Two data gaps were identified in the 2003 Risk Profile:

- Incidence of *V. parahaemolyticus* infection in the New Zealand population; and
- Prevalence of *V. parahaemolyticus* in New Zealand seafood.

There are still insufficient data to estimate the incidence of *V. parahaemolyticus* infection in the New Zealand population. More data are now available on the prevalence (and concentration) of *V. parahaemolyticus* in New Zealand seafood, although these data are mainly for Pacific oysters harvested from North Island waters. Data for other BMS are scarce and there are no data on *V. parahaemolyticus* in BMS at retail/food service.

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

- The ability to identify strains of *V. parahaemolyticus* that will cause infection from those that will not, considering any differences in susceptibility between people with immunocompromising health conditions and the general population;
- Uncertainty over dose-response;
- Environmental surveys for New Zealand to better evaluate the relationship (if any) between environmental variables (e.g. water temperature and salinity) and *V. parahaemolyticus* concentrations in water, sediment and BMS;

- The consumption patterns for recreationally harvested BMS; and
- Post-harvest time/temperature profiles for BMS harvested in New Zealand intended for sale as live or raw product, and their effect on the numbers of total and pathogenic *V. parahaemolyticus* in the BMS.

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. parahaemolyticus* in BMS but temperature requirements will help minimise growth of this bacterium. A BMS processor may choose to include monitoring for *Vibrio* spp. as part of their Risk Management Programme. There are no regulatory microbiological standards for *V. parahaemolyticus* in BMS but guideline standards are available.

Current seafood safety advice for New Zealand consumers advises them to cook seafood thoroughly, which will reduce the risk of *Vibrio* spp. infection.

The 2003 Risk Profile stated: "For raw seafood, processing and storage at low temperature is essential to prevent growth of *V. parahaemolyticus* to high numbers. Heat treatment will readily destroy the organism, but prevention of cross contamination from raw to cooked product is important." This statement is still relevant, and additional controls are introduced in the following sections. Current regulatory controls are also discussed, since the 2003 Risk Profile was prepared during a transitional phase in New Zealand food law (introduction of the *Animal Products Act 1999* and the Australia New Zealand Food Standards Code).

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"[25] 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).[26] 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 grown or harvested in New Zealand 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).[27] The microbiological monitoring requirements do not include standards for *Vibrio* spp.

[25] 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.
[26] http://www.foodsafety.govt.nz/industry/sectors/seafood/bms/index.htm (accessed 18 July 2016).
[27] 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).

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 meters, river gauges 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. 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"). 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. parahaemolyticus*), 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).[28] Generic RMPs for half-shell mussels and oysters are available and these list *Vibrio* spp. among the possible microbiological hazards to be considered.[29] 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

[28] http://www.foodsafety.govt.nz/industry/sectors/seafood/bms/processors.htm (accessed 18 July 2016).
[29] http://www.foodsafety.govt.nz/elibrary/industry/code-practice-seafood/generic-rmp-model.pdf (accessed 18 July 2016).

the most recent version of this notice came into effect on 1 April 2016.[30] Sections 14.12 to 14.34 set out specific requirements; none are specific to *Vibrio* spp.

A revised Australia New Zealand Food Standards Code came into effect on 1 March 2016.[31] 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 regulatory 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, 2016b). 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.[32]

5.1.3 Voluntary industry controls

Microbiological standards for *V. parahaemolyticus* in ready-to-eat BMS are available in two guidance documents available to industry.[33] These are not regulatory requirements. The standards are:

- Shellfish (processed, requiring no further cooking) (Ministry of Health, 1995): Of five samples (n=5), no more than two samples shall contain more than 10^3 *V. parahaemolyticus*/g (c=2, M=10^3). A concentration greater than 10^2 *V. parahaemolyticus*/g is considered marginally acceptable (m=10^2).
- Ready-to-eat foods (FSANZ, 2016): Criteria are "satisfactory" (<3 CFU/g), "marginal" (<3-10^2 CFU/g), "unsatisfactory" (10^2-10^4 CFU/g) and "potentially hazardous" (≥10^4 CFU/g). For ready-to-eat seafoods that are raw, a higher satisfactory level may be applied (<10^2 CFU/g). *V. parahaemolyticus* should not be present in seafoods that have been cooked.

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.[34] 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

[30] http://www.foodsafety.govt.nz/elibrary/industry/animal-products-specifications-asd/index.htm (accessed 18 July 2016).
[31] http://www.foodstandards.gov.au/code/Pages/default.aspx (accessed 18 July 2016).
[32] 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).
[33] http://www.foodsafety.govt.nz/elibrary/industry/Which_Microbiological-Outlines_Four.htm (accessed 13 October 2016).
[34] 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).

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 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, particularly for oysters where the preference is for raw consumption.

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. parahaemolyticus* in oysters include electrolysed oxidising water, antimicrobial photodynamic therapy 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. parahaemolyticus* 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 hydrostatic pressure, individual quick freezing (IQF) with extended storage, and irradiation 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 levels (defined as <30 MPN/g) (USFDA, 2011).

Predictive modelling using water quality parameters (temperature, salinity) is also being investigated as a way to predict the presence, abundance and potential virulence of *V. parahaemolyticus*, with the intention that such models can be used to identify harvest days with potentially increased human health risks (Froelich *et al.*, 2013). However, such models need to be site specific and well validated, and do not appear to have been used as part of regulatory controls in any country, as yet.

There are strain-dependant differences in resistance to control methods, and the level of resistance may also change depending on other stressors the cells were exposed to prior to a control intervention (Burnham *et al.*, 2009; Calik *et al.*, 2002; Drake *et al.*, 2007; Wong *et al.*, 2004a). Studies have identified that the pandemic *V. parahaemolyticus* strain O3:K6 is more resistant to controls such as low temperature pasteurisation and HPP (Andrews *et al.*, 2003b; Cook, 2003).

5.2.1 Management techniques

As concluded in the 2003 Risk Profile, depuration can reduce the concentration of *V. parahaemolyticus* inside BMS but is not a reliable method for eliminating these bacteria from BMS. Depuration can also spread *V. parahaemolyticus* from contaminated oysters to those that are not (Ramos *et al.*, 2012a). The effectiveness of depuration on removing bioaccumulated *V. parahaemolyticus* can be improved by using UV light and chlorine to control microbes in the water (Ramos *et al.*, 2012a).

There is limited information on the success of relaying as a treatment step to remove *V. parahaemolyticus* from BMS. The concentration of *V. parahaemolyticus* in *Crassostrea commercialis* oysters was shown to reduce from 18 per gram to 5 per gram after being relayed from a harvest area to a pollution free waterway (Son and Fleet, 1980).

A quantitative risk assessment model was used to investigate interventions that might reduce the number of cases of *V. parahaemolyticus* infection in the USA population arising as a result of eating raw oysters containing pathogenic *V. parahaemolyticus* (USFDA, 2005a, 2005b). The model predicted that immediate refrigeration of oysters after harvesting would reduce the annual number of cases approximately seven-fold, with this intervention being more effective in regions where warmer conditions encouraged growth of the pathogen after harvest. The model also predicted that a number of other interventions would be effective at reducing the risk of illness. Harvesting oysters from intertidal regions into baskets and allowing them to be re-submerged by the tide, then retrieving the baskets just before the oysters are exposed again reduced the risk of illness by approximately 90%. Diverting oysters with $>1x10^4$ *V. parahaemolyticus*/g away from the raw market was also effective, but not necessarily practical nor achievable.

5.2.2 Temperature controls

Experiments with oysters artificially contaminated with *V. parahaemolyticus* found that treatment of 50ºC for 10 minutes was needed to reduce the concentration by >5 $\log_{10}$ MPN/g (Ye *et al.*, 2012). Treatment at 50ºC for only 5 minutes or treatment at 45ºC for 20 minutes only achieved reductions of 3.9 and 2.6 $\log_{10}$ MPN/g, respectively. An earlier study (Andrews *et al.*, 2000) had measured a 5-log reduction of *V. parahaemolyticus* in oysters after 5 minutes at 50ºC. The difference may be due to different strains or methods (e.g. Andrews *et al.* used a kettle at 55ºC to initially heat the oysters to 50ºC, while Ye *et al*. used a water bath at 50ºC).

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 total *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 found that on-board and dockside icing did not predictably reduce the concentration of *V. parahaemolyticus* in oysters, and icing significantly and negatively affected oyster survival (Melody *et al.*, 2008).

As demonstrated by data in Section 2.4, *V. parahaemolyticus* 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.

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). Combining HPP with low temperature pasteurisation has a synergistic effect on killing *V. parahaemolyticus* (Ye *et al.*, 2012).

An HPP of 293 MPa for two minutes at 8ºC reduced the concentration of *V. parahaemolyticus* in Pacific oysters by >3.52 $\log_{10}$ MPN/g (Ma and Su, 2011). Oysters processed in this way had a shelf life of 6-8 days when stored at 5ºC or 16-18 days when stored in ice. A treatment of 275 MPa or more for two minutes at 21ºC achieved the same *V. vulnificus* reduction (>3.52 $\log_{10}$ MPN/g) in Atlantic oysters (*Crassostrea virginica*) (Ye *et al.*, 2012). A pressure of 300 MPa was required to achieve a reduction of >5 $\log_{10}$ MPN/g.

Two studies found that lowering the temperature of the HPP process improved its effectiveness against *V. parahaemolyticus*, but the experimental conditions were not realistic (one study used inoculated oyster homogenates, the other pre-sterilised oysters) (Kural and

Chen, 2008; Phuvasate and Su, 2015). Another study, using shucked oysters, did not identify the HPP temperature as being important (Ye *et al.*, 2013).

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, and that HPP followed by cold storage was more effective at reducing the concentration of *V. parahaemolyticus* in oysters than HPP after cold storage (Ye *et al.*, 2013):

- In the first experiment, oysters were artificially-contaminated with a pressure-resistant strain of *V. parahaemolyticus*, stored under various conditions (air, seawater, frozen), then shucked, and the meat subjected to 12 different HPP regimes (225-300 MPa, 2 minutes at 4, 21 or 35ºC). HPP at 300 MPa was most effective at reducing the number of *V. parahaemolyticus*, 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. parahaemolyticus* survivors. However, frozen storage was the most effective pre-HPP storage condition for reducing the concentration of *V. parahaemolyticus*.
- The second experiment found that inactivation of *V. parahaemolyticus* was greater when the oysters were subjected to HPP before storage (in an ice slurry or freezer) (Ye *et al.*, 2013). An HHP treatment of 250 MPa followed by 10-day ice storage, or 300 MPa followed by 5-day ice storage, reduced the concentration of *V. parahaemolyticus* in whole-shell oysters by >7 $\log_{10}$ MPN/g.

Thus the combination of HPP and frozen storage is 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.*, 2003a; Drake *et al.*, 2007; Jakabi *et al.*, 2003; Thupila *et al.*, 2011). However, irradiation has been reported to decrease shelf-life of oysters (Dixon and Rodrick, 1998).

An ionising irradiation dose of 1.0 kGy reduced *V. parahaemolyticus* artificially bioaccumulated in whole shell oysters by 4-6 $\log_{10}$ MPN/g (Jakabi *et al.*, 2003). A 4-log reduction of *V. parahaemolyticus* O3:K6 in whole shell oysters was achieved with an ionising irradiation dose of 1.5 kGy (Andrews *et al.*, 2003a).

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

5.2.5 Other treatments

A number of chemical controls have been investigated for reducing *V. parahaemolyticus* in BMS. The concentration of artificially bioaccumulated *V. parahaemolyticus* in live clams and mussels reduced by up to 1.5 $\log_{10}$ CFU/g when the shellfish were submerged in acidic electrolysed water for 1-2 hours (Al-Qadiri *et al.*, 2016). Citric acid and lactic acid effectively reduced *V. parahaemolyticus* in shucked, pre-sterilised oysters, but the effect of these organic acids on *V. parahaemolyticus* in non-sterilised oysters was not investigated (Mahmoud, 2014). Other treatment agents that have demonstrated antimicrobial activity towards *V. parahaemolyticus* in BMS include green tea extract (Xi *et al.*, 2012) and chlorine dioxide (Wang *et al.*, 2010b).

Antimicrobial photodynamic therapy (aPDT) treatment involves delivering visible light of an appropriate wavelength to a photosensitive additive, and exciting this additive to undertake a photochemical reaction with oxygen to produce radicals (type 1 reaction) or singlet oxygen (type 2 reaction) (Wu *et al.*, 2016). The reaction destroys bacterial cells. When oysters were submerged in a solution of the photosensitive additive cucumin and *V. parahaemolyticus*, then opened and exposed to a light source for 60 seconds, the concentration of *V. parahaemolyticus* was reduced by approximately 5 $\log_{10}$ CFU/g (Wu *et al.*, 2016).

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

5.3 CONTROL MEASURES IN OTHER COUNTRIES

KEY FINDINGS

General food hygiene measures (including cooling) are internationally recognised as being important for controlling growth of *Vibrio* spp. in BMS, plus controlling cross-contamination.

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 available 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 *V. parahaemolyticus* illnesses are linked to a BMS harvesting area, when elevated water temperatures are measured or on the basis of a risk evaluation. The USA have guideline levels for *V. parahaemolyticus* in ready-to-eat fishery products ($1x10^4$ MPN/g) and for BMS carrying the label "processed to reduce *Vibrio parahaemolyticus* to non-detectable levels" (<30 MPN/g).

Canada has set a microbiological guideline for *V. parahaemolyticus* in live oysters intended for raw consumption; of five sample units, none may exceed 100 MPN/g *V. parahaemolyticus*. In addition, oysters harvested from Canadian waters during the summer months, and intended for sale in-shell, should only be harvested from sites where the concentration of *V. parahaemolyticus* in the oysters is ≤100 MPN/g, unless a validated post-harvest processing step is applied that will reduce *V. parahaemolyticus* to this level.

Japan has had the same standard (≤100 MPN/g) in place since 2001 for all seafood intended for raw consumption, and has also issued advisories aiming to minimise the time between seafood being taken from the last point in the cool-chain (e.g. a consumer's fridge) and being eaten.

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

6. REFERENCES

Aagesen A M and Hase C C (2014) Seasonal effects of heat shock on bacterial populations, including artificial *Vibrio parahaemolyticus* exposure, in the Pacific oyster, *Crassostrea gigas*. Food Microbiology; 38: 93-103.

Aagesen A M, Phuvasate S, Su Y C and Hase C C (2013) Persistence of *Vibrio parahaemolyticus* in the Pacific oyster, *Crassostrea gigas*, is a multifactorial process involving pili and flagella but not type III secretion systems or phase variation. Applied and Environmental Microbiology; 79: 3303-3305.

Al-Qadiri H M, Al-Holy M A, Shiroodi S G, Ovissipour M, Govindan B N, Al-Alami N, Sablani S S and Rasco B (2016) Effect of acidic electrolyzed water-induced bacterial inhibition and injury in live clam (*Venerupis philippinarum*) and mussel (*Mytilus edulis*). International Journal of Food Microbiology; 231: 48-53.

Anacleto P, Pedro S, Nunes M L, Rosa R and Marques A (2013) Microbiological composition of native and exotic clams from Tagus estuary: Effect of season and environmental parameters. Marine Pollution Bulletin; 74: 116-124.

Andrews L, Jahncke M and Millikarjunan K (2003a) Low-dose gamma irradiation to reduce pathogenic vibrios in live oysters (*Crassostrea virginica*). Journal of Aquatic Food Product Technology; 12: 71-82.

Andrews L S, DeBlanc S, Veal C D and Park D L (2003b) Response of *Vibrio parahaemolyticus* 03:K6 to a hot water/cold shock pasteurization process. Food Additives and Contaminants; 20: 331-334.

Andrews L S, Park D L and Chen Y P (2000) Low temperature pasteurization to reduce the risk of *Vibrio* infections from raw shell-stock oysters. Food Additives and Contaminants; 17: 787-791.

Anonymous (2015) Communicable Diseases Quarterly, Issue 10 Q4 2015. Department of Health and Human Services, Tasmanian Government, Tasmania. http://www.dhhs.tas.gov.au/publichealth/communicable_diseases_prevention_unit (accessed: 7 July 2016).

Ansaruzzaman M, Lucas M, Deen J, Bhuiyan N, Wang X-Y, Safa A, Sultana M, Chowdhury A, Nair G, Sack D, von Seidlein L, Puri M, Ali M, Chaignat C-L, Clemens J and Barreto A (2005) Pandemic serovars (O3:K6 and O4:K68) of *Vibrio parahaemolyticus* associated with diarrhea in Mozambique: Spread of the pandemic into the African Continent. Journal of Clinical Microbiology; 43: 2559-2562.

ANSES (2012) Évaluation du risque lié à *Vibrio parahaemolyticus* lors de la consommation de coquillages vivants. French Agency for Food, Environmental and Occupational Health & Safety (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail), Maisons-Alfort Cedex. https://www.anses.fr/fr/system/files/BIORISK2010sa0301Ra.pdf (accessed: 15 December 2016).

ANZFA (2001) Raw commodity consumption figures. Australia New Zealand Food Authority, Canberra.

Aquaculture New Zealand (2012) New Zealand Aquaculture: A sector overview with key facts, statistics and trends. Aquaculture New Zealand, Nelson. http://www.aquaculture.org.nz/wp-content/uploads/2012/05/NZ-Aquaculture-Facts-2012.pdf (accessed: 31 May 2016).

Aranda C P, Yevenes M, Rodriguez-Benito C, Godoy F A, Ruiz M and Cachicas V (2015) Distribution and growth of *Vibrio parahaemolyticus* in southern Chilean clams (*Venus antiqua*) and blue mussels (*Mytilus chilensis*). Foodborne Pathogens and Disease; 12: 1-7.

Baker-Austin C, Trinanes J A, Taylor N G H, Hartnell R, Siitonen A and Martinez-Urtaza J (2013) Emerging *Vibrio* risk at high latitudes in response to ocean warming. Nature Climate Change; 3: 73-77.

Balter S, Hanson H, Kornstein L, Lee L, Reddy V, Sahl S, Stavinsky F, Fage M, Johnson G, Bancroft J, Keene W, Koepsell J, Williams M, MacDonald K, Napolilli N, Hofmann J, Bopp C, Lynch M, Moore K, Painter J, Puhr N and Yu P (2006) *Vibrio parahaemolyticus* infections associated with consumption of raw shellfish - three states, 2006. Morbidity and Mortality Weekly Report; 55: 854-856.

Banerjee S K, Kearney A K, Nadon C A, Peterson C L, Tyler K, Bakouche L, Clark C G, Hoang L, Gilmour M W and Farber J M (2014) Phenotypic and genotypic characterization of Canadian clinical isolates of *Vibrio parahaemolyticus* collected from 2000 to 2009. Journal of Clinical Microbiology; 52: 1081-1088.

Barile N B, Scopa M, Nerone E, Mascilongo G, Recchi S, Cappabianca S and Antonetti L (2009) Study of the efficacy of a closed cycle depuration system on bivalve molluscs. Veterinaria Italiana; 45: 555-566.

Baross J and Liston J (1970) Occurrence of *Vibrio parahaemolyticus* and related hemolytic vibrios in marine environments of Washington State. Applied Microbiology; 20: 179-186.

Baross J A, Liston J and Morita R Y (1978) Incidence of *Vibrio parahaemolyticus* bacteriophages and other *Vibrio* bacteriophages in marine samples. Applied and Environmental Microbiology; 36: 492-499.

Bates T C and Oliver J D (2004) The viable but nonculturable state of Kanagawa positive and negative strains of *Vibrio parahaemolyticus*. Journal of Microbiology; 42: 74-79.

Bauer A, Østensvik Ø, Florvåg M, Ørmen Ø and Rørvik L M (2006) Occurrence of *Vibrio parahaemolyticus*, *V. cholerae*, and *V. vulnificus* in Norwegian Blue Mussels (*Mytilus edulis*). Applied and Environmental Microbiology; 72: 3058-3061.

Baumann P and Baumann L (1977) Biology of the marine enterobacteria: Genera *Beneckea* and *Photobacterium*. Annual Reviews in Microbiology; 31: 39-61.

Bechlars S, Jaeckel C, Diescher S, Wuestenhagen D A, Kubick S, Dieckmann R and Strauch E (2015) Characterization of trh2 harbouring *Vibrio parahaemolyticus* strains isolated in Germany. Plos One; 10: pii=e0118559 [28 pp.] doi: 10.1371/journal.pone.0118559.

Beuchat L R (1976) Sensitivity of *Vibrio parahaemolyticus* to spices and organic acids. Journal of Food Science; 41: 899-902.

Bisha B, Simonson J, Janes M, Bauman K and Goodridge L D (2012) A review of the current status of cultural and rapid detection of *Vibrio parahaemolyticus*. International Journal of Food Science and Technology; 47: 885-899.

Borazjani A, Andrews L S and Veal C D (2003) Novel nonthermal methods to reduce *Vibrio vulnificus* in raw oysters. Journal of Food Safety; 23: 179-187.

Boyd E F, Cohen A L V, Naughton L M, Ussery D W, Binnewies T T, Stine O C and Parent M A (2008) Molecular analysis of the emergence of pandemic *Vibrio parahaemolyticus*. BMC Microbiology; 8: pii=110 [14 pp.] doi: 10.1186/1471-2180-8-110.

Burnham V E, Janes M E, Jakus L A, Supan J, DePaola A and Bell J (2009) Growth and survival differences of *Vibrio vulnificus* and *Vibrio parahaemolyticus* strains during cold storage. Journal of Food Science; 74: M314-M318.

Butler A J, Thomas M K and Pintar K D (2015) Expert elicitation as a means to attribute 28 enteric pathogens to foodborne, waterborne, animal contact, and person-to-person transmission routes in Canada. Foodborne Pathogens Disease; 12: 335-344.

Cabello F C, Espejo R T, Hernandez M C, Rioseco M L, Ulloa J and Vergara J A (2007) *Vibrio parahaemolyticus* O3:K6 epidemic diarrhea, Chile, 2005. Emerging Infectious Diseases; 13: 655-656.

Cabrera-Garcia M E, Vazquez-Salinas C and Quinones-Ramirez E I (2004) Serologic and molecular characterization of *Vibrio parahaemolyticus* strains isolated from seawater

and fish products of the Gulf of Mexico. Applied and Environmental Microbiology; 70: 6401-6406.
Calik H, Morrissey M T, Reno P W and An H (2002) Effect of high-pressure processing on *Vibrio parahaemolyticus* strains in pure culture and Pacific oysters. Journal of Food Science; 67: 1506-1510.
Cantet F, Hervio-Heath D, Caro A, Le Mennec C, Monteil C, Quemere C, Jolivet-Gougeon A, Colwell R R and Monfort P (2013) Quantification of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* in French Mediterranean coastal lagoons. Research in Microbiology; 164: 867-874.
Cartwright S (2006) Animal Products (Regulated Control Scheme - Bivalve Molluscan Shellfish) Regulations 2006 (SR 2006/38). Reprint as at 1 April 2008. New Zealand Government, Wellington. http://www.legislation.govt.nz/regulation/public/2006/0038/latest/DLM369366.html?search=ts_regulation_bivalve_resel (accessed: 18 July 2016).
Castinel A, Fletcher L, Dhand N, Rubio A, Whittington R and Taylor M (2015) OSHV-1 mortalities in Pacific oysters in Australia and New Zealand: The farmer's story. Cawthron Institute, Nelson. http://www.cawthron.org.nz/media_new/publications/pdf/2015_09/CR2567printable.pdf (accessed: 9 August 2016).
CFIA (2016) Fish Products Standards and Methods Manual. Appendix 2 - Bacteriological Guidelines for Fish and Fish Products (end product). Canadian Food Inspection Agency, Ontario. http://www.inspection.gc.ca/food/fish-and-seafood/manuals/standards-and-methods/eng/1348608971859/1348609209602 (accessed: 25 July 2016).
Chiou C S, Hsu S Y, Chiu S I, Wang T K and Chao C S (2000) *Vibrio parahaemolyticus* serovar O3:K6 as cause of unusually high incidence of food-borne disease outbreaks in Taiwan from 1996 to 1999. Journal of Clinical Microbiology; 38: 4621-4625.
Chu F L E (1988) Humoral defense factors in marine bivalves. American Fisheries Society Special Publication; 18: 178-188.
Codex Alimentarius (2010) 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, Rome. http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/ (accessed: 19 July 2016).
Cole K M, Supan J, Ramirez A and Johnson C N (2015) Suspension of oysters reduces the populations of *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Letters in Applied Microbiology; 61: 209-213.
Collin B and Rehnstam-Holm A-S (2011) Occurrence and potential pathogenesis of *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* on the South Coast of Sweden. FEMS Microbiology Ecology; 78: 306-313.
Colwell R R (1984) Vibrios in the environment. John Wiley and Sons. New York.
Comeau A M, Buenaventura E and Suttle C A (2005) A persistent, productive, and seasonally dynamic vibriophage population within Pacific oysters (*Crassostrea gigas*). Applied and Environmental Microbiology; 71: 5324-5331.
Cook D W (2003) Sensitivity of *Vibrio* species in phosphate-buffered saline and in oysters to high-pressure processing. Journal of Food Protection; 66: 2276-2282.
Cook D W and Ruple A D (1989) Indicator bacteria and Vibrionaceae multiplication in post-harvest shellstock oysters. Journal of Food Protection; 52: 343-349.
Cordova J L, Astorga J, Silva W and Riquelme C (2002) Characterization by PCR of *Vibrio parahaemolyticus* isolates collected during the 1997-1998 Chilean outbreak. Biological Research; 35: 433-440.
Cox A M and Gomez-Chiarri M (2012) *Vibrio parahaemolyticus* in Rhode Island coastal ponds and the estuarine environment of Narragansett Bay. Applied and Environmental Microbiology; 78: 2996-2999.
Cressey P (2012) Risk ranking: Updated estimates of the burden of foodborne disease for New Zealand 2011. Institute of Environmental Science and Research Limited,

Christchurch. http://www.foodsafety.govt.nz/elibrary/industry/updated-estimate-foodborne-disease-2011.pdf (accessed: 1 August 2016).
Cressey P (2013) Food consumption data for risk assessments. Institute of Environmental Science and Research, Christchurch.
Cressey P, King N and Lake R (2006) Food consumption data for risk assessments. Institute of Environmental Science and Research, Christchurch.
Cressey P and Lake R (2007) Risk ranking: Estimates of the burden of foodborne disease for New Zealand. Institute of Environmental Science and Research Limited, Christchurch. http://www.foodsafety.govt.nz/elibrary/industry/Risk_Ranking_Estimates-Science_Research.pdf (accessed: 1 August 2016).
Cressey P and Lake R (2008) Risk ranking: Estimates of the cost of foodborne disease for New Zealand. Institute of Environmental Science and Research Limited, Christchurch. http://www.foodsafety.govt.nz/elibrary/industry/risk-ranking-estimates-research-projects/FW07102_COI_estimates_final.pdf (accessed: 1 August 2016).
Cressey P and Lake R (2009) Risk ranking: DALY estimates for selected foodborne diseases in New Zealand using revised Dutch disability weights. Institute of Environmental Science and Research Limited, Christchurch. http://www.foodsafety.govt.nz/elibrary/industry/Risk_Ranking_Daly-Science_Research.pdf (accessed: 1 August 2016).
Cressey P and Lake R (2011) Estimated incidence of foodborne illness in New Zealand: Application of overseas models and multipliers. Institute of Environmental Science and Research Limited, Christchurch. http://www.foodsafety.govt.nz/elibrary/industry/estimates-burden-foodborne-disease-2011.pdf (accessed: 31 August 2016).
Crim S, Griffin P, Tauxe R, Marker E, Gilliss D, Cronquist A, Cartter M, Tobin-D'Angelo M, Blythe D, Smith K, Lathrop S, Zansky S, Cieslak P, Dunn J, Holt K, Wolpert B and Henao O (2015) Preliminary incidence and trends of infection with pathogens transmitted commonly through food - Foodborne Diseases Active Surveillance Network, 10 U.S. sites, 2006–2014. Morbidity and Mortality Weekly Report; 64: 495-499.
Croci L, Serratore P, Cozzi L, Stacchini A, Milandri S, Suffredini E and Toti L (2001) Detection of Vibrionaceae in mussels and in their seawater growing area. Letters in Applied Microbiology; 32: 57-61.
Croci L, Suffredini E, Cozzi L and Toti L (2002) Effects of depuration of molluscs experimentally contaminated with *Escherichia coli, Vibrio cholerae* O1 and *Vibrio parahaemolyticus*. Journal of Applied Microbiology; 92: 460-465.
Cruz C, Chycka M, Gannabathula S, Dsa G, Wei N and Fletcher G (2015a) The effect of storage temperature on *Vibrio parahaemolyticus* numbers in Pacific oysters (*Crassostrea gigas*): Evaluating a predictive model. 60th New Zealand Microbiological Society Conference, 2-5 November, Rotorua.
Cruz C D, Hedderley D and Fletcher G C (2015b) Long-term study of *Vibrio parahaemolyticus* prevalence and distribution in New Zealand shellfish. Applied and Environmental Microbiology; 81: 2320-2327.
Daniels N A (2011) Vibrio vulnificus oysters: Pearls and perils. Clinical Infectious Diseases; 52: 788-792.
Daniels N A, Ray B, Easton A, Marano N, Kahn E, McShan A L, Del Rosario L, Baldwin T, Kingsley M A, Puhr N D, Wells J G and Angulo F J (2000) Emergence of a new *Vibrio parahaemolyticus* serotype in raw oysters - A prevention quandary. Journal of the American Medical Association; 284: 1541-1545.
Daniels N A and Shafaie A (2000) A review of pathogenic *Vibrio* infections for clinicians. Infections in Medicine; 17: 665-685.
Davidson V J, Ravel A, Nguyen T N, Fazil A and Ruzante J M (2011) Food-specific attribution of selected gastrointestinal illnesses: Estimates from a Canadian expert elicitation survey. Foodborne Pathogens and Disease; 8: 983-995.

Davis C R, Heller L C, Peak K K, Wingfield D L, Goldstein-Hart C L, Bodager D W, Cannons A C, Amuso P T and Cattani J (2004) Real-time PCR detection of the thermostable direct hemolysin and thermolabile hemolysin genes in a *Vibrio parahaemolyticus* cultured from mussels and mussel homogenate associated with a foodborne outbreak. Journal of Food Protection; 67: 1005-1008.

de Sousa O V, Vieira R H, de Menezes F G, dos Reis C M and Hofer E (2004) Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae* in oyster, *Crassostrea rhizophorae*, collected from a natural nursery in the Coco river estuary, Fortaleza, Ceara, Brazil. Revista do Instituto de Medicina Tropical de Sao Paulo; 46: 59-62.

Deepanjali A, Kumar H S, Karunasagar I and Karunasagar I (2005) Seasonal variation in abundance of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters along the southwest coast of India. Applied and Environmental Microbiology; 71: 3575-3580.

DePaola A, Capers G and Alexander D (1994) Densities of *Vibrio vulnificus* in the intestines of fish from the US Gulf Coast. Applied and Environmental Microbiology; 60: 984-988.

DePaola A, Hopkins L H, Peeler J T, Wentz B and McPhearson R M (1990) Incidence of *Vibrio parahaemolyticus* in United States coastal waters and oysters. Applied and Environmental Microbiology; 56: 2299-2302.

DePaola A, Jones J L, Noe K E, Byars R H and Bowers J C (2009) Survey of postharvest-processed oysters in the United States for levels of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. Journal of Food Protection; 72: 2110-2113.

DePaola A, Ulaszek J, Kaysner C A, Tenge B J, Nordstrom J L, Wells J, Puhr N and Gendel S M (2003) Molecular, serological, and virulence characteristics of *Vibrio parahaemolyticus* isolated from environmental, food, and clinical sources in North America and Asia. Applied and Environmental Microbiology; 69: 3999-4005.

Desmarchelier P M (2003) Pathogenic vibrios. In: Foodborne microorganisms of public health significance Sixth Edition, Ed: A. D. Hocking, pp.333-358. Australian Institute of Food Science and Technology (NSW Branch) Food Microbiology Group: Waterloo, NSW.

Deter J, Lozach S, Veron A, Chollet J, Derrien A and Hervio-Heath D (2010) Ecology of pathogenic and non-pathogenic *Vibrio parahaemolyticus* on the French Atlantic coast. Effects of temperature, salinity, turbidity and chlorophyll *a*. Environmental Microbiology; 12: 929-937.

Di Pinto A, Clecarese G, De Corato R, Novello L and Terio V (2008) Detection of pathogenic *Vibrio parahaemolyticus* in southern Italian shellfish. Food Control; 19: 1037-1041.

Dixon D W and Rodrick G E (1998) Effect of gamma radiation on shellstock oysters - Extension of shelf-life and reduction in bacterial numbers, with particular reference to *Vibrio vulnificus*. pp.97-110 In Proceedings of Combination processes for food irradiation: Proceedings of the final research co-ordination meeting of the coordinated research programme on Irradiation in Combination with Other Processes for Improving Food Quality organised by the joint FAO/IAEA division of Nuclear Techniques in Food and Agriculture, Pretoria, South Africa.

Drake S L, DePaola A and Jaykus L-A (2007) An overview of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. Comprehensive Reviews in Food Science and Food Safety; 6: 120-144.

Draper A (2016) Enteric disease in the Northern Territory in 2015. The Northern Territory Disease Control Bulletin; 23: 17-25.

Duan J Y and Su Y C (2005) Occurrence of *Vibrio parahaemolyticus* in two Oregon oyster-growing bays. Journal of Food Science; 70: M58-M63.

Ellis C N, Schuster B M, Striplin M J, Jones S H, Whistler C A and Cooper V S (2012) Influence of seasonality on the genetic diversity of *Vibrio parahaemolyticus* in New Hampshire shellfish waters as determined by multilocus sequence analysis. Applied and Environmental Microbiology; 78: 3778-3782.

Elmahdi S, DaSilva L V and Parveen S (2016) Antibiotic resistance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various countries: A review. Food Microbiology; 57: 128-134.
Escalante-Maldonado O, Kayali A Y, Yamazaki W, Vuddhakul V, Nakaguchi Y and Nishibuchi M (2015) Improvement of the quantitation method for the tdh(+) *Vibrio parahaemolyticus* in molluscan shellfish based on most-probable-number, immunomagnetic separation, and loop-mediated isothermal amplification. Frontiers in Microbiology; 6: pii=270 [10 pp.] doi: 10.3389/fmicb.2015.00270.
ESR (2001) *Vibrio parahaemolyticus*. Institute of Environmental Science and Research Limited, Christchurch. http://www.foodsafety.govt.nz/science-risk/hazard-data-sheets/pathogen-data-sheets.htm (accessed: 28 June 2016).
Esteves K, Hervio-Heath D, Mosser T, Rodier C, Tournoud M G, Jumas-Bilak E, Colwell R R and Monfort P (2015) Rapid proliferation of *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio cholerae* during freshwater flash floods in French Mediterranean coastal lagoons. Applied and Environmental Microbiology; 81: 7600-7609.
European Commission (2001) Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on *Vibrio vulnificus* and *Vibrio parahaemolyticus* (in raw and undercooked seafood).
European Food Safety Authority and European Centre for Disease Prevention and Control (2015) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA Journal; 13: 4329 [91 pp.] doi: 10.2903/j.efsa.2015.4329.
Eyles M J and Davey G R (1984) Microbiology of commercial depuration of the Sydney rock oyster, *Crassostrea commercialis*. Journal of Food Protection; 47: 703-712.
FAO/WHO (2005) Risk assessment of *Vibrio vulnificus* in raw oysters. Microbiological risk assessment series No. 8. Food and Agriculture Organization of the United Nations and the World Health Organization, Rome. http://www.who.int/foodsafety/publications/mra8/en/ (accessed: 26 July 2016).
FAO/WHO (2011) Risk assessment of *Vibrio parahaemolyticus* in seafood. Microbiological risk assessment series No. 16. Food and Agriculture Organization of the United Nations and the World Health Organization, Rome. http://www.fao.org/3/a-i2225e.pdf (accessed: 26 July 2016).
FAO/WHO (2016) Selection and application of methods for the detection and enumeration of human-pathogenic halophilic *Vibrio* spp. in seafood. Food and Agriculture Organization of the United Nations, World Health Organization, Rome. http://www.fao.org/publications/card/en/c/004643ce-e2eb-4498-8f44-53adc00f1f0e/ (accessed: 12 October 2016).
Fatima M R, Dinesh S, Mekata T, Itami T and Sudhakaran R (2016) Therapeutic efficiency of *Portieria hornemannii* (Rhodophyta) against *Vibrio parahaemolyticus* in experimentally infected *Oreochromis mossambicus*. Aquaculture; 450: 369-374.
Feng S Y (1988) Cellular defense mechanisms of oysters and mussels. American Fisheries Society Special Publication; 18: 153-168.
Fisher W S (1986) Structure and functions of oyster hemocytes. In: Immunity in invertebrates, Ed: M. Brehélin, pp.25-35. Springer: Berlin.
Fisher W S and DiNuzzo A R (1991) Agglutination of bacteria and erythrocytes by serum from six species of marine molluscs. Journal of Invertebrate Pathology; 57: 380-394.
Fletcher G and Wei N (2016) Monitoring *Vibrio* numbers in North Island Pacific oysters - summer 2016. Plant & Food Research, Mount Albert.
Fletcher G C (1985) The potential food poisoning hazard of *Vibrio-parahaemolyticus* in New-Zealand Pacific oysters. New Zealand Journal of Marine and Freshwater Research; 19: 495-505.
Flores-Primo A, Pardio-Sedas V, Lizarraga-Partida L, Lopez-Hernandez K, Uscanga-Serrano R and Flores-Hernandez R (2014) Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* isolated from American oysters harvested in the

Mandinga lagoon system, Veracruz, Mexico: Implications for food safety. Journal of Food Protection; 77: 1069-1077.
Frischer M E, Thurmond J M and Paul J H (1990) Natural plasmid transformation in a high-frequency-of-transformation marine *Vibrio* strain. Applied and Environmental Microbiology; 56: 3439-3444.
Froelich B, Bowen J, Gonzalez R, Snedeker A and Noble R (2013) Mechanistic and statistical models of total *Vibrio* abundance in the Neuse River Estuary. Water Research; 47: 5783-5793.
Froelich B A and Noble R T (2016) *Vibrio* bacteria in raw oysters: managing risks to human health. Philosophical Transactions of the Royal Society B-Biological Sciences; 371: pii=20150209 [6 pp.] doi: 10.1098/rstb.2015.0209.
FSANZ (2016) Compendium of Microbiological Criteria for Food. Food Standards Australia New Zealand, Canberra. http://www.foodstandards.gov.au/publications/Pages/Compendium-of-Microbiological-Criteria-for-Food.aspx?utm_source=Food+Standards+News&utm_campaign=923884dfc8-Food_Standards_News_September_2016&utm_medium=email&utm_term=0_71d71e1fc3-923884dfc8-310067457n (accessed: 30 October 2016).
Fuenzalida L, Armijo L, Zabala B, Hernandez C, Rioseco M L, Riquelme C and Espejo R T (2007) *Vibrio parahaemolyticus* strains isolated during investigation of the summer 2006 seafood related diarrhea outbreaks in two regions of Chile. International Journal of Food Microbiology; 117: 270-275.
Fyfe M, Kelly M T, Yeung S T, Daly P, Schallie K, Buchanan S, Waller P, Kobayashi J, Therien N, Guichard M, Lankford S, Stehr-Green P, Harsch R, DeBess E, Cassidy M, McGivern T, Mauvais S, Fleming D, Lippmann M, Pong L, McKay R W, Cannon D E, Werner S B, Abbott S, Hernandez M, Wojee C, Waddell J, Waterman S, Middaugh J, Sasaki D, Effler P, Groves C, Curtis N, Dwyer D, Dowdle G and Nichols C (1998) Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters - Pacific Northwest, 1997. Morbidity and Mortality Weekly Report; 47: 457-462.
Fyfe M, Yeung S T, Daly P, Schallie K, Kelly M T and Buchanan S (1997) Outbreak of *Vibrio parahaemolyticus* related to raw oysters in British Columbia. Canadian Communicable Disease Report; 23: 145-148.
Gadiel D (2010) The economic cost of foodborne disease in New Zealand. Applied Economics, Sydney. http://www.foodsafety.govt.nz/elibrary/industry/economic-cost-foodborne-disease/index.htm (accessed: 1 August 2016).
Garcia K, Bastias R, Higuera G, Torres R, Mellado A, Uribe P and Espejo R T (2013) Rise and fall of pandemic *Vibrio parahaemolyticus* serotype O3:K6 in southern Chile. Environmental Microbiology; 15: 527-534.
Garrido-Maestu A, Lozano-Leon A, Rodriguez-Souto R R, Vieites-Maneiro R, Chapela M-J and Cabado A G (2016) Presence of pathogenic *Vibrio* species in fresh mussels harvested in the southern Rias of Galicia (NW Spain). Food Control; 59: 759-765.
Genovese G, Faggio C, Gugliandolo C, Torre A, Spano A, Morabito M and Maugeri T L (2012) In vitro evaluation of antibacterial activity of *Asparagopsis taxiformis* from the Straits of Messina against pathogens relevant in aquaculture. Marine Environmental Research; 73: 1-6.
Genthner F J, Volety A K, Oliver L M and Fisher W S (1999) Factors influencing *in vitro* killing of bacteria by hemocytes of the eastern oyster (*Crassostrea virginica*). Applied and Environmental Microbiology; 65: 3015-3020.
Gilbert S, Whyte R, Bayne G, Lake R and van der Logt P (2007) Survey of internal temperatures of New Zealand domestic refrigerators. British Food Journal; 109: 323-329.
Givens C E, Bowers J C, DePaola A, Hollibaugh J T and Jones J L (2014) Occurrence and distribution of *Vibrio vulnificus* and *Vibrio parahaemolyticus* - potential roles for fish, oyster, sediment and water. Letters in Applied Microbiology; 58: 503-510.

Gjerde J and Boe B, 1981. Acta veterinaria scandinavica (1981) Isolation and characterization of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* from the Norwegian coastal environment. Acta Veterinaria Scandinavica; 22: 331-343.

Gonzalez-Escalona N, Cachicas V, Acevedo C, Rioseco M L, Vergara J A, Cabello F, Romero J and Espejo R T (2005) *Vibrio parahaemolyticus* diarrhea, Chile, 1998 and 2004. Emerging Infectious Diseases; 11: 129-131.

Gonzalez-Escalona N, Gavilan R G, Toro M, Zamudio M L and Martinez-Urtaza J (2016) Outbreak of *Vibrio parahaemolyticus s*equence type 120, Peru, 2009. Emerging Infectious Diseases; 22: 1235-1237.

Gooch J A, DePaola A, Bowers J and Marshall D L (2002) Growth and survival of *Vibrio parahaemolyticus* in postharvest American oysters. Journal of Food Protection; 65: 970-974.

Greenberg E P, Dubois M and Palhof B (1982) The survival of marine vibrios in *Mercenaria mercenaria*, the hardshell clam. Journal of Food Safety; 4: 113-123.

Hackney C R, Ray B and Speck M L (1980) Incidence of *Vibrio parahaemolyticus* in and the microbiological quality of seafood in North Carolina. Journal of Food Protection; 43: 769-772.

Haendiges J, Jones J, Myers R A, Mitchell C S, Butler E, Toro M and Gonzalez-Escalona N (2016) A nonautochthonous US strain of *Vibrio parahaemolyticus* isolated from Chesapeake Bay oysters caused the outbreak in Maryland in 2010. Applied and Environmental Microbiology; 82: 3208-3216.

Haendiges J, Timme R, Allard M W, Myers R A, Brown E W and Gonzalez-Escalona N (2015) Characterization of *Vibrio parahaemolyticus* clinical strains from Maryland (2012-2013) and comparisons to a locally and globally diverse *V. parahaemolyticus* strains by whole-genome sequence analysis. Frontiers in Microbiology; 6: pii=125 [11 pp.] doi: 10.3389/fmicb.2015.00125.

Han H, Li F, Yan W, Guo Y, Li N, Liu X, Zhu J, Xu J, Chen Y, Li X, Lv H, Zhang Y, Cai T and Chen Y (2015) Temporal and spatial variation in the abundance of total and pathogenic *Vibrio parahaemolyticus* in shellfish in China. Plos One; 10: pii=e0130302 [13 pp.] doi: 10.1371/journal.pone.0130302.

Hara-Kudo Y and Kumagai S (2014) Impact of seafood regulations for *Vibrio parahaemolyticus* infection and verification by analyses of seafood contamination and infection. Epidemiology and Infection; 142: 2237-2247.

Harlock M (2012) A look at enteric disease in the NT during 2011 from the OzFoodNet perspective. The Northern Territory Disease Control Bulletin; 19: 14-18.

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

Hay B E, Grant C M and McCoubrey D J (2000) A review of the marine biotoxin monitoring programme for non-commercially harvested shellfish. AquaBio Consultants Ltd., Auckland.

Hazen T H, Lafon P C, Garrett N M, Lowe T M, Silberger D J, Rowe L A, Frace M, Parsons M B, Bopp C A, Rasko D A and Sobecky P A (2015) Insights into the environmental reservoir of pathogenic *Vibrio parahaemolyticus* using comparative genomics. Frontiers in Microbiology; 6: pii=204 [14 pp.] doi: 10.3389/fmicb.2015.00204.

Henigman U, Biasizzo M, Vadnjal S, Kirbis A, Toplak I and Barlic-Maganja D (2011) Detection of *Vibrio parahaemolyticus* in Mediterranean mussels (*Mytilus galloprovincialis*) in Slovenia. Acta Veterinaria Hungarica; 59: 155-164.

Hernroth B, Lothigius A and Bolin I (2010) Factors influencing survival of enterotoxigenic *Escherichia coli*, *Salmonella enterica* (serovar Typhimurium) and *Vibrio parahaemolyticus* in marine environments. FEMS Microbiology Ecology; 71: 272-280.

Hervio-Heath D, Colwell R R, Derrien A, Robert-Pillot A, Fournier J M and Pommepuy M (2002) Occurrence of pathogenic vibrios in coastal areas of France. Journal of Applied Microbiology; 92: 1123-1135.

Hiyoshi H, Kodama T, Iida T and Honda T (2010) Contribution of *Vibrio parahaemolyticus* virulence factors to cytotoxicity, enterotoxicity, and lethality in mice. Infection and Immunity; 78: 1772-1780.

Hondo S, Goto I, Minematsu I, Ikeda N, Asano N, Ishibashi M, Kinoshita Y, Nishibuchi N, Honda T and Miwatani T (1987) Gastroenteritis due to Kanagawa negative *Vibrio parahaemolyticus*. Lancet; 1: 331-332.

Hsiao H-I, Jan M-S and Chi H-J (2016) Impacts of climatic variability on *Vibrio parahaemolyticus* outbreaks in Taiwan. International Journal of Environmental Research and Public Health; 13: pii=188 [15 pp.] doi: 10.3390/ijerph13020188.

Huehn S, Eichhorn C, Urmersbach S, Breidenbach J, Bechlars S, Bier N, Alter T, Bartelt E, Frank C, Oberheitmann B, Gunzer F, Brennholt N, Boeer S, Appel B, Dieckmann R and Strauch E (2014) Pathogenic vibrios in environmental, seafood and clinical sources in Germany. International Journal of Medical Microbiology; 304: 843-850.

Hurley C C, Quirke A, Reen F J and Boyd E F (2006) Four genomic islands that mark post-1995 pandemic *Vibrio parahaemolyticus* isolates. BMC Genomics; 7: pii=104 [19 pp.] doi: 10.1186/1471-2164-7-104.

Ichige A, Matsutani S, Oishi K and Mizushima S (1989) Establishment of gene transfer systems for and construction of the genetic map of a marine *Vibrio* strain. Journal of Bacteriology; 171: 1825-1834.

ICMSF (1996) Microorganisms in Food. Volume 5: Microbiological specifications of food pathogens. International Commission on Microbiological Specifications for Foods. Blackie Academic and Professional. London.

Iida T, Park K S, Suthienkul O, Kozawa J, Yamaichi Y, Yamamoto K and Honda T (1998) Close proximity of the *tdh, trh* and *ure* genes on the chromosome of *Vibrio parahaemolyticus*. Microbiology; 144: 2517-2523.

Iwamoto M, Ayers T, Mahon B E and Swerdlow D L (2010) Epidemiology of seafood-associated infections in the United States. Clinical Microbiology Reviews; 23: 399-411.

Jakabi M, Gelli D S, Torre J, Rodas M A B, Franco B, Destro M T and Landgraf M (2003) Inactivation by ionizing radiation of *Salmonella enteritidis, Salmonella infantis*, and *Vibrio parahaemolyticus* in oysters (*Crassostrea brasiliana*). Journal of Food Protection; 66: 1025-1029.

Johnson C N, Bowers J C, Griffitt K J, Molina V, Clostio R W, Pei S, Laws E, Paranjpye R N, Strom M S, Chen A, Hasan N A, Huq A, Noriea N F, III, Grimes D J and Colwell R R (2012) Ecology of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in the coastal and estuarine waters of Louisiana, Maryland, Mississippi, and Washington (United States). Applied and Environmental Microbiology; 78: 7249-7257.

Johnson C N, Flowers A R, Noriea N F, III, Zimmerman A M, Bowers J C, DePaola A and Grimes D J (2010) Relationships between environmental factors and pathogenic vibrios in the Northern Gulf of Mexico. Applied and Environmental Microbiology; 76: 7076-7084.

Jones J L, Kinsey T P, Johnson L W, Porso R, Friedman B, Curtis M, Wesighan P, Schuster R and Bowers J C (2016) Effects of intertidal harvest practices on *Vibrio parahaemolyticus* and *Vibrio vulnificus* levels in oysters. Applied and Environmental Microbiology; 82: 4517-4522.

Jones J L, Ludeke C H, Bowers J C, DeRosia-Banick K, Carey D H and Hastback W (2014) Abundance of *Vibrio cholerae*, *V. vulnificus*, and *V. parahaemolyticus* in oysters (*Crassostrea virginica*) and clams (*Mercenaria mercenaria*) from Long Island sound. Applied and Environmental Microbiology; 80: 7667-7672.

Jones J L, Ludeke C H, Bowers J C, Garrett N, Fischer M, Parsons M B, Bopp C A and DePaola A (2012) Biochemical, serological, and virulence characterization of clinical and oyster *Vibrio parahaemolyticus* isolates. Journal of Clinical Microbiology; 50: 2343-2352.

Joseph S W, Colwell R R and Kaper J B (1982) *Vibrio parahaemolyticus* and related halophilic vibrios. CRC Critical Reviews in Microbiology; 10: 77-124.

Jun J W, Kim H J, Yun S K, Chai J Y and Park S C (2014) Eating oysters without risk of vibriosis: Application of a bacteriophage against *Vibrio parahaemolyticus* in oysters. International Journal of Food Microbiology; 188: 31-35.
Kaneko T and Colwell R R (1973) Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. Journal of Bacteriology; 113: 24-32.
Kaneko T and Colwell R R (1975) Adsorption of *Vibrio parahaemolyticus* onto chitin and copepods. Applied Microbiology; 29: 269-274.
Kaneko T and Colwell R R (1977) The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. Microbial Ecology; 4: 135-155.
Kang C H, Shin Y, Kim W, Kim Y, Song K, Oh E G, Kim S, Yu H and So J S (2016) Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from oysters in Korea. Environmental Science and Pollution Research International; 23: 918-926.
Kaufman G E, Bej A K, Bowers J and DePaola A (2003) Oyster-to-oyster variability in levels of *Vibrio parahaemolyticus*. Journal of Food Protection; 66: 125-129.
Khouadja S, Suffredini E, Spagnoletti M, Croci L, Colombo M M and Amina B (2013) Presence of pathogenic *Vibrio parahaemolyticus* in waters and seafood from the Tunisian Sea. World Journal of Microbiology & Biotechnology; 29: 1341-1348.
Kim Y B, Okuda J, Matsumoto C, Takahashi N, Hashimoto S and Nishibuchi M (1999) Identification of *Vibrio parahaemolyticus* strains at the species level by PCR targeted to the *toxR* gene. Journal of Clinical Microbiology; 37: 1173-1177.
King N and Lake R (2013) Bivalve shellfish harvesting and consumption in New Zealand, 2011: Data for exposure assessment. New Zealand Journal of Marine and Freshwater Research; 47: 62-72.
King N, McCoubrey D-J and Cressey P (2016) Risk Profile: *Vibrio vulnificus* in bivalve molluscan shellfish (with a specific focus on *V. vulnificus* in Pacific oysters). Institute of Environmental Science and Research, Christchurch.
Kinsey T P, Lydon K A, Bowers J C and Jones J L (2015) Effects of dry storage and resubmersion of oysters on total *Vibrio vulnificus* and total and pathogenic (*tdh*+/*trh*+) *Vibrio parahaemolyticus* levels. Journal of Food Protection; 78: 1574-1580.
Kirs M, Depaola A, Fyfe R, Jones J L, Krantz J, Van Laanen A, Cotton D and Castle M (2011) A survey of oysters (*Crassostrea gigas*) in New Zealand for *Vibrio parahaemolyticus* and *Vibrio vulnificus*. International Journal of Food Microbiology; 147: 149-153.
Kirs M, van Laanen A, Cotton D, DePaola Jr A, Jones J, Krantz J, Heuberger A, van Loon N and Fyfe R (2010) A survey of commercially harvested North Island oysters (*Crassostrea gigas*) for *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Prepared for the New Zealand Food Safety Authority by Cawthron (Report No. 1749). Ministry for Primary Industries, Wellington. http://www.foodsafety.govt.nz/elibrary/industry/north-island-oysters.pdf (accessed: 20 June 2016).
Knox T (2006) Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006. Ministry of Agriculture and Forestry (New Zealand Food Safety Authority), Wellington. http://www.foodsafety.govt.nz/elibrary/industry/Animal_Products-Applies_Anyone.pdf (accessed: 18 July 2016).
Kural A G and Chen H (2008) Conditions for a 5-log reduction of *Vibrio vulnificus* in oysters through high hydrostatic pressure treatment. International Journal of Food Microbiology; 122: 180-187.
Lake R, Hudson J and Cressey P (2003) Risk Profile: *Vibrio parahaemolyticus* in seafood. Instutute of Environmental Science and Research Limited, Christchurch. http://www.foodsafety.govt.nz/science-risk/risk-assessment/risk-profiles/ (accessed: 28 June 2016).
Lake R, King N, Sexton K, Bridgewater P and Campbell D (2009) Acute gastrointestinal illness in New Zealand: information from a survey of community and hospital laboratories. New Zealand Medical Journal; 122: 48-54.

Lee J-K, Jung D-W, Eom S-Y, Oh S-W, Kim Y, Kwak H-S and Kim Y-H (2008) Occurrence of *Vibrio parahaemolyticus* in oysters from Korean retail outlets. Food Control; 19: 990-994.

Letchumanan V, Chan K-G and Lee L-H (2014) *Vibrio parahaemolyticus*: A review on the pathogenesis, prevalence, and advance molecular identification techniques. Frontiers in Microbiology; 5: pii=705 [13 pp.] doi: 10.3389/fmicb.2014.00705.

Letchumanan V, Pusparajah P, Tan L T-H, Yin W-F, Lee L-H and Chan K-G (2015) Occurrence and antibiotic resistance of *Vibrio parahaemolyticus* from shellfish in Selangor, Malaysia. Frontiers in Microbiology; 6: pii=1417 [11 pp.] doi: 10.3389/fmicb.2015.01417.

Li H, Liu C, Chen L, Zhang X and Cai J (2011) Biological characterization of two marine *Bdellovibrio*-and-like organisms isolated from Daya bay of Shenzhen, China and their application in the elimination of *Vibrio parahaemolyticus* in oyster. International Journal of Food Microbiology; 151: 36-43.

Liao Y, Li Y, Wu S, Mou J, Xu Z, Cui R, Klena J D, Shi X, Lu Y, Qiu Y, Lin Y, Xie X, Ma H, Li Z, Yu H, Varma J K, Ran L, Hu Q and Cheng J (2015) Risk factors for *Vibrio parahaemolyticus* infection in a southern coastal region of China. Foodborne Pathogens and Disease; 12: 881-886.

Liu B, Liu H, Pan Y, Xie J and Zhao Y (2016) Comparison of the effects of environmental parameters on the growth variability of *Vibrio parahaemolyticus* coupled with strain sources and genotypes analyses. Frontiers in Microbiology; 7: pii= 994 [11 pp.] doi: 10.3389/fmicb.2016.00994.

Liu C, Lu J and Su Y-C (2009) Effects of flash freezing, followed by frozen storage, on reducing *Vibrio parahaemolyticus* in Pacific Raw Oysters (*Crassostrea gigas*). Journal of Food Protection; 72: 174-177.

Lopez-Hernandez K M, Pardio-Sedas V T, Lizarraga-Partida L, Williams Jde J, Martinez-Herrera D, Flores-Primo A, Uscanga-Serrano R and Rendon-Castro K (2015) Environmental parameters influence on the dynamics of total and pathogenic *Vibrio parahaemolyticus* densities in *Crassostrea virginica* harvested from Mexico's Gulf coast. Marine Pollution Bulletin; 91: 317-329.

Lopez-Joven C, de Blas I, Dolores Furones M and Roque A (2015) Prevalences of pathogenic and non-pathogenic *Vibrio parahaemolyticus* in mollusks from the Spanish Mediterranean Coast. Frontiers in Microbiology; 6: pii=736 [10 pp.] doi: 10.3389/fmicb.2015.00736.

Loyola D E, Navarro C, Uribe P, Garcia K, Mella C, Diaz D, Valdes N, Martinez-Urtaza J and Espejo R T (2015) Genome diversification within a clonal population of pandemic *Vibrio parahaemolyticus* seems to depend on the life circumstances of each individual bacteria. BMC Genomics; 16: pii=176 [10 pp.] doi: 10.1186/s12864-015-1385-8.

Lozano-Leon A, Torres J, Osorio C R and Martinez-Urtaza J (2003) Identification of *tdh*-positive *Vibrio parahaemolyticus* from an outbreak associated with raw oyster consumption in Spain. FEMS Microbiology Letters; 226: 281-284.

Lüdeke C H M, Gonzalez-Escalone N, Fischer M and Jones J L (2015) Examination of clinical and environmental *Vibrio parahaemolyticus* isolates by multi-locus sequence typing (MLST) and multiple-locus variable-number tandem-repeat analysis (MLVA). Frontiers in Microbiology; 6: pii=564 [10 pp.] doi: doi: 10.3389/fmicb.2015.00564.

Lydon K A, Farrell-Evans M and Jones J L (2015) Evaluation of ice slurries as a control for postharvest growth of *Vibrio* spp. in oysters and potential for filth contamination. Journal of Food Protection; 78: 1375-1379.

Ma L and Su Y C (2011) Validation of high pressure processing for inactivating *Vibrio parahaemolyticus* in Pacific oysters (*Crassostrea gigas*). International Journal of Food Microbiology; 144: 469-474.

MAF Biosecurity (2004) Import health standard for the importation into New Zealand of marine fisheries products for human consumption from the European Community. Ministry of Agriculture and Forestry Biosecurity New Zealand, Wellington.

http://www.mpi.govt.nz/importing/food/seafood/requirements/ (accessed: 19 July 2016).
MAF Biosecurity (2008) Import health standard for the importation of marine fisheries products for human consumption from all countries. Ministry of Agriculture and Forestry Biosecurity New Zealand, Wellington. http://www.mpi.govt.nz/importing/food/seafood/requirements/ (accessed: 19 July 2016).
Mahmoud B S and Burrage D D (2009) Inactivation of *Vibrio parahaemolyticus* in pure culture, whole live and half shell oysters (*Crassostrea virginica*) by x-ray. Letters in Applied Microbiology; 48: 572-578.
Mahmoud B S M (2014) The efficacy of grape seed extract, citric acid and lactic acid on the inactivation of *Vibrio parahaemolyticus* in shucked oysters. Food Control; 41: 13-16.
Mahoney J C, Gerding M J, Jones S H and Whistler C A (2010) Comparison of the pathogenic potentials of environmental and clinical *Vibrio parahaemolyticus* strains indicates a role for temperature regulation in virulence. Applied and Environmental Microbiology; 76: 7459-7465.
Malcolm T T H, Cheah Y K, Radzi C W J W M, Abu Kasim F, Kantilal H K, John T Y H, Martinez-Urtaza J, Nakaguchi Y, Nishibuchi M and Son R (2015) Detection and quantification of pathogenic *Vibrio parahaemolyticus* in shellfish by using multiplex PCR and loop-mediated isothermal amplification assay. Food Control; 47: 664-671.
Manaaki Taha Moana Research Team (2012) Factors affecting populations of toheroa (*Paphies ventricosa*): A literature review. Massey University, Palmerston North. http://www.mtm.ac.nz/wp-content/uploads/2015/03/factors-affecting-toheroa-lit-review_taiao-raukawa.pdf (accessed: 28 July 2016).
Mannas H, Mimouni R, Chaouqy N, Hamadi F and Martinez-Urtaza J (2014) Occurrence of *Vibrio* and *Salmonella* species in mussels (*Mytilus galloprovincialis*) collected along the Moroccan Atlantic coast. Springerplus; 3: pii=265 [11 pp.] doi: 10.1186/2193-1801-3-265.
Marques A, Nunes M L, Moore S K and Strom M S (2010) Climate change and seafood safety: Human health implications. Food Research International; 43: 1766-1779.
Martinez-Urtaza J, Baker-Austin C, Jones J L, Newton A E, Gonzalez-Aviles G D and DePaola A (2013) Spread of Pacific Northwest *Vibrio parahaemolyticus* strain. New England Journal of Medicine; 369: 1573-1574.
Martinez-Urtaza J, Bowers J C, Trinanes J and DePaola A (2010) Climate anomalies and the increasing risk of *Vibrio parahaemolyticus* and *Vibrio vulnificus* illnesses. Food Research International; 43: 1780-1790.
Martinez-Urtaza J, Huapaya B, Gavilan R G, Blanco-Abad V, Ansede-Bermejo J, Cadarso-Suarez C, Figueiras A and Trinanes J (2008a) Emergence of Asiatic *Vibrio* diseases in South America in phase with El Niño. Epidemiology; 19: 829-837.
Martinez-Urtaza J, Lozano-Leon A, Varela-Pet J, Trinanes J, Pazos Y and Garcia-Martin O (2008b) Environmental determinants of the occurrence and distribution of *Vibrio parahaemolyticus* in the rias of Galicia, Spain. Applied and Environmental Microbiology; 74: 265-274.
Mathurand P and Schaffner D W (2013) Effect of lime juice on *Vibrio parahaemolyticus* and *Salmonella enterica* inactivation during the preparation of the raw fish dish ceviche. Journal of Food Protection; 76: 1027-1030.
McLaughlin J B, DePaola A, Bopp C A, Martinek K A, Napolilli N P, Allison C G, Murray S L, Thompson E C, Bird M M and Middaugh J P (2005) Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. New England Journal of Medicine; 353: 1463-1470.
Melody K, Senevirathne R, Janes M, Jaykus L A and Supan J (2008) Effectiveness of icing as a postharvest treatment for control of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in the eastern oyster (*Crassostrea virginica*). Journal of Food Protection; 71: 1475-1480.

Miles D W, Ross T, Olley J and McMeekin T A (1997) Development and evaluation of a predictive model for the effect of temperature and water activity on the growth rate of *Vibrio parahaemolyticus*. International Journal of Food Microbiology; 38: 133-142.
Ministry of Health (1995) Microbiological reference criteria for food. New Zealand Ministry of Health, Wellington. http://www.foodsafety.govt.nz/elibrary/industry/Microbiological_Reference-Guide_Assess.pdf (accessed: 13 October 2016).
Ministry of Health (2003) NZ Food NZ Children. Key results of the 2002 National Children's Nutrition Survey. Ministry of Health, Wellington. https://www.health.govt.nz/system/files/documents/publications/nzfoodnzchildren.pdf (accessed: 27 July 2016).
Ministry of Health (2013) List of diseases notifiable to the Medical Officer of Health. New Zealand Ministry of Health, Wellington. http://www.health.govt.nz/our-work/diseases-and-conditions/notifiable-diseases (accessed: 11 July 2016).
Mizunoe Y, Wai S N, Ishikawa T, Takade A and Yoshida S (2000) Resuscitation of viable but nonculturable cells of *Vibrio parahaemolyticus* induced at low temperature under starvation. FEMS Microbiology Letters; 186: 115-120.
Moore J G, Ruple A, Ballenger-Bass K, Bell S, Pennington P L and Scott G I (2014) Snapshot of *Vibrio parahaemolyticus* densities in open and closed shellfish beds in Coastal South Carolina and Mississippi. Environmental Monitoring and Assessment; 186: 7949-7960.
Morris J G (2003) Cholera and other types of vibriosis: A story of human pandemics and oysters on the half shell. Clinical Infectious Diseases; 37: 272-280.
MPI (2014) Fisheries (Conversion Factors) Notice 2014 (Notice No: MPI 392). New Zealand Gazette (supplement); 115: 3249-3297.
MPI (2016a) Importing food. Food Notice. Issued 1 March 2016. Ministry for Primary Industries, Wellington. https://www.mpi.govt.nz/document-vault/10685 (accessed: 19 July 2016).
MPI (2016b) Importing food into New Zealand. Guidance document. Issued 22 February 2016. Ministry for Primary Industries, Wellington. http://www.mpi.govt.nz/document-vault/11416 (accessed: 19 July 2016).
MPI (2016c) Meeting requirements as a registered food importer. Guidance document. Issued 23 February 2016. Ministry for Primary Industries, Wellington. http://www.mpi.govt.nz/document-vault/11413 (accessed: 19 July 2016).
MPI (2016d) Application of annual levies to primary processors of fish and bivalve molluscan shellfish. Ministry for Primary Industries, Wellington. http://www.foodsafety.govt.nz/elibrary/industry/Application_Revised-Describes_Requirements.htm (accessed: 1 June 2016).
Mudoh M F, Parveen S, Schwarz J, Rippen T and Chaudhuri A (2014) The effects of storage temperature on the growth of *Vibrio parahaemolyticus* and organoleptic properties in oysters. Frontiers in Public Health; 2: pii=45 [7 pp.] doi: 10.3389/fpubh.2014.00045.
Muntada-Garriga J M, Rodriguez-Jerez J J, Lopez-Sabater E I and Mora-Ventura M T (1995) Effect of chill and freezing temperatures on survival of *Vibrio parahaemolyticus* inoculated in homogenates of oyster meat. Letters in Applied Microbiology; 20: 225-227.
Nair G B, Abraham M and Natarajan R (1980) Distribution of *Vibrio parahaemolyticus* in finfish harvested from Porto Novo (S. India) environs: A seasonal study. Canadian Journal of Microbiology; 26: 1264-1269.
Nair G B, Ramamurthy T, Bhattacharya S K, Dutta B, Takeda Y and Sack D A (2007) Global dissemination of *Vibrio parahaemolyticus* serotype O3:K6 and its serovariants. Clinical Microbiology Reviews; 20: 39-48.
Natarajan R, Abraham M and Nair G B (1980) Distribution of *Vibrio parahaemolyticus* in Porto Novo environment. Indian Jounral of Medical Research; 71: 679-687.
National Shellfish Sanitation Program (2011) Guide for the control of molluscan shellfish. 2011 revision. United States Food and Drug Administration, Silver Stream, MD.

http://www.fda.gov/downloads/Food/GuidanceRegulation/FederalStateFoodPrograms/UCM350344.pdf (accessed: 19 July 2016).
NC Division of Public Health (2012) North Carolina Communicable Disease Manual. Diseases and conditions reportable in North Carolina. Disease notes: Vibrio infection (other than cholera and vulnificus). North Carolina Department of Health and Human Services, North Carolina Public Health, Raleigh, NC. http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/toc.html (accessed: 15 September 2016).
Newton A E, Garrett N, Stroika S G, Halpin J L, Turnsek M and Mody R K (2014) Increase in *Vibrio parahaemolyticus* infections associated with consumption of Atlantic Coast shellfish - 2013. Morbidity and Mortality Weekly Report; 63: 335-336.
Nigro O D and Steward G F (2015) Differential specificity of selective culture media for enumeration of pathogenic vibrios: Advantages and limitations of multi-plating methods. Journal of Microbiological Methods; 111: 24-30.
Nilsson W B and Turner J W (2016) The thermostable direct hemolysin-related hemolysin (*trh*) gene of *Vibrio parahaemolyticus*: Sequence variation and implications for detection and function. Journal of Microbiological Methods; 126: 1-7.
Nishibuchi M and Kaper J B (1995) Thermostable direct hemolysin gene of *Vibrio parahaemolyticus*: a virulence gene acquired by a marine bacterium. Infection and Immunity; 63: 2093-2099.
NIWA (2011) Risk assessment of contaminants in traditional food sources. National Institute of Water and Atmospheric Research, Hamilton. https://www.niwa.co.nz/freshwater-and-estuaries/research-projects/risk-assessment-of-contaminants-in-traditional-food-sources (accessed: 27 July 2016).
Nordstrom J L, Kaysner C A, Blackstone G M, Vickery M C, Bowers J C and DePaola A (2004) Effect of intertidal exposure on *Vibrio parahaemolyticus* levels in Pacific Northwest oysters. Journal of Food Protection; 67: 2178-2182.
Noriea N F, III, Johnson C N, Griffitt K J and Grimes D J (2010) Distribution of type III secretion systems in *Vibrio parahaemolyticus* from the northern Gulf of Mexico. Journal of Applied Microbiology; 109: 953-962.
Normanno G, Parisi A, Addante N, Quaglia N C, Dambrosio A, Montagna C and Chiocco D (2006) *Vibrio parahaemolyticus, Vibrio vulnificus* and microorganisms of fecal origin in mussels (*Mytilus galloprovincialis*) sold in the Puglia region (Italy). International Journal of Food Microbiology; 106: 219-222.
Nydam S D, Shah D H and Call D R (2014) Transcriptome analysis of *Vibrio parahaemolyticus* in type III secretion system 1 inducing conditions. Frontiers in Cellular and Infection Microbiology; 4: pii=1 [15 pp.] doi: 10.3389/fcimb.2014.00001.
Odeyemi O A (2016) Incidence and prevalence of *Vibrio parahaemolyticus* in seafood: a systematic review and meta-analysis. Springerplus; 5: pii=464 [17 pp.] doi: 10.1186/s40064-016-2115-7.
Ottaviani D, Leoni F, Rocchegiani E, Canonico C, Potenziani S, Santarelli S, Masini L, Mioni R and Carraturo A (2010a) Prevalence, serotyping and molecular characterization of *Vibrio parahaemolyticus* in mussels from Italian growing areas, Adriatic Sea. Environmental Microbiology Reports; 2: 192-197.
Ottaviani D, Leoni F, Rocchegiani E, Canonico C, Potenziani S, Santarelli S, Masini L, Scuota S and Carraturo A (2010b) *Vibrio parahaemolyticus*-associated gastroenteritis in Italy: Persistent occurrence of O3:K6 pandemic clone and emergence of O1:KUT serotype. Diagnostic Microbiology and Infectious Disease; 66: 452-455.
Ottaviani D, Leoni F, Serra R, Serracca L, Decastelli L, Rocchegiani E, Masini L, Canonico C, Talevi G and Carraturo A (2012) Nontoxigenic *Vibrio parahaemolyticus* strains causing acute gastroenteritis. Journal of Clinical Microbiology; 50: 4141-4143.
Ottaviani D, Santarelli S, Bacchlocchi S, Masini L, Ghittino C and Bacchiocchi I (2005) Presence of pathogenic *Vibrio parahaemolyticus* strains in mussels from the Adriatic Sea, Italy. Food Microbiology; 22: 585-590.

Paranjpye R, Hamel O S, Stojanovski A and Liermann M (2012) Genetic diversity of clinical and environmental *Vibrio parahaemolyticus* strains from the Pacific Northwest. Applied and Environmental Microbiology; 78: 8631-8638.

Parveen S, DaSilva L, DePaola A, Bowers J, White C, Munasinghe K A, Brohawn K, Mudoh M and Tamplin M (2013) Development and validation of a predictive model for the growth of Vibrio parahaemolyticus in post-harvest shellstock oysters. International Journal of Food Microbiology; 161: 1-6.

Parveen S, Hettiarachchi K A, Bowers J C, Jones J L, Tamplin M L, McKay R, Beatty W, Brohawn K, Dasilva L V and Depaola A (2008) Seasonal distribution of total and pathogenic *Vibrio parahaemolyticus* in Chesapeake Bay oysters and waters. International Journal of Food Microbiology; 128: 354-61.

Passalacqua P L, Zavatta E, Bignami G, Serraino A and Serratore P (2016) Occurrence of *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus* in the clam *Ruditapes philippinarum* (Adams & Reeve, 1850) from Emilia Romagna and Sardinia, Italy. Italian Journal of Food Safety; 5: pii=5709 [6 pp.] doi: 10.4081/ijfs.2016.5709.

Payinda G (2008) Necrotizing fasciitis due to *Vibrio parahaemolyticus*. New Zealand Medical Journal; 121: 99-101.

Paz S, Bisharat N, Paz E, Kidar O and Cohen D (2007) Climate change and the emergence of *Vibrio vulnificus* disease in Israel. Environmental Research; 103: 390-396.

Pereira M A, Nunes M M, Nuernberg L, Schulz D and Batista C R V (2006) Microbiological quality of oysters (*Crassostrea gigas*) produced and commercialized in the coastal region of Florianopolis - Brazil. Brazilian Journal of Microbiology; 37: 159-163.

Phuvasate S and Su Y C (2015) Efficacy of low-temperature high hydrostatic pressure processing in inactivating *Vibrio parahaemolyticus* in culture suspension and oyster homogenate. International Journal of Food Microbiology; 196: 11-15.

Pien F, Lee K and Higa H (1977) *Vibrio alginolyticus i*nfections in Hawaii. Journal of Clinical Microbiology; 5: 670-672.

Powell A, Baker-Austin C, Wagley S, Bayley A and Hartnell R (2013) Isolation of pandemic *Vibrio parahaemolyticus* from UK water and shellfish produce. Microbial Ecology; 65: 924-927.

Pradhan J, Das B K, Sahu S, Marhual N P, Swain A K, Mishra B K and Eknath A E (2012) Traditional antibacterial activity of freshwater microalga *Spirulina platensis* to aquatic pathogens. Aquaculture Research; 43: 1287-1295.

Qi X L, Wang H X, Bu S R, Xu X G, Wu X Y and Lin D F (2016) Incidence rates and clinical symptoms of *Salmonella*, *Vibrio parahaemolyticus*, and *Shigella* infections in China, 1998–2013. The Journal of Infection in Developing Countries; 10: 127-133.

Raghunath P (2011) Genetic markers of pandemic *Vibrio parahaemolyticus*: Are they truly unique? Foodborne Pathogens and Disease; 8: 653-654.

Raghunath P, Karunasagar I and Karunasagar I (2009) Improved isolation and detection of pathogenic *Vibrio parahaemolyticus* from seafood using a new enrichment broth. International Journal of Food Microbiology; 129: 200-203.

Ramos R J, Miotto L A, Miotto M, Silveira Junior N, Cirolini A, da Silva H S, Rodrigues D d P and Werneck Vieira C R (2014) Occurrence of potentially pathogenic *Vibrio* in oysters (*Crassostrea gigas*) and waters from bivalve mollusk cultivations in the South Bay of Santa Catarina. Revista Da Sociedade Brasileira De Medicina Tropical; 47: 327-333.

Ramos R J, Miotto M, Lagreze Squella F J, Cirolini A, Ferreira J F and Werneck Vieira C R (2012a) Depuration of oysters (*Crassostrea gigas*) contaminated with *Vibrio parahaemolyticus* and *Vibrio vulnificus* with UV light and chlorinated seawater. Journal of Food Protection; 75: 1501-1506.

Ramos R J, Pereira M A, Miotto L A, Faria R D A, Silveira Junior N and Werneck Vieira C R (2012b) Ocurrence of *Vibrio* spp., positive coagulase staphylococci and enteric bacteria in oysters (*Crassostrea gigas*) harvested in the south bay of Santa Catarina island, Brazil. Ciencia E Tecnologia De Alimentos; 32: 478-484.

Richards G P, Fay J P, Dickens K A, Parent M A, Soroka D S and Boyd E F (2012) Predatory bacteria as natural modulators of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in seawater and oysters. Applied and Environmental Microbiology; 78: 7455-7466.

Richards G P, Fay J P, Uknalis J, Olanya O M and Watson M A (2016) Purification and host specificity of predatory *Halobacteriovorax i*solates from seawater. Applied and Environmental Microbiology; 82: 922-927.

Robert-Pillot A, Copin S, Himber C, Gay M and Quilici M-L (2014) Occurrence of the three major *Vibrio* species pathogenic for human in seafood products consumed in France using real-time PCR. International Journal of Food Microbiology; 189: 75-81.

Rosec J-P, Causse V, Cruz B, Rauzier J and Carnat L (2012) The international standard ISO/TS 21872-1 to study the occurence of total and pathogenic *Vibrio parahaemolyticus* and *Vibrio cholerae* in seafood: ITS improvement by use of a chromogenic medium and PCR. International Journal of Food Microbiology; 157: 189-194.

Russell D G, Parnell W R, Wilson N C, Faed J, Ferguson E, Herbison P, Horwath C, Nye T, Reid P, Walker R, Wilson B and Tukuitonga C (1999) NZ Food: NZ People. Ministry of Health, Wellington. http://www.moh.govt.nz/NoteBook/nbbooks.nsf/0/62C5D9D4C418C4E74C2567D9007186C2/$file/nns.pdf (accessed: 27 July 2016).

Rykovskaya O A, Mazrukho A B, Smolikova L M, Monakhova E V, Chemisova O S, Podoinitsyna O A, Allenov A V, Khomenko T V, Zhirov A Y, Sanamyants E M, Gaevskaya N E, Kudryakova T A, Dalikova R R and Sagakyants M M (2014) O3:K6 serogroup *Vibrio parahaemolyticus* - the causative agent of food toxic infection outbreaks in Primorsky region of Russian federation. Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii; (4): 57-61.

Sagoo S K, Little C L and Greenwood M (2007) Microbiological study of cooked crustaceans and molluscan shellfish from UK production and retail establishments. International Journal of Environmental Health Research; 17: 219-230.

Saito S, Iwade Y, Tokuoka E, Nishio T, Otomo Y, Araki E, Konuma H, Nakagawa H, Tanaka H, Sugiyama K, Hasegawa A, Sugita-Konishi Y and Hara-Kudo Y (2015) Epidemiological evidence of lesser role of Thermostable Direct Hemolysin (TDH)-Related Hemolysin (TRH) than TDH on *Vibrio parahaemolyticus* pathogenicity. Foodborne Pathogens and Disease; 12: 131-138.

Scallan E, Hoekstra R, Angulo F, Tauxe R, Widdowson M, Roy S, Jones J and Griffin P (2011) Foodborne illness acquired in the United States - major pathogens. Emerging Infectious Diseases; 17: 7-15.

Seafood New Zealand (2015) New Zealand Seafood Exports: Report 10a, seafood exports by species by country, calendar year to December 2015 (final). Seafood New Zealand, Wellington. http://www.seafoodnewzealand.org.nz/publications/export-information/export-statistics/item/january-december-2015/ (accessed: 1 June 2016).

Shaw K S, Jacobs J M and Crump B C (2014) Impact of hurricane Irene on *Vibrio vulnificus* and *Vibrio parahaemolyticus* concentrations in surface water, sediment, and cultured oysters in the Chesapeake Bay, MD, USA. Frontiers in Microbiology; 5: pii=204 [10 pp.] doi: 10.3389/fmicb.2014.00204.

Shen X, Cai Y, Liu C, Liu W, Hui Y and Su Y-C (2009) Effect of temperature on uptake and survival of *Vibrio parahaemolyticus* in oysters (*Crassostrea plicatula*). International Journal of Food Microbiology; 136: 129-132.

Silvester R, Alexander D, Santha S and Hatha M (2016) RAPD PCR discloses high genetic heterogeneity among *Vibrio parahaemolyticus* from various environments along the southwest coast of India. Annals of Microbiology; 66: 925-929.

Simmons G, Garbutt C, Hewitt J and Greening G (2007) A New Zealand outbreak of norovirus gastroenteritis linked to the consumption of imported raw Korean oysters. New Zealand Medical Journal; 120: U2773.

Sims J N, Isokpehi R D, Cooper G A, Bass M P, Brown S D, St John A L, Gulig P A and Cohly H H (2011) Visual analytics of surveillance data on foodborne vibriosis, United States, 1973-2010. Environmental Health Insights; 5: 71-85.
Slayton R B, Newton A E, Depaola A, Jones J L and Mahon B E (2014) Clam-associated vibriosis, USA, 1988–2010. Epidemiology and Infection; 142: 1083-1088.
Sobrinho P S C, Destro M T, Franco B D and Landgraf M (2010) Correlation between environmental factors and prevalence of *Vibrio parahaemolyticus* in oysters harvested in the southern coastal area of Sao Paulo State, Brazil. Applied and Environmental Microbiology; 76: 1290-1293.
Sobrinho P S C, Destro M T, Franco B D and Landgraf M (2014) A quantitative risk assessment model for *Vibrio parahaemolyticus* in raw oysters in Sao Paulo State, Brazil. International Journal of Food Microbiology; 180: 69-77.
Son N T and Fleet G H (1980) Behavior of pathogenic bacteria in the oyster, *Crassostrea commercialis*, during depuration, re-laying, and storage. Applied and Environmental Microbiology; 40: 994-1002.
Strom M S and Paranjpye R N (2000) Epidemiology and pathogenesis of *Vibrio vulnificus*. Microbes and Infection; 2: 177-188.
Su Y C, Yang Q and Hase C (2010) Refrigerated seawater depuration for reducing *Vibrio parahaemolyticus* contamination in Pacific oyster (*Crassostrea gigas*). Journal of Food Protection; 73: 1111-1115.
Suffredini E, Mioni R, Mazzette R, Bordin P, Serratore P, Fois F, Piano A, Cozzi L and Croci L (2014) Detection and quantification of *Vibrio parahaemolyticus* in shellfish from Italian production areas. International Journal of Food Microbiology; 184: 14-20.
Sun Y and Oliver J D (1995) Hot sauce - no elimination of *Vibrio-vulnificus* in oysters. Journal of Food Protection; 58: 441-442.
Tamplin M L (1994) The seasonal occurrence of *Vibrio vulnificus* in shellfish, seawater, and sediment of United States coastal waters and the inffuence of environmental factors on survival and virulence. Final report to Salstonstall-Kennedy Grant Program. Project NA27FD0117-01. United States Department of Commerce, Seattle.
Tamura N, Kobayashi S, Hashimoto H and Hirose S (1993) Reactive arthritis induced by *Vibrio-parahaemolyticus*. Journal of Rheumatology; 20: 1062-1063.
Tang L, Wang H, Luo Y, Huang L, Gong H, Zhang H and Wang J (2012) Surveillance and molecular characterization of *Vibrio parahaemolyticus* from different sources in Zhoushan Islands, China. Journal of Animal and Veterinary Advances; 11: 3635-3640.
Terzi G, Buyuktanir O and Yurdusev N (2009) Detection of the tdh and trh genes in *Vibrio parahaemolyticus* isolates in fish and mussels from Middle Black Sea Coast of Turkey. Letters in Applied Microbiology; 49: 757-763.
Thornton V, Hazell W and Simmons G (2002) Acute gastroenteritis associated with seafood privately imported from the Pacific Islands. New Zealand Medical Journal; 115: 234-236.
Thupila N, Ratana-arporn P and Wilaipun P (2011) Radiation resistances and decontamination of common pathogenic bacteria contaminated in white scar oyster (*Crassostrea belcheri*) in Thailand. Radiation Physics and Chemistry; 80: 828-832.
Turner J W, Paranjpye R N, Landis E D, Biryukov S V, Gonzalez-Escalona N, Nilsson W B and Strom M S (2013) Population structure of clinical and environmental *Vibrio parahaemolyticus* from the Pacific Northwest coast of the United States. Plos One; 8: pii=e55726 [10 pp.] doi:10.1371/journal.pone.0055726.
Turner N, Cressey P, Lake R and Whyte R (2005) Review of non-commercial wild food in New Zealand. Report to the New Zealand Food Safety Authority from the Institute of Environmental Science & Research Ltd. New Zealand Food Safety Authority, Wellington. (accessed:
Ueno H, Tomari K, Kikuchi K, Kobori S and Miyazaki M (2016) The first report of *Vibrio parahaemolyticus* strain O10:K60 in Japan, a new combination of O and K serotypes

isolated from a patient with gastroenteritis. Japanese Journal of Infectious Diseases; 69: 28-32.
University of Otago and Ministry of Health (2011) A focus on nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey. Ministry of Health, Wellington. http://www.health.govt.nz/publication/focus-nutrition-key-findings-2008-09-nz-adult-nutrition-survey (accessed: 27 July 2016).
Urquhart E A, Jones S H, Yu J W, Schuster B M, Marcinkiewicz A L, Whistler C A and Cooper V S (2016) Environmental conditions associated with elevated *Vibrio parahaemolyticus* concentrations in Great Bay Estuary, New Hampshire. Plos One; 11: pii=e0155018 [15 pp.] doi: 10.1371/journal.pone.0155018.
USFDA (1994) Proceedings of the 1994 *Vibrio vulnificus* workshop. June 15-26, 1994, Washington DC. United States Food and Drug Administration and the National Marine Fisheries Service.
USFDA (2005a) Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters (interpretive summary). United States Food and Drug Administration, Silver Spring, MD. http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm050421.htm (accessed: 26 July 2016).
USFDA (2005b) Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters. United States Food and Drug Administration, Silver Spring, MD. http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm050421.htm (accessed: 26 July 2016).
USFDA (2011) Fish and fishery products hazards and controls guidance. Fourth edition, April 2011. United States Department of Health and Human Services, United States Food and Drug Administration, Silver Spring, MD. http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM251970.pdf (accessed: 19 July 2016).
USFDA (2012) Bad bug book. Handbook of foodborne pathogenic microorganisms and natural toxins (2nd edition). United States Food and Drug Administration, Silverspring, MD. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/default.htm (accessed: 28 June 2016).
Vasconcelos G J, Stang W J and Laidlaw R H (1975) Isolation of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* from estuarine areas of Southeastern Alaska. Applied Microbiology; 29: 557-559.
Veenstra J, Rietra P J G M, Coster J M, Slaats E and Dirks-Go S (1994) Seasonal variations in the occurrence of *Vibrio vulnificus* along the Dutch coast. Epidemiology and Infection; 112: 285-290.
Venkateswaran K, Kiiyukia C, Nakanishi K, Nakano H, Matsuda O and Hashimoto H (1990) The role of sinking particles in the overwintering process of *Vibrio parahaemolyticus* in a marine environment. FEMS Microbiology Ecology; 6: 159-166.
Vernocchi P, Maffei M, Lanciotti R, Suzzi G and Gardini F (2007) Characterization of Mediterranean mussels (*Mytilus galloprovincialis*) harvested in Adriatic Sea (Italy). Food Control; 18: 1575-1583.
Vezzulli L, Grande C, Reid P C, Hélaouët P, Edwards M, Höfle M G, Brettar I, Colwell R R and Pruzzo C (2016) Climate influence on *Vibrio* and associated human diseases during the past half-century in the coastal North Atlantic. Proceedings of the National Academy of Sciences; 113: E5062-E5071.
Volety A K, McCarthy S A, Tall B D, Curtis S K, Fisher W S and Genthner F J (2001) Responses of oyster *Crassostrea virginica* hemocytes to environmental and clinical isolates of *Vibrio parahaemolyticus*. Aquatic Microbial Ecology; 25: 11-20.
Volety A K, Oliver L M, Genthner F J and Fisher W S (1999) A rapid tetrazolium dye reduction assay to assess the bactericidal activity of oyster (*Crassostrea virginica*) hemocytes against *Vibrio parahaemolyticus*. Aquaculture; 172: 205-222.

Vuddhakul V, Chowdhury A, Laohaprertthisan V, Pungrasamee P, Patararungrong N, Thianmontri P, Ishibashi M, Matsumoto C and Nishibuchi M (2000) Isolation of a pandemic O3:K6 clone of a *Vibrio parahaemolyticus* strain from environmental and clinical sources in Thailand. Applied and Environmental Microbiology; 66: 2685-2689.

Wagley S, Koofhethile K, Wing J B and Rangdale R (2008) Comparison of *V. parahaemolyticus* isolated from seafoods and cases of gastrointestinal disease in the UK. International Journal of Environmental Health Research; 18: 283-293.

Wang C-Y, Huang H-W, Hsu C-P, Shyu Y-T and Yang B B (2013) Inactivation and morphological damage of *Vibrio parahaemolyticus* treated with high hydrostatic pressure. Food Control; 32: 348-353.

Wang D, Yu S, Chen W, Zhang D and Shi X (2010a) Enumeration of *Vibrio parahaemolyticus* in oyster tissues following artificial contamination and depuration. Letters in Applied Microbiology; 51: 104-108.

Wang D, Zhang D, Chen W, Yu S and Shi X (2010b) Retention of *Vibrio parahaemolyticus* in oyster tissues after chlorine dioxide treatment. International Journal of Food Microbiology; 137: 76-80.

Wang H Z, Wong M M, O'Toole D, Mak M M, Wu R S and Kong R Y (2006) Identification of a DNA methyltransferase gene carried on a pathogenicity island-like element (VPAI) in *Vibrio parahaemolyticus* and its prevalence among clinical and environmental isolates. Applied and Environmental Microbiology; 72: 4455-4460.

Wang Y, Li D, Wang Y, Li K and Ye C (2016) Rapid and sensitive detection of *Vibrio parahaemolyticus* and *Vibrio vulnificus* by Multiple Endonuclease Restriction Real-Time Loop-Mediated Isothermal Amplification Technique. Molecules; 21: pii=E111 [16 pp.] doi: 10.3390/molecules21010111.

Weis K E, Hammond R M, Hutchinson R and Blackmore C G M (2011) *Vibrio* illness in Florida, 1998-2007. Epidemiology and Infection; 139: 591-598.

Whyte A L, Hook G R, Greening G E, Gibbs-Smith E and Gardner J P (2009) Human dietary exposure to heavy metals via the consumption of greenshell mussels (*Perna canaliculus* Gmelin 1791) from the Bay of Islands, northern New Zealand. Science of the Total Environment; 407: 4348-4355.

Wong H C, Chang C N and Chen M Y (2004a) Effects of heat, acid, and freeze-thaw challenges on survival of starved *Vibrio parahaemolyticus* in minimal salt medium, tryptic soy broth, and filtered oyster homogenate medium. Journal of Food Protection; 67: 1243-1246.

Wong H C, Shen C T, Chang C N, Lee Y S and Oliver J D (2004b) Biochemical and virulence characterization of viable but nonculturable cells of *Vibrio parahaemolyticus*. Journal of Food Protection; 67: 2430-2435.

Wong H C and Wang P (2004) Induction of viable but nonculturable state in *Vibrio parahaemolyticus* and its susceptibility to environmental stresses. Journal of Applied Microbiology; 96: 359-366.

Wu J, Mou H, Xue C, Leung A W, Xu C and Tanga Q J (2016) Photodynamic effect of curcumin on *Vibrio parahaemolyticus*. Photodiagnosis and Photodynamic Therapy; 15: 34-39.

Wu Y, Wen J, Ma Y, Ma X and Chen Y (2014) Epidemiology of foodborne disease outbreaks caused by *Vibrio parahaemolyticus*, China, 2003-2008. Food Control; 46: 197-202.

Wynne-Jones J, Gray A, Hill L and Heinemann A (2014) National panel survey of marine recreational fishers 2011-12: Harvest estimates. New Zealand Fisheries Assessment Report 2014/67. Ministry for Primary Industries, Wellington. http://fs.fish.govt.nz/Doc/23718/FAR_2014_67_2847_MAF2010-01.pdf.ashx (accessed: 1 June 2016).

Xi D, Liu C and Su Y-C (2012) Effects of green tea extract on reducing *Vibrio parahaemolyticus* and increasing shelf life of oyster meats. Food Control; 25: 368-373.

Xu F, Ilyas S, Hall J A, Jones S H, Cooper V S and Whistler C A (2015) Genetic characterization of clinical and environmental *Vibrio parahaemolyticus* from the

Northeast USA reveals emerging resident and non-indigenous pathogen lineages. Frontiers in Microbiology; 6: pii=272 [15 pp.] doi: 10.3389/fmicb.2015.00272.
Yamamoto A, Iwahori J, Vuddhakul V, Charernjiratragul W, Vose D, Osaka K, Shigematsu M, Toyofuku H, Yamamoto S, Nishibuchi M and Kasuga F (2008) Quantitative modeling for risk assessment of *Vibrio parahaemolyticus* in bloody clams in southern Thailand. International Journal of Food Microbiology; 124: 70-78.
Yan W X, Dai Y, Zhou Y J, Liu H, Duan S G, Han H H and Chen Y (2015) Risk factors for sporadic *Vibrio parahaemolyticus* gastroenteritis in east China: a matched case-control study. Epidemiology and Infection; 143: 1020-1028.
Yanez R, Bastias R, Higuera G, Salgado O, Katharios P, Romero J, Espejo R and Garcia K (2015) Amplification of *tlh* gene in other Vibrionaceae specie by specie-specific multiplex PCR of *Vibrio parahaemolyticus*. Electronic Journal of Biotechnology; 18: 459-463.
Ye M, Huang Y and Chen H (2012) Inactivation of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in oysters by high-hydrostatic pressure and mild heat. Food Microbiology; 32: 179-184.
Ye M, Huang Y, Gurtler J B, Niemira B A, Sites J E and Chen H (2013) Effects of pre- or post-processing storage conditions on high-hydrostatic pressure inactivation of *Vibrio parahaemolyticus* and *V. vulnificus* in oysters. International Journal of Food Microbiology; 163: 146-152.
Yeung P S and Boor K J (2004) Effects of acid stress on *Vibrio parahaemolyticus* survival and cytotoxicity. Journal of Food Protection; 67: 1328-1334.
Yeung P S, Hayes M C, DePaola A, Kaysner C A, Kornstein L and Boor K J (2002) Comparative phenotypic, molecular, and virulence characterization of *Vibrio parahaemolyticus* O3:K6 isolates. Applied and Environmental Microbiology; 68: 2901-2909.
Yeung P S M, Wiedmann M and Boor K J (2007) Evaluation of a tissue culture-based approach for differentiating between virulent and avirulent *Vibrio parahaemolyticus* strains based on cytotoxicity. Journal of Food Protection; 70: 348-354.
Young I, Gropp K, Fazil A and Smith B A (2015) Knowledge synthesis to support risk assessment of climate change impacts on food and water safety: A case study of the effects of water temperature and salinity on *Vibrio parahaemolyticus* in raw oysters and harvest waters. Food Research International; 68: 86-93.
Yu W-T, Jong K-J, Lin Y-R, Tsai S-E, Tey Y H and Wong H-C (2013) Prevalence of *Vibrio parahaemolyticus* in oyster and clam culturing environments in Taiwan. International Journal of Food Microbiology; 160: 185-192.
Zhang Y, Zhao Y, Ding K, Wang X, Chen X, Liu Y and Chen Y (2014) Analysis of bacterial pathogens causing acute diarrhea on the basis of sentinel surveillance in Shanghai, China, 2006-2011. Japanese Journal of Infectious Diseases; 67: 264-268.
Zimmerman A M, DePaola A, Bowers J C, Krantz J A, Nordstrom J L, Johnson C N and Grimes D J (2007) Variability of total and pathogenic *Vibrio parahaemolyticus* densities in northern Gulf of Mexico water and oysters. Applied and Environmental Microbiology; 73: 7589-7596.

APPENDIX A: HAZARD AND FOOD

A.1 *V. parahaemolyticus* growth and survival

V. parahaemolyticus is a motile, Gram-negative, curved rod-shaped bacteria with a single flagellum and two circular chromosomes (Drake *et al.*, 2007; Joseph *et al.*, 1982). It does not form spores. *V. parahaemolyticus* 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 (including plankton) or sediments, and marine biotic surfaces (e.g. on BMS shells). *V. parahaemolyticus* in the water or attached to suspected biotic/abiotic matter can be taken up by marine animals including mammals, fish, shellfish, crustaceans and plankton. Their presence is not due to faecal pollution.

General information on *V. parahaemolyticus* 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 minimum growth temperature for *V. parahaemolyticus* is often cited as 10ºC (Codex Alimentarius, 2010; Odeyemi, 2016), but broth-based experiments measured a minimum growth temperature of 5.35ºC and a maximum of 47.85ºC (Miles *et al.*, 1997). The apparent inconsistency probably arises as a result of strain variability, culture conditions and the ability of *V. parahaemolyticus* to move into the VBNC state under nutrient poor, cool conditions. The concentration of *V. parahaemolyticus* inoculated into an oyster meat homogenate decreased at 4ºC and below (Muntada-Garriga *et al.*, 1995).

Differences in temperature sensitivity between *V. parahaemolyticus* strains have been observed in broth experiments that used both clinical and environmental isolates (Burnham *et al.*, 2009). Over 10 days, eight *V. parahaemolyticus* strains survived but were unable to grow at 5°C, while 5/8 strains grew at 8°C and 7/8 strains grew at 10°C (the concentration of the remaining strain did not significantly change). Growth at 10ºC was confirmed in another study of ten *V. parahaemolyticus* isolates, where the populations had increased by 2 log after 72 hours (Hara-Kudo and Kumagai, 2014). Growth was also found to be significantly faster with incubation at 12ºC, i.e. an increase of only 2ºC.

Optimum growth of *V. parahaemolyticus* was observed in broth in the range 37-39ºC (Miles *et al.*, 1997). Temperatures above 20ºC are known to favour growth in seawater (Cantet *et al.*, 2013). Growth is very rapid under optimum conditions, and the generation time can be as low as 8 minutes (European Commission, 2001; Miles *et al.*, 1997).

A mathematical model to describe the effect of temperature and water activity (a_w) on the growth rate of *V. parahaemolyticus* in food has been developed (European Commission, 2001). The model predicted a minimum growth temperature of 8.3ºC and a maximum of 45.3ºC. The optimum was 37-39ºC.

It has been observed that pre-stressed (starvation-adapted or starvation-low salinity-adapted) *V. parahaemolyticus* were better able to resist subsequent temperature stressors (heat, freeze-thaw) in laboratory media (Wong *et al.*, 2004a). However, this stress adaptation was

not observed when the pre-stressed cells were exposed to temperature stressors in oyster homogenate medium.

Salinity

V. parahaemolyticus can grow in sodium chloride (NaCl) concentrations ranging from 5 to 100‰ with optimum levels between 10 and 30‰ (Colwell, et al., 1984)

The aforementioned mathematical model of the effect of temperature and water activity (a_w) on the growth rate of *V. parahaemolyticus* in food predicted that *V. parahaemolyticus* would grow in the range 4-96‰ NaCl (aw 0.995-0.936) (European Commission, 2001).

Temperature and salinity

Experiments in laboratory broth identified the optimal conditions for growth as being 37ºC/30‰, based on 50 *V. parahaemolyticus* isolates from freshwater (n=24), humans (n=2) and seawater (n=24) (Liu *et al.*, 2016). These conditions were considered the most optimal when growth (as measured by optical density) was compared between 20 combinations of temperature (10, 20, 30, 37ºC) and salinity (5, 30, 50, 70 and 90‰ NaCl). The most sub-optimal combination was 10ºC/90‰. There was considerable difference in the maximum growth rate between isolates at all temperature/salinity combinations. Most isolates grew at 10ºC/30‰, and the maximum growth rate doubled at 20ºC/30‰.

A.2 *V. parahaemolyticus* testing and typing

Testing and typing methods for *V. parahaemolyticus* have been recently reviewed and evaluated (Bisha *et al.*, 2012; Drake *et al.*, 2007; 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 thermolabile haemolysin gene, *tlh*, is specific to *V. parahaemolyticus* and is commonly used to identify an isolate to species level in addition to (or instead of) biochemical tests. The regulatory gene *tox*R may also be targeted (Kim *et al.*, 1999). The presence of *tdh* and/or *trh* is most commonly used to identify isolates most likely to cause illness.

Serotyping depends on the antigenic properties of the somatic (O) and capsular (K) antigens (Nair *et al.*, 2007). All *V. parahaemolyticus* strains share a common H (flagellar) antigen (Drake *et al.*, 2007). While some serotypes are more associated with clinical cases (Appendix A.3), serotyping does not predict virulence so is not routinely used. There are multiple serotypes, for example, 27 serotypes were detected among 178 *V. parahaemolyticus* isolates from seafood, sediments and humans (DePaola *et al.*, 2003).

Culture-based techniques are still important for obtaining a bacterial isolate, thus there are efforts to improve culturing techniques (Escalante-Maldonado *et al.*, 2015; Nigro and Steward, 2015; Raghunath *et al.*, 2009; Rosec *et al.*, 2012). Tissue culture-based tests have been investigated as alternatives to molecular tests for detecting virulence (Yeung *et al.*, 2007). These methods examined cell toxicity rather than targeting specific virulence genes, and the researchers found that *V. parahaemolyticus* clinical isolates were generally more cytotoxic than food isolates, although the limit of detection was high (>10^5 cells).

Efforts have been directed towards developing better molecular-based detection to improve sensitivity, shorten testing time and indicate *V. parahaemolyticus* concentration and/or pathogenicity (Malcolm *et al.*, 2015; Wang *et al.*, 2016). A recent paper showed that PCR methods targeting the *tlh* gene need careful design to avoid false-positives (Yanez *et al.*, 2015). A range of molecular methods have been used to investigate genetic relatedness between *V. parahaemolyticus* isolates (e.g. (Haendiges *et al.*, 2015; Hazen *et al.*, 2015; Lüdeke *et al.*, 2015; Silvester *et al.*, 2016). These methods, including whole genome sequencing, pulsed field gel electrophoresis, multi-locus sequence typing and random

amplified polymorphic DNA, can all potentially be used to investigate relatedness of *V. parahaemolyticus* strains during outbreak investigations.

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

A.3 Virulence factors associated with pathogenic *V. parahaemolyticus*

Kanagawa phenomenon (KP), TDH, TRH and *ure*

As explained in the 2003 Risk Profile, the Kanagawa reaction is a traditional method used to indicate whether an isolate of *V. parahaemolyticus* is potentially pathogenic to humans, based on the finding that clinical isolates are often positive for the Kanagawa phenomenon (KP+) and environmental isolates are usually negative (KP-). The association between KP-positivity of a strain and its ability to cause gastroenteritis is well established (Hondo *et al.*, 1987; Nishibuchi and Kaper, 1995). KP+ isolates are able to produce TDH, which lyses red blood cells in Wagatsuma blood agar. Haemolysins are associated with virulence in a number of pathogenic bacterial species, and there is evidence from animal models for the role of haemolysin in virulence of *V. parahaemolyticus* (Hiyoshi *et al.*, 2010). The ability to produce TDH is more easily measured through detection of the *tdh* gene.

Clinical isolates lacking *tdh* (that were KP-) were found to possess *trh*, coding for TDH-related haemolysis (Letchumanan *et al.*, 2014). The *trh* gene plays a similar role to *tdh*. *V. parahaemolyticus* may possess the *tdh* or *trh* gene, or both.

Possession of the *tdh* and *trh* genes is the pathogenicity indicator most commonly used in current studies of *V. parahaemolyticus*. However, there is evidence to show that the absence of these genes does not definitively indicate avirulence, for example:

- Of 100 clinical *V. parahaemolyticus* isolates from patients in Canada isolated between 2000 and 2009, 15% were *tdh*+, 22% were *trh*+, 59% were *tdh*+/*trh*+, but 4% were negative for both virulence markers by PCR (Banerjee *et al.*, 2014). The authors suggested the existence of unknown virulence factors or the emergence of new virulence traits.
- Of 94 clinical *V. parahaemolyticus* isolates, most obtained from gastrointestinal cases in the North East US, 13 (14%) did not possess the *tdh* or *trh* genes (Xu *et al.*, 2015).
- Of 35 *V. parahaemolyticus* isolates from mussels, only one was *tdh*+ and two were *trh*+, but bacterial filtrates from all 35 were cytotoxic towards CHO K1 cells, Intestine 407 cells and Vero cells, confirming the presence of other virulence factors (Ottaviani *et al.*, 2005).
- Of 77 clinical isolates from USA patients, 21 (27%) were negative for *tdh* and *trh* (Jones *et al.*, 2012). These 21 isolates also lacked genes for T3SS2 (see below), but the authors cautioned that information on these 21 isolates was not complete and they could not all definitively be connected with foodborne illness.

There are five subtypes of *tdh* (*tdh*1-*tdh*5) and two subtypes of *trh* (*trh1* and *trh2*) (Haendiges *et al.*, 2015). Researchers do not usually seek to identify the specific subtype possessed by a *V. parahaemolyticus* isolate.

A recent paper discussed how developing PCR-based assays for the reliable detection of *trh* has been challenging due to the sequence variation of this gene, and provided evidence to show how established methods gave false-negative results (Nilsson and Turner, 2016). This complicates the findings reported in the bullet points above, as it is possible that *trh* was not detected by the PCR protocols employed in these studies.

Nilsson and Turner (2016) identified 13 sequence variants of *trh* from 81 *V. parahaemolyticus* isolates of environmental and clinical origin, which clustered into the *trh1* and *trh2* groups.

They predicted that other sequence variants exist and it might not be possible to design a single PCR that will reliably amplify all variants of the *trh* gene. Instead, they suggested focussing on a regulator of the urease gene cluster, *ureR*, which is genetically linked to *trh* and highly conserved. The *ure*R gene might be used as a proxy for the presence of *trh*, based on the current view that both genes almost always exist together. They cautioned that urease activity should not be used as an indicator for the urease gene cluster since the genes might be present but not expressed. For example, 3/98 *V. parahaemolyticus trh*+ isolates from clinical and oyster samples did not produce urease (Jones *et al.*, 2012).

Efforts in Germany suggest that the combination of *trh2*, urease gene and T3SS2β might be important for human infection, at least in that country (Huehn *et al.*, 2014).

T3SS1, T3SS2α and T3SS2β

T3SSs are needle-like structures bacteria use to inject chemicals directly into the membrane and cytoplasm of eukaryotic (host) cells, and are made from multiple proteins that can be linked back to gene targets (Letchumanan *et al.*, 2014). T3SS1 is present in environmental and clinical *V. parahaemolyticus* strains, so is not a useful pathogenicity marker. T3SS2 is encoded on a pathogenicity island on chromosome 2 and is commonly found in clinical isolates. There are two types, called T3SS2α and T3SS2β. Identifying possession of these T3SSs usually involves detecting a suite of genes that encode various proteins (Jones *et al.*, 2012; Noriea *et al.*, 2010; Ueno *et al.*, 2016).

As has been observed with *tdh* and *trh*, possession of T3SS2 is not always reliable as a pathogenicity indicator. For example, none of the *V. parahaemolyticus* isolates from four cases of acute gastroenteritis in Italy possessed genes encoding TDH, TRH, T3SS2α or T3SS2β, yet all were cytotoxic towards Caco-2 cells and adhesive on Hep-2 cells, but were not invasive on HT29 cells (Ottaviani *et al.*, 2012). Consumption of contaminated mussels was the most probable cause of infection for these cases and no other pathogenic bacteria were isolated from their clinical samples. In another study, some environmental *V. parahaemolyticus* isolates lacking *tdh*, *trh* and T3SS2 were more cytotoxic to human CaCo-2 cells than most clinical strains when assayed at 28ºC, and the majority were as cytotoxic at 37ºC (Mahoney *et al.*, 2010). It is possible that cytotoxicity assays are not an effective measure of human virulence for *V. parahaemolyticus* (Mahoney *et al.*, 2010). Alternatively, these studies suggest the presence of other virulence factors and advocate caution over the assumption that absence of these genes means a *V. parahaemolyticus* isolate is not pathogenic towards humans (Ottaviani *et al.*, 2012).

A.4 Pandemic *V. parahaemolyticus* clones

Occasionally a specific serotype becomes dominant amongst cases of *V. parahaemolyticus* infection and can spread to other regions or countries. These are called pandemic strains because of their geographic spread, but lack the characteristics of a truly pandemic microorganism which, but definition, infects a high proportion of the population (Nair *et al.*, 2007). A recent paper has described a succession of dominant serotypes in Peru and linked these to Asian *V. parahaemolyticus* clones and the arrival of El Niño waters (Gonzalez-Escalona *et al.*, 2016).

O3:K6

While first recognised as a cause of a cluster of clinical cases in India 1996, O3:K6 was perhaps first isolated from a traveller returning from Indonesia to Japan in 1995 (Nair *et al.*, 2007). The O3:K6 clone emerged in Japan during 1995 and by 1998 was the dominant serotype isolated from humans (Hara-Kudo and Kumagai, 2014). O3:K6 was the first recognised pandemic strain and it increased hospitalisations from *V. parahaemolyticus* infection where ever it prevailed.

The first outbreak of the O3:K6 clone in United States was reported in 1998 and the vehicle of infection was oysters (Daniels *et al.*, 2000). The O3:K6 clone was first isolated in England in 2012, from Pacific oysters and water (Powell *et al.*, 2013), but was isolated earlier from travellers returning the UK (Wagley *et al.*, 2008). The scientific literature contains multiple reports of the O3:K6 clone isolated from clinical and environmental samples as the clone spread to different countries, causing sporadic disease and outbreaks (Ansaruzzaman *et al.*, 2005; Chiou *et al.*, 2000; Ottaviani *et al.*, 2010b; Rykovskaya *et al.*, 2014; Vuddhakul *et al.*, 2000).

Surveillance in Chile has provided an interesting picture of how the O3:K6 clone dominated and spread, then later declined. The clone first caused outbreaks in northern Chile in 1998, but was then rarely reported until 2004 (Fuenzalida *et al.*, 2007). Between January and March 2005, a total of 10,783 cases of *V. parahaemolyticus* infection were reported in southern Chile, beginning in a region that was the source of approximately 75% of the seafood consumed in Chile, and spreading throughout the country (Cabello *et al.*, 2007). Serotyping of isolates from 60 patients confirmed all were the pandemic clone O3:K6 and the most common vehicles of disease were clams and mussels. Further surveillance in 2006 confirmed the clone was still causing illness, but now largely in southern Chile where it was persistent in shellfish isolated from these southern waters, despite the waters being 5°C colder (11-16ºC year round) than the northern Chilean waters (Fuenzalida *et al.*, 2007). By 2010, cases had declined to less than 10, and it was hypothesised that bacteriophage had a role in the decline of the pandemic clone in Chile (Garcia *et al.*, 2013).

The O3:K6 pandemic clone is distinguished from other O3:K6 serotypes by the following characteristics (Ansaruzzaman *et al.*, 2005; Nair *et al.*, 2007; Ueno *et al.*, 2016):

- *tdh*+/*trh*-
- urease negative
- T3SS1+/T3SS2α+/T3SS2β-
- A group-specific PCR (GS-PCR) detecting seven base changes in the *toxRS* gene sequences and open-reading-frame (ORF) 8 from the f237 phage.

Initially, an open-reading-frame from the f237 phage, ORF8, was also included in the GS-PCR as it was thought to be unique to the O3:K6 clone. However, GS-PCR positive strains lacking ORF8 have been reported among the pandemic strains (Ansaruzzaman et al., 2005).

O3:K6 serovariants

Since the first reports of the O3:K6 pandemic clone, other serotypes have emerged that have been shown to be related to the pandemic clone by molecular techniques (Ansaruzzaman *et al.*, 2005). These have been collectively called the O3:K6 serovariants, and a 2014 review reported 20 recognised serovariants of the O3:K6 clone (Letchumanan *et al.*, 2014).

Examples of the serovariants reported possess the serotype O4:K8, O4:K12, O4:K68 and O1:KUT (Ansaruzzaman *et al.*, 2005).

A.5 Additional information on BMS in New Zealand

TABLE 9: 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)
Trough shell, large	*Mactra murchisoni*	Subtidal surf zone	Widespread (more common around southern NZ)

COMMON NAMES[2]	SCIENTIFIC NAME	COMMON TIDAL HABITAT	NEW ZEALAND DISTRIBUTION
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.6 *V. parahaemolyticus* in BMS overseas

Data presented in the 2003 Risk Profile showed that *V. parahaemolyticus* were usually detected in BMS sampled from growing sites and retail, but the prevalence ranged from approximately 2% to 92%. Similarly, the concentrations of *V. parahaemolyticus* varied (concentrations exceeding 10^4 MPN/g were reported). The proportion of isolates positive for KP+ (or *tdh*+) was commonly <1%, but higher proportions were measured in surveys in Hong Kong and the USA.

Data from surveys published post 2003 have been summarised in TABLE 10. 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. (Lee *et al.*, 2008; Letchumanan *et al.*, 2015; Normanno *et al.*, 2006; Robert-Pillot *et al.*, 2014; Sagoo *et al.*, 2007; Tang *et al.*, 2012)) 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. parahaemolyticus* 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. parahaemolyticus*; 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 is for each study as a whole.

TABLE 10 shows:

- *V. parahaemolyticus* have been detected in BMS (oysters, mussels and clams) harvested from waters around the American continent and countries in the European, Middle Eastern, North African and Asian regions.
- The prevalence of *V. parahaemolyticus* is variable, with up to 100% of samples positive.
- The concentration of *V. parahaemolyticus* was as high as 10^5 cells/g in some studies (the spread of these data has not been reported well in most studies).
- The prevalence of *tdh*+ and/or *trh*+ *V. parahaemolyticus* is variable and can be >50% (e.g. 69% (47/68) of oyster samples were *trh*+ in one USA study. This disagrees with the common opinion in the scientific literature, that the prevalence of *V. parahaemolyticus* carrying these pathogenicity genes is quite low relative to total *V. parahaemolyticus*, e.g.

0.2-3.0% of environmental *V. parahaemolyticus* isolates carry the *tdh* and/or *trh* genes (Kirs *et al.*, 2011). The prevalence of *V. parahaemolyticus* carrying these virulence markers does not appear to be correlated with the prevalence of total *V. parahaemolyticus* (the ratio between the two varies between studies).

- Concentration data for *tdh*+ and/or *trh*+ *V. parahaemolyticus* are few, but suggest that in most cases the concentrations within BMS are <100 cells/g. However, 10^2 *tdh*+ cells/g was reported in a study from India. It has been reported that the total concentration of *V. parahaemolyticus* in oysters does not appear to be correlated with the concentration of strains carrying these virulence markers, suggesting the latter subgroup occupies a niche different from that of the species as a whole (Froelich and Noble, 2016). The available data appear to agree with this statement.

- Most studies observed increased prevalence and/or concentration of *V. parahaemolyticus* in BMS with increasing water temperature, and often the correlation was statistically significant. One study identified a negative correlation with temperature. Potentially pathogenic *V. parahaemolyticus* isolates were recovered from BMS at 16ºC.

- The effect of water salinity on *V. parahaemolyticus* prevalence and/or concentration appears variable, with studies finding no correlation, negative correlation or positive correlation. One study found the concentration of *V. parahaemolyticus* was positively correlated to water salinity, but the concentration of potentially pathogenic *V. parahaemolyticus* was negatively correlated.

TABLE 10 does not include studies where the number of samples was <30, since data from small sample sets may not represent the wider population, nor studies where it was difficult to extract the data needed for the table. However, a review of these studies supports the findings above (Anacleto *et al.*, 2013; Aranda *et al.*, 2015; Cabrera-Garcia *et al.*, 2004; Cantet *et al.*, 2013; Collin and Rehnstam-Holm, 2011; de Sousa *et al.*, 2004; Flores-Primo *et al.*, 2014; Henigman *et al.*, 2011; Hervio-Heath *et al.*, 2002; Johnson *et al.*, 2012; Kang *et al.*, 2016; Khouadja *et al.*, 2013; Lopez-Hernandez *et al.*, 2015; Shaw *et al.*, 2014; Suffredini *et al.*, 2014; Vernocchi *et al.*, 2007; Yu *et al.*, 2013).

A systematic review and meta-analysis of 48 studies investigating the prevalence of *V. parahaemolyticus* in seafood has recently been published (Odeyemi, 2016).[35] The overall prevalence were:

- Oysters (n=951): 63.4% (95% CI 0.59-0.67)
- Clam and cockles (combined, n=830): 52.9% (95% CI 0.49-0.57)
- Mussels, scallops and periwinkles (combined, n=1670): 28.0% (95% CI 0.26-0.31).

Studies included in this analysis were from environmental surveys (shellfish collected from environmental sites) and retail surveys (shellfish collected from retail stores, markets, etc.), and the analytical methods included culture and molecular approaches.

[35] The review incorporated peer reviewed studies published between 2003 and 2015. Of 6,876 studies identified, 48 met inclusion criteria. Only studies published in English were included.

TABLE 10: Prevalence and concentration of *V. parahaemolyticus* measured in raw BMS sampled from growing waters in other countries (studies published in the scientific literature from 2003 onwards; surveys with 30 or more samples only)

COUNTRY, SAMPLING SITES	SURVEY YEAR(S)	WATER TEMP. (°C)[A]	WATER SALINITY (‰/ppt)[A]	*V. PARAHAEMO-LYTICUS* PREVALENCE (%)[B]	*V. PARAHAEMO-LYTICUS* CONCENTRATION IN POSITIVE SAMPLES (MPN/g or CFU/g)	PATHOGENIC *V. PARAHAEMO-LYTICUS* PREVALENCE	COMMENTS ON *V. PARAHAEMOLYTICUS* RESULTS	REF.[D]
Pacific oysters (*C. gigas*)								
Brazil (3 coastal sites)	2003-2004	NR	NR	0/45	NR	NR		*1*
Brazil (6 coastal sites)	2006-2007	18-29	NR	8/180 (4)	4-7	NR		*2*
Brazil (6 coastal sites)	2008-2009	20-29	NR	18/60 (30)	Mean 16 (max. $2x10^2$)	Isolates: 4/48 *tdh*+ 23/48 *trh*+	Highest counts in summer months but no significant correlation between temperature and concentration. Negative correlation with salinity.	*3*
Spain (delta, Mediterranean)	2006, 2008 - 2010	25-28	35-37	Tested individually (not pooled): 88/613 (14)	NR	Tested individually: 26/613 (4%) *tdh*+ and/or *trh*+	Negative correlation between temperature and *V. parahaemolyticus*; positive correlation between salinity and *V. parahaemolyticus* but negative correlation between salinity and pathogenic *V. parahaemolyticus*.	*4*
Oysters (*C. virginica*, other *Crassostrea* spp. or species not identified)								
Brazil (estuary)	2004-2005	14-29	5-30	122/123 (99)	6-$1.1x10^5$	1/123 (1%) *tdh*+ 0/123 *trh*+	Concentrations correlated significantly with water temperature but not with salinity.	*5*
China (aquafarms, two provinces)	2013-2014	7.0-29.0	1-38	123/180 (68)	<10 n=8 >10-10^2 n=3 >10^2-10^3 n=35 >10^3-10^4 n=58 >10^4-10^5 n=19	NR	Concentration significantly associated with water temperature but not salinity.	*6*
India (estuaries)	2002	25-35	0.8-31	46/49 (94)[C]	10^2-10^4 (max. $6.7x10^4$)[C]	5/49 (10%) *tdh*+ Max. $1.3x10^2$ CFU/g	Concentration not correlated with temperature or salinity.	*7*
USA (5 sites, South Carolina)	2001-2003	6-30	21-38	79/85 (93)	7-$2.2x10^4$	NR	Significant correlation between temperature and concentrations but only for some sites.	*8*

COUNTRY, SAMPLING SITES	SURVEY YEAR(S)	WATER TEMP. (°C)[A]	WATER SALINITY (‰/ppt)[A]	*V. PARAHAEMO-LYTICUS* PREVALENCE (%)[B]	*V. PARAHAEMO-LYTICUS* CONCENTRATION IN POSITIVE SAMPLES (MPN/g or CFU/g)	PATHOGENIC *V. PARAHAEMO-LYTICUS* PREVALENCE	COMMENTS ON *V. PARAHAEMOLYTICUS* RESULTS	REF.[D]
USA (4 sites, Mississippi)	2001-2003	7-34	3-34	53/62 (85)	3-1.3x10^4	NR	Significant correlation between temperature and concentrations but only for some sites.	*8*
USA (2 sites, Oregon)	2002-2003	5.6-21.4	1.5-33.3	6/40 (15)	Max. 27	3/40 (8%) *tdh*+ and/or *trh*+ Max. 3.0 MPN/g	Concentrations correlated with temperature but not salinity.	*9*
USA (2 sites, Gulf of Mexico)	2004	22-34	4-28	32/32 (100)	0.2-3x10^3	16/32 (50%) *tdh*+ and/or *trh*+ Max. 30 MPN/g	Correlation between concentration and salinity at 1 site. No correlation with temperature at either site (all temperatures >22ºC).	*10*
USA (3 sites, Chesapeake Bay)	2004-2005	3-29	5-14	26/33 (79)	10-6x10^2	1/33 (3%) *tdh*+ 10 CFU/g	Concentration positively correlated with water temperature. Not detected in oysters from waters <9ºC.	*11*
USA (Great Bay Estuary)	2007-2013	3-26	9-32	91/140 (65)	Median 7.3 (0.04-4,600)	NR	Concentration positively correlated with water temperature, salinity, dissolved oxygen, total dissolved nitrogen and chlorophyll *a.*	*12*
USA (Rhode Island coast)	2009-2010	NR	NR	46/48 (96)[A]	Max. 9x10^3	Average 2.5-31.9% *tdh*+ and/or *trh*+ per sampling	Concentration increased rapidly 2-3 weeks after water temperatures reached approx. 18ºC.	*13*
USA (Long Island Sound)	2012	20-26	23-28	68/68 (100)	Median 78 (max. 1x10^4)	39/68 (57%)*tdh*+ Max. 43 MPN/g 47/68 (69%)*trh*+ Max. 76 MPN/g	Positive correlation between temperature and pathogenic *V. parahaemolyticus*; negative correlation between salinity and pathogenic *V. parahaemolyticus*.	*14*
Mussels (*M. edulis*, *M. galloprovincialis*, other *Mytilus* spp.)								
France (Pertuis Breton)	2008-2009	6-23 (mean values)	NR	32/48 (67)	21-110	Isolates: 34/223 *trh*2+	Mean temperature when total *V. parahaemolyticus* were detected was 20°C, compared with 13°C when they were not.	*15*
Italy (6 sites, Adriatic Sea)	2002-2004	NR	NR	35/144 (24)	NR	Isolates: 1/35 *tdh*+ 3/35 *trh*+		*16*

COUNTRY, SAMPLING SITES	SURVEY YEAR(S)	WATER TEMP. (°C)[A]	WATER SALINITY (‰/ppt)[A]	*V. PARAHAEMO-LYTICUS* PREVALENCE (%)[B]	*V. PARAHAEMO-LYTICUS* CONCENTRATION IN POSITIVE SAMPLES (MPN/g or CFU/g)	PATHOGENIC *V. PARAHAEMO-LYTICUS* PREVALENCE	COMMENTS ON *V. PARAHAEMOLYTICUS* RESULTS	REF.[D]
Italy	2006-2007	NR	NR	9/144 (6)	NR	3/144 (2%) *tdh*+		*17*
Italy (3 sites, Adriatic Sea)	2007	NR	NR	65/559 (12)	NR	0/559 *tdh*+ 5/559 (1%) *trh*+		*18*
Morocco (4 coastal sites)	2010-2011	18-25	30-37	4/52 (8)	NR	NR		*19*
Spain (delta, Mediterranean)	2006, 2008 - 2010	25-28	35-37	Tested individually (not pooled): 73/606 (12)	NR	Tested individually: 19/606 (3%) *tdh*+ and/or *trh*+	Negative correlation between temperature and *V. parahaemolyticus*; positive correlation between salinity and *V. parahaemolyticus* but negative correlation between salinity and pathogenic *V. parahaemolyticus*.	*4*
Spain (Southern Galicia Rias)	2013	NR	NR	69/101 (68)[C]	NR	50/101 (50%) *tdh*+ and/or *trh*+		*20*
Turkey (6 coastal sites)	2005-2006	16-27	14-16	24/60 (40)	NR	24/60 (40%) *tdh*+ and/or *trh*+	Potentially pathogenic isolates obtained from shellfish at 16.0 and 26.6ºC.	*21*
Clams (*Venus* spp., *Ruditapes* spp., *Venerupis* spp., *Mercenaria* spp.)								
Italy (Emilia Romagna)	2011-2014	NR	NR	22/79 (28)	NR	NR		*22*
Spain (delta, Mediterranean)	2006, 2008 - 2010	25-28	35-37	Tested individually (not pooled): 46/240 (19)	NR	Tested individually: 11/240 (5%) *tdh*+ and/or *trh*+	Negative correlation between temperature and *V. parahaemolyticus*; positive correlation between salinity and *V. parahaemolyticus* but negative correlation between salinity and pathogenic *V. parahaemolyticus*.	*4*
USA (Long Island Sound)	2012	20-26	23-28	30/30 (100)	Median 12 (max. $1x10^2$)	3/30 (10%) *tdh*+ Max. 10 MPN/g 7/30 (23%) *trh*+ Max. 2 MPN/g	Positive correlation between temperature and pathogenic *V. parahaemolyticus*; negative correlation between salinity and pathogenic *V. parahaemolyticus*.	*14*

See over for table notes.

Notes to Table 10

NR, not reported.

[A] Estimated from graph if data not specified.

[B] 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).

[C] As indicated by PCR, targeting the *tlh* or *toxR* gene.

[D] References:

1 (Pereira *et al.*, 2006)
2 (Ramos *et al.*, 2012b)
3 (Ramos *et al.*, 2014)
4 (Lopez-Joven *et al.*, 2015)
5 (Sobrinho *et al.*, 2010)
6 (Han *et al.*, 2015)
7 (Deepanjali *et al.*, 2005)
8 (Moore *et al.*, 2014)
9 (Duan and Su, 2005)
10 (Zimmerman *et al.*, 2007)
11 (Parveen *et al.*, 2008)
12 (Urquhart *et al.*, 2016)
13 (Cox and Gomez-Chiarri, 2012)
14 (Jones *et al.*, 2014)
15 (Deter *et al.*, 2010)
16 (Ottaviani *et al.*, 2005)
17 (Di Pinto *et al.*, 2008)
18 (Ottaviani *et al.*, 2010a)
19 (Mannas *et al.*, 2014)
20 (Garrido-Maestu *et al.*, 2016)
21 (Terzi *et al.*, 2009)
22 (Passalacqua *et al.*, 2016)

APPENDIX B: EVALUATION OF ADVERSE HEALTH EFFECTS

B.1 Dose-response

As described in the 2003 Risk Profile, modelling of data from human feeding trials have predicted doses in the 5-9 $\log_{10}$ *V. parahaemolyticus* range.[36] It is important to reiterate that, as with other dose-response models, there is not a threshold dose that must be consumed before disease will occur, i.e. there is no safe dose other than zero cells, but modelling studies predict that the probability of illness decreases with decreasing dose.

Data from three human feeding trials that used Kanagawa-positive strains (i.e. those able to produce TDH) were used as the basis for dose-response estimates in two risk assessments published since 2003 (FAO/WHO, 2011; USFDA, 2005b). These risk assessments used the same beta-Poisson dose-response model. It was observed that the dose-response model overestimated of the incidence of disease in the population (based on USA epidemiological data prior to *V. parahaemolyticus* becoming a notifiable disease) and a 27-fold adjustment (increase in the ID_{50}) was applied to make the predictions more realistic.[37] The adjustment accounted for consumption of *V. parahaemolyticus* within a food matrix (e.g. in oysters) and normal stomach acidity (human feeding trial volunteers were administered antacids), both which shift the dose-response graph to the right (i.e. more cells are ingested for the same probability of illness). After adjustment, non-parametric bootstrapping was performed to account for uncertainty in the model, and this produced a weighted set of 21 dose-response curves that were randomly selected (based on their weighting) for model simulations.

The initial, adjusted model estimates a 50% probability of illness with consumption of $8x10^7$ *V. parahaemolyticus* cells (i.e. approximately 10^8 cells). After uncertainty weighting, the 21 dose-response curves estimated a 50% probability of illness within the range 10^7-10^{10} cells.

At exposure levels of approximately 10^4 cells, the probability of illness is relatively low (<0.001, or <0.1%). The probability of illness approaches 1.0 (i.e. 100% certainty of illness) at exposure levels around 10^9 cells.

The dose-response model is based on a number of assumptions, of which the most important are (FAO/WHO, 2011; USFDA, 2005b):

- The responses of the healthy volunteers who participated in the feeding trials are representative of the general population;
- All individuals are equally susceptible to gastroenteritis (host susceptibility does not vary); and
- The TDH-producing *V. parahaemolyticus* strains used in the feeding trials are representative of all pathogenic *V. parahaemolyticus* strains (i.e. virulence does not vary)

A key uncertainty is the effect of food matrix on the dose-response relationship (FAO/WHO, 2011).

Limited data from outbreaks suggests that the dose required to cause illness may be lower than that predicted by the above model. Investigators of three outbreaks of *V. parahaemolyticus* infection aboard the same cruise ship identified raw oysters as the vehicle

[36] These feeding trials are described in USFDA (2005b).
[37] The adjustment factor of 27 corresponded to a difference of 1.4 $\log_{10}$ between the ID_{50} of the unadjusted and adjusted dose-response curves.

of disease (McLaughlin *et al.*, 2005). Oysters collected from the implicated oyster farm during the outbreak and the following weeks contained *V. parahaemolyticus* at a median concentration of 3.5 MPN/g (range 0.3-430.0). The median number of oysters consumed by patients was one (range 1-6) and the attack rate for people who ate oysters was 29%. In Japan, a concentration of ≤1 *V. parahaemolyticus*/g was reported for leftover tuna that caused an outbreak, but no further details are available (Hara-Kudo and Kumagai, 2014). A paper by Daniels *et al.* (2000) regarding a large USA outbreak is often cited as evidence that a lower dose of the O3:K6 serotype may cause illness (Daniels *et al.*, 2000). While it is possible that this is true, the strain responsible for the majority of outbreak cases was not isolated from any oysters during the environmental investigation. Moreover, while the median concentration of *V. parahaemolyticus* in these oysters was 15 MPN/g, it ranged up to 4,600 MPN/g.

B.2 *V. parahaemolyticus* infection in other countries

The first major *V. parahaemolyticus* outbreak in the United States occurred in Maryland in 1971 and was associated with improperly cooked crabs (European Commission, 2001). Since then *V. parahaemolyticus* illnesses have been linked to locally harvested seafood throughout the world including Asia, Australia, Canada, Europe, Mexico, South America and USA (FAO/WHO, 2011; Lake *et al.*, 2003).

Data presented in the 2003 Risk Profile show that *V. parahaemolyticus* infection is more prevalent in countries where people consume large quantities of seafood and/or frequently consume raw seafood. This has not changed. *V. parahaemolyticus* causes 20-30% of foodborne illnesses in Japan and is the leading cause of human gastroenteritis associated with seafood consumption in the United States and Asian countries (Elmahdi *et al.*, 2016; Letchumanan *et al.*, 2014). A study of 2,243 cases of acute diarrhoea in Shanghai (China) found that 82% were infected with *V. parahaemolyticus* (Zhang *et al.*, 2014).

V. parahaemolyticus 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.[38] 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)" are notifiable in the Northern Territory, "*Vibrio* infection" in Tasmania) and "*Vibrio parahaemolyticus*" in Western Australia.[39]

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).[40]

B.2.1 Incidence of *V. parahaemolyticus* infection

USA

TABLE 11 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. parahaemolyticus* cases, including wound infections. Based on the USA's annual estimated resident population for 2014 (319 million), the incidence for 2014 was 0.2 per 100,000.[41] Some notable trends were

[38] https://wwwn.cdc.gov/nndss/conditions/vibriosis/ (accessed 5 July 2016).
[39] http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-casedefs-statedis.htm (accessed 6 July 2015).
[40] 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).
[41] United States Census Bureau population data, http://www.census.gov/popest/data/national/totals/2015/index.html (accessed 6 July 2016).

observed for vibriosis cases each year (data on these trends was not specific to *V. parahaemolyticus*):

- 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.

Clams have also been reported as vehicles of *V. vulnificus* infection in the USA (Slayton *et al.*, 2014).

TABLE 11: Reported cases of *V. parahaemolyticus* 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	605	47 (4-96)	66	86/575 (15)	4/389 (1)	84
2013	594	48 (0-95)	65	112/543 (21)	4/546 (<1)	80
2012	431	49 (1-93)	69	101/403 (25)	6/390 (2)	45
2011	334	45 (1-94)	67	75/315 (24)	7/304 (2)	72
2010	421	47.5 (1-90)	62	84/383 (22)	2/366 (1)	77

[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. parahaemolyticus* was exclusively isolated. Additional cases were reported with *V. parahaemolyticus* in combination with another *Vibrio* spp.

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

[3] Estimated from graph.

Australia

Data assembled for Western Australia showed 14-16 cases of *V. parahaemolyticus* infection each year during the period 2012-2014 (rate 0.6 per 100,000), and seven cases in 2015 (rate 0.3 per 100,000).[42]

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).

One case of *Vibrio* spp. infection was reported in Tasmania during 2015 but the *Vibrio* species was not identified (Anonymous, 2015).

[42] http://www.public.health.wa.gov.au/3/1535/3/vibrio_parahaemolyticus.pm (accessed 7 July 2016).

[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).

Other countries

V. parahaemolyticus have been the dominant cause of foodborne infections in Japan but both the number of reported outbreaks and cases has been decreasing since controls were introduced from 1999 (see Appendix C.2.6) (Hara-Kudo and Kumagai, 2014). Prior to this, in 1997, the number of outbreaks per year peaked at 839, and the number of cases exceeded 12,000. The number of cases and outbreaks of *V. parahaemolyticus* infections decreased by 99- and 93-fold, respectively, from 1998 to 2012.

Hospital-based surveillance programmes covering patients with acute diarrhoea in Shanghai, China, show that *V. parahaemolyticus* is the dominant cause of gastrointestinal disease relative to other identified bacterial pathogens (Qi *et al.*, 2016; Zhang *et al.*, 2014). For example, in 2011, 84% (315/374) of patients with acute diarrhoea caused by an identified pathogen were infected with *V. parahaemolyticus* (Zhang *et al.*, 2014). The infection rate of *V. parahaemolyticus* was much higher in summer months.

Of 134 non-choleraic vibrio strains responsible for human infections and received at France's National Reference Center between 1995 and 2009, 23 were *V. parahaemolyticus* (ANSES, 2012). Eighteen of these were from cases of gastroenteritis (all were positive for the *tdh* and or *trh* genes).

B.2.2 Outbreaks of *V. parahaemolyticus* infection associated with BMS

The 2003 Risk Profile presented information from eight outbreaks linked to seafood. TABLE 12 lists outbreaks of *V. parahaemolyticus* infection linked to consumption of BMS that were identified from the scientific literature. Additional outbreaks of *V. parahaemolyticus* infection have been reported in:

- The ProMED-mail database, e.g. closure of a specific shellfish bed in New York during 2014 due to cases of foodborne illness linked to consumption of oysters and clams from the area.[44]
- Outbreak and annual surveillance reports or alerts made available by governmental agencies such as the US Centers for Disease Control and Prevention and the Public Health Agency of Canada, e.g. During 2015, 67 cases of *V. parahaemolyticus* infection were reported in Canada and all cases reported consumption of raw shellfish, primarily oysters.[45]
- Searchable databases such as those made available by the USCDC and EFSA.[46] For example, the USA online database records 144 outbreaks of confirmed *V. parahaemolyticus* infection reported in the USA for the period 1998-2015 where the implicated food vehicle was BMS. These outbreaks involved a total of 980 cases, of whom 38 were hospitalised (no deaths were reported). Oysters were implicated in all but three of these outbreaks, mostly "oysters, raw". The largest outbreak involved 400 cases (1998, in Texas).

Analyses of reported outbreaks have also been published in the scientific literature:

- USA: Of 188 seafood-associated outbreaks for the period 1973-2006, 33 (18%) were *V. parahaemolyticus* infection linked to molluscs, with an associated 1,159 illnesses and 23 hospitalisations (Iwamoto *et al.*, 2010).

[44] ProMED-mail (http://www.promedmail.org/, accessed 8 September 2016): Archive number 20140913.2768717.
[45] http://www.phac-aspc.gc.ca/phn-asp/2015/vibrioparahaemolyticus-eng.php (accessed 7 July 2016).
[46] USCDC Food Outbreak Online Database, http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx. EFSA Rapid Alert System for Food and Feed, https://webgate.ec.europa.eu/rasff-window/portal/?event=searchForm&cleanSearch=1. Accessed 28 June 2016.

- China: Of 187 outbreaks of *V. parahaemolyticus* gastrointestinal infection during the period 2003-2008, where a single food commodity was implicated based on laboratory or epidemiological evidence, three were attributed to “molluscs” (Wu *et al.*, 2014).

EFSA’s Rapid Alert System for Food and Feed contains 41 alerts issued between January 2008 and March 2016 for food poisoning associated with “bivalve molluscs and products thereof”, but none of these were for contamination with *Vibrio* spp. However, outbreaks of *V. parahaemolyticus* infection are occasionally reported as part of the category “other bacterial agents” in EFSA’s annual surveillance reports. In 2014, four outbreaks caused by *V. parahaemolyticus* were reported, all in France (European Food Safety Authority and European Centre for Disease Prevention and Control, 2015). Only one had strong evidence linking cases to the food, and this was caused by the consumption of ‘crustaceans, shellfish, molluscs and products thereof’.

TABLE 12: Outbreaks of *V. parahaemolyticus* infection overseas linked to BMS (reported in the scientific literature)

YEAR	COUNTRY	SHELLFISH IMPLICATED	NUMBER OF CASES (NUMBER HOSPITALISED)[1]	EVIDENCE LINKING FOOD TO CASES	CONDITIONS SUPPORTING PRESENCE OF *V. PARAHAEMOLYTICUS*	REFERENCE
1997	USA	Oysters (raw)	209 (2; 1 death)	Case histories, oysters from implicated harvest sites contained *V. parahaemolyticus*	Higher-than-normal sea temperatures	(Fyfe *et al.*, 1998)
1997	Canada	Oysters (raw, undercooked)	43C 14P (1)	Case histories	NR	(Fyfe *et al.*, 1997)
1998	USA	Oysters (raw, undercooked)	416 (12)	Cohort studies, *V. parahaemolyticus* isolated from oysters but not same strains as clinical isolates (O3:K6)	Higher-than-normal sea temperatures and salinities, up to 11 hours between harvest and refrigeration	(Daniels *et al.*, 2000)
1997-1998	Chile	"shellfish"	298	Case histories, same strains identified in shellfish and clinical samples	El Niño event, higher-than-normal sea temperatures, bacterial bloom	(Cordova *et al.*, 2002)
1999	Spain	Oysters (raw)	64 (9)	Case histories	Rainy season and summer (decreased salinity, increased water temperature)	(Lozano-Leon *et al.*, 2003)
2002	USA	Mussels (raw)	2 (1)	*V. parahaemolyticus* isolated from mussels (*tdh*+)[2]	NR	(Davis *et al.*, 2004)
2004	Cruise ship (Alaska)	Oysters (raw)	62 (0)	Genetically similar *V. parahaemolyticus* isolated from oysters and patients (*tdh*+; serotypes O6:K18, O1:K56)	Oysters harvested when mean daily temperatures >15ºC	(McLaughlin *et al.*, 2005)
2005	Chile	Clams, mussels	10,783 (1 death)	O3:K6 clone isolated from clinical and samples of mussels and clams	higher-than-normal sea temperatures, arrival of O3:K6 clone, algal blooms	(Cabello *et al.*, 2007)

YEAR	COUNTRY	SHELLFISH IMPLICATED	NUMBER OF CASES (NUMBER HOSPITALISED)[1]	EVIDENCE LINKING FOOD TO CASES	CONDITIONS SUPPORTING PRESENCE OF *V. PARAHAEMOLYTICUS*	REFERENCE
2006	USA	Oysters, clams	72C 105P	Case histories	NR	(Balter *et al.*, 2006)
2010	USA	Oysters	2	Outbreak strain isolated from oysters	NR	(Haendiges *et al.*, 2016)
2012	USA	"shellfish"	28	NR	NR	(Martinez-Urtaza *et al.*, 2013)
2013	USA	"shellfish" (raw)	104	Traceback investigation	NR	(Newton *et al.*, 2014)

[1] C, confirmed; P, probable.

[2] Investigators determined that cross-contamination of fresh produce by raw mussel liquid was the probable cause.

B.2.3 Case control studies

The 2003 Risk Profile reported three case control studies, none of which considered BMS or shellfish consumption as a risk factor for *V. parahaemolyticus* infection. Very few case control studies investigating consumption of BMS as a risk factor for *V. parahaemolyticus* infection were located, possibly because the link between this hazard and food is so well established.

Two case control studies were identified from the literature where consumption of "shellfish" were considered, sometimes as part of consumption of seafoods (TABLE 13). Both of these studies were conducted in China, one over the period 2010/11, the other during 2012. Note that refrigeration of "aquatic products" was protective.

TABLE 13: Case control studies considering *V. parahaemolyticus* infection and shellfish in China, 2010-2012 (significant odds ratios in bold)

RISK FACTOR(S)	NUMBER OF PARTICIPANTS		ODDS RATIO (95% CONFIDENCE INTERVAL) BY:[1]		REF.*
	CASES	CONTROLS	UNIVARIATE ANALYSIS	MULTIVARIATE ANALYSIS	
Eating raw (undercooked) seafood Eating cooked shellfish	83	249	**11.0 (2.2-55.8)** 2.1 (0.9-4.7)	**8.0 (1.3-50.4)** NR	*a*
Ate shellfish in the past five days Keep aquatic products refrigerated in the past 5 days	71	142	**3.1(1.2-8.1)** **0.3 (0.1-0.7)**	**3.2 (1.0-9.9)** **0.4 (0.1-0.9)**	*b*

* References:
a. (Liao *et al.*, 2015)
b. (Yan *et al.*, 2015)

B.2.4 Attribution studies

A Canadian expert elicitation process during 2014 estimated that the median proportion of *Vibrio parahaemolyticus* enteric infections attributed to foodborne transmission was 82.8% (90% Credible Interval (CI) 46.0-94.6%) (Butler *et al.*, 2015). The estimate for waterborne transmission (11.0%) also had wide 90% CIs (0.9-50.2%), which may have been a result of the experts having difficulty separating infection from drinking contaminated water with the non-enteric route of infection (i.e. wound infection). Experts were asked to only consider enteric routes of infection. Smaller proportions were assigned to the other transmission routes of animal contact (2.0%, presumably contact with contaminated marine animals) and person-to-person (2.8%). An earlier Canadian expert elicitation study had attributed the majority of *Vibrio* spp. infections to the food category "seafood" (Davidson *et al.*, 2011).

APPENDIX C: CONTROL MEASURES IN OTHER COUNTRIES

C.1 International controls

C.1.1 Codex Alimentarius

The 2003 Risk Profile described activities being undertaken at the time by Codex Alimentarius. This included a 2002 discussion paper on risk management strategies for *Vibrio* spp. in seafood, and efforts of the Codex Committee on Fish and Fisheries Products (CCFFP) towards preparing standards and codes of practice relevant to managing risk from *V. parahaemolyticus*.

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. parahaemolyticus* and *V. vulnificus* in bivalve molluscs intended for consumption in a live, raw or partially treated state.[47] 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. The Annex cautions that *V. parahaemolyticus* are generally more resistant than *V. vulnificus* to any given treatment, so a process that is effective for *V. vulnificus* may not be as effective for *V. parahaemolyticus*.

C.1.2 European Union

There are no microbiological criteria for *V. parahaemolyticus* in fishery products placed on the market in the EU (Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs).[48] 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.[49]

[47] "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.
[48] (EC) No 2073/2005 is available at http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R2073 (accessed 19 July 2016).
[49] (EU) 2015/2285 is available at http://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1468917512617&uri=CELEX:32015R2285 (accessed 19 July 2016).

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

The European Centre for Disease Prevention and Control 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.[50] The model driving the mapping software has been calibrated to the Baltic Region in Northern Europe.

C.2 Country-specific controls

Monitoring *V. parahaemolyticus* 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.[51] 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 2003 Risk Profile listed measures for Shellfish Control Authorities to undertake that would minimise the risk of BMS being contaminated with pathogenic *V. parahaemolyticus* and explained that processors of fish and fishery products were required to have a HACCP system in place.

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 purpose 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. parahaemolyticus* and *V. vulnificus* illnesses, conducting annual risk evaluations, and (if necessary) implementing Control Plans for *V. parahaemolyticus* and *V. vulnificus*. A Control Plan is implemented for a State if the annual risk evaluation concludes that *V. parahaemolyticus* infection from the consumption of oysters from that State is "reasonably likely to occur" (i.e. the risk constitutes an annual occurrence). The risk evaluation considers:

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

[50] https://e3geoportal.ecdc.europa.eu/SitePages/Vibrio%20Map%20Viewer.aspx (accessed 19 July 2016).
[51] https://www.legislation.gov.au/Series/F2012L00291 (accessed 19 July 2016).

A Control Plan must also be implemented if a State has a shellfish growing area that was the source of oysters that were epidemiologically linked to an outbreak of *V. parahaemolyticus* within the prior five years.

The Control Plan includes identifying one or more triggers (e.g. water temperature) that indicate when control measures are needed and specifying the controls to be implemented.

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.[52] 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 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. parahaemolyticus* and *V. vulnificus* to non-detectable levels (defined as <30 MPN/g).

The guidance specifically describes circumstances where it is reasonably likely that shellfish will be contaminated with *V. parahaemolyticus* at an unsafe level (so kill-steps should be implemented). This is when oysters are harvested from an area that meets any one of the following conditions:

- The shellfish control authority has conducted a risk evaluation and determined that the risk of *V. parahaemolyticus* illness from the consumption of oysters harvested from that growing area is reasonably likely to occur;
- The shellfish control authority has determined that harvesting occurs in the growing area at a time when average monthly daytime water temperatures exceed 60°F (15.6ºC) for waters bordering the Pacific Ocean and 81°F (27.2ºC) for waters bordering the Gulf of Mexico and the Atlantic Ocean (New Jersey and south), except where a more rigorous risk evaluation has led the shellfish control authority to conclude that the risk of *V. parahaemolyticus* illness from the consumption of oysters harvested from that growing area is not reasonably likely to occur; or
- The growing area has been confirmed as the original source of oysters associated with two or more *V. parahaemolyticus* illnesses in the past three years.

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

- Ready-to-eat fishery products (minimal cooking by consumer): $1x10^4$ MPN/g.
- Post-harvest processed BMS that make a label claim of "processed to reduce *Vibrio parahaemolyticus* to non-detectable levels": <30 MPN/g.

A 2004 survey of post-harvest processed oysters carrying the label claim detected *V. parahaemolyticus* in 18/61 samples, but the concentration of *V. parahaemolyticus* only exceeded 30 MPN/g in two samples (DePaola *et al.*, 2009). These samples did not make the label claim for *V. parahaemolyticus* (only for *V. vulnificus*).

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* in the oysters is ≤100 MPN/g, unless a validated post-harvest processing step is applied that will reduce *V. parahaemolyticus* to this level (FAO/WHO, 2016).

[52] http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334115.htm (accessed 19 July 2016).

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.[53] If deemed necessary, BMS processors are also required to undertake occasional testing of end-product BMS for *V. parahaemolyticus*. A microbiological guideline has been established for *V. parahaemolyticus* in live oysters (CFIA, 2016): Of five sample units, none may exceed 100 MPN/g *V. parahaemolyticus*.

The *V. parahaemolyticus* microbiological guidelines for live oysters intended for raw consumption were under review as of May 2016, when a call for data to support the review was announced.[54]

C.2.6 Japan

A description of Japanese controls for *V. parahaemolyticus* in seafood has recently been published (Hara-Kudo and Kumagai, 2014). Four main controls were introduced by 2001:

- Use disinfected or artificial seawater, or potable water, for washing and processing seafood;
- Maintaining seafood temperature at or below 10ºC during distribution and storage;
- Microbiological standards for *V. parahaemolyticus*: ≤100 MPN/g for seafood intended for raw consumption, not detected/25 g for ready-to-eat boiled seafood;[55] and
- Advice that consumer should consume seafood within 2 h of it being removed from the fridge, and restaurants should serve it immediately.

The microbiological standards were based on the assumptions that an infectious dose of TDH-producing *V. parahaemolyticus* is 100 cells/serving and a raw seafood serving is 100 g, and informed by outbreaks and studies of the ratio of total *V. parahaemolyticus*:*tdh*+ *V. parahaemolyticus* in seawater (Hara-Kudo and Kumagai, 2014).

A substantial reduction in incidence of reported *V. parahaemolyticus* infection in Japan was credited to these regulations (Hara-Kudo and Kumagai, 2014).

[53] http://www.inspection.gc.ca/food/fish-and-seafood/communiques/archive/2013-07-23/eng/1371488770625/1371488872212 (accessed 25 July 2016).
[54] http://www.hc-sc.gc.ca/fn-an/consult/2016-oyster-huitre/document-consultation-eng.php (accessed 25 July 2016).
[55] Note that the standard for "seafood for raw consumption" is reported as "not detectable in 25 g" in FAO/WHO (2016).

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