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NZ-RLO& *T. maritimum* 2015 response

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1 Glossary

AHL	Animal Health Laboratory
CAN	Controlled Area Notice
FY	Financial Year
ITS PCR	Internal Transcribed Spacer Polymerase Chain Reaction
KCSRA	Kenepuru & Central Sounds Residents Association
MLST	Multilocus Sequence Typing
MPI	Ministry for Primary Industries
NZSFA	New Zealand Salmon Farming Association
NZKS	New Zealand King Salmon Ltd.
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
RLO	*Rickettsia-Like* Organism
TAG	Technical Advisory Group

2 Foreword

This report provides information on findings and summarises the actions and decisions undertaken as part of the NZ-RLO & *T. maritimum* 2015 biosecurity response to unusually high mortality rates in marine-farmed salmon and the subsequent confirmation of two new-to-New Zealand bacteria thought to be a contributing factor in fish morbidity and mortality.

It should be noted that since the bulk of this report was written, various actions have been undertaken to address recommendations by the Technical Advisory Group, and to provide context and explanation around some of the issues and observations highlighted. Not all of these actions are covered in this report. Additionally, due to the timeframe of the response, certain details presented within this report may now be out of date.

3 Executive summary

Between 2012 and 2015, higher than usual rates of fish mortality were observed during summer at some salmon farms in the Marlborough Sounds. The Ministry for Primary Industries (MPI) has been working closely with the New Zealand King Salmon (NZKS) company to investigate the situation by looking at a number of factors that could be involved, including the environment, farm management practices, and the presence of bacterial agents, all of which could have a relationship to the increased mortalities.

During 2012 MPI tested tissue samples from dead fish but could not find a definitive cause of mortality. The Ministry concluded that there were likely to be multiple factors at play including the farm site, water temperature and flow, and feed.

Following a 2015 mortality event and subsequent detection of a *Rickettsia-like* organism (RLO), an unwanted organism, MPI initiated a biosecurity response. The biosecurity response has included the imposition of movement controls and notices of direction pursuant to the Biosecurity Act 1993, extensive sampling and testing of salmon at the Animal Health Laboratory (AHL) in Wellington, a multivariate analysis of the possible causes of increased mortalities, and the formation of a Technical Advisory Group.

MPI's scientists developed and validated new tests and, through this work, detected the presence of two bacteria in the Marlborough Sounds that had not been confirmed as present in New Zealand before; one being a *Rickettsia-like* organism and the other a bacterium called *Tenacibaculum maritimum*. Wider testing has also detected the presence of these bacteria in salmon farming areas outside of the

Marlborough Sounds. As such, MPI believes that the recent mortality event has a range of causes, with the bacteria possibly being involved in the fish deaths in combination with other factors.

Rickettsia-like organisms (RLOs) are very small bacteria that live inside the cells of fish some of which are harmless, while others may cause disease and death. MPI's genetic testing shows the bacteria found in New Zealand are different to *Piscirickettsia salmonis*; a form of *Rickettsia* found in Chilean salmon farms which is known to have caused problems for salmon farming overseas.

Testing of historical tissue samples from the 2012 using a suite of improved techniques has shown the *Rickettsia-like* organism was present then, and is likely to have been in New Zealand for some years and may be widespread in our marine environment.

4 Introduction

4.1 Aquaculture in NZ

The NZ Government supports well-planned and sustainable aquaculture growth in New Zealand and is committed to enabling industry to achieve its goal of $1 billion in annual sales by 2025. A vital part of this commitment is to ensure aquaculture growth takes place under safe and sound biosecurity management and within acceptable environmental limits.

4.2 Salmon Farming

Chinook (king) salmon is farmed in three regions of New Zealand: Marlborough Sounds (64% of New Zealand's king salmon), Southland (32% is grown in Big Glory Bay, off Stewart Island) and Canterbury (4% – including a marine farm off Akaroa, and some small freshwater farms in the McKenzie Country hydro canals).

The vast majority of king salmon farmed in New Zealand are raised in ocean pens in the Marlborough Sounds, Stewart Island and Akaroa.

The farms are located in areas that were originally used for farming mussels and were available to convert to salmon farms. Farm site selection is so critical that only a handful of areas around the country are considered suitable for salmon farming operations. Government and industry are currently exploring the feasibility of relocating existing salmon farms in the Marlborough Sounds to alternative locations.

A New Zealand farmed king salmon starts life in a fresh water hatchery where it will remain until around 6-13 months before being transferred to a seawater farm. After being placed in a seawater farm, a young fish will generally take 12-18 months of further nurturing to grow to an optimum market size of around 3.5-4 kg and ranging up to around 6 kg. The pens are around 20m deep and offer improved growing conditions, allowing the fish to move below the surface water and away from surface related stress. Densities range from less than 1 kg/m^3 to around 25 kg/m^3 (depending on the life stage of the salmon).

New Zealand farmed salmon are fed food pellets specially formulated for king salmon.

4.3 Biosecurity response

Following unusually high mortality events on New Zealand King Salmon (NZKS) farms in February 2015, NZKS supplied salmon samples to determine whether an infectious agent could have been causing the mortalities. From samples provided to AHL, two new-to-New Zealand detections were confirmed in king salmon (*Oncorhynchus tshawytscha*): a New Zealand *Rickettsia-like* organism (NZ-RLO1) and *Tenacibaculum maritimum*. This was the first time the presence of these two bacterial agents had been confirmed in New Zealand. Of the two bacteria, the NZ-RLO is of particular interest because it is listed as an unwanted organism under the Biosecurity Act 1993.

The biosecurity response team believes these bacterial organisms could be a factor in the unusual salmon mortalities experienced by the affected farming operations although, based on a multivariate analysis, a direct causal link is unknown and difficult to determine. In response, MPI issued a Notice of Direction under section 122 of the Biosecurity Act to NZKS in October 2015, directing the company to comply with their own Biosecurity Management Plan and controlling movements between their marine farming operations. Subsequent surveillance has revealed that all of the NZKS farms tested in the Marlborough Sounds contained these organisms.

Subsequently a second NZ-RLO (NZ-RLO2) was detected at a NZKS farm in the Marlborough Sounds which may have also contributed to mortality events. A third NZ-RLO (NZ-RLO3) was detected at a farm in Akaroa but did not appear to cause increased mortalities.

In April 2016, MPI revoked the Notice of Direction and imposed controls on the movement of salmon, salmon products, and salmon farming-related equipment within the Marlborough Sounds using a Declaration of Controlled Area and Notice of Movement Controls under section 131 of the Biosecurity Act (“Controlled Area Notice”) with the intention of limiting the spread of NZ-RLOs to other marine salmon farming areas.

5 Operations and unusual mortality in marine salmon farms in the Marlborough Sounds

5.1 NZKS operations

The New Zealand King Salmon Company Ltd (NZKS) is one of the largest producers of farmed king (Chinook) salmon (*Onchorhynchus tshawytscha*) worldwide with a yield of approximately 6,300 metric tonnes in FY16 of which about half is exported contributing around $60M to New Zealand's GDP. This represents over 55% of the global market for king salmon and approximately 54% of the total salmon production in New Zealand. Smolt are raised in land-based hatcheries in the South Island and then transferred into several sea pens located throughout the Marlborough Sounds region (Figure 1). The time from transfer until harvest ranges from 54 weeks to 72 weeks depending on growth rates and market demand.

NZKS operates three freshwater hatcheries in the South Island. The majority of NZKS production broodstock is held at their Takaka (Golden Bay) hatchery, whilst some backup broodstock is held at Waiau. There is a smolt hatchery (Tentburn) and a backup hatchery (Waiau) in Canterbury. The MPI Spatial Allocations Team issues authorisations to move brood stock from marine farm sites to the Takaka freshwater farm and smolt from land based hatcheries to marine sites.

NZKS moves smolt from hatcheries to the seafarms during spring and autumn. In spring, smolt from Tentburn are transferred to Ngamahau, Te Pangu, and Clay Point. Between March and May fish from these farms are transferred to Otanerau, a farm which is only stocked in the cooler winter months due to high water temperatures in summer. In autumn, smolt from Tentburn are transferred to Waitata, Ruakaka, and Te Pangu. In order to meet harvest demands, some fish are counted and size graded about 6 months after seawater entry.

Otanerau and Waitata are fallowed post harvesting for about 2-3 months prior to stocking with the next new year class. No other farms are laid fallow on a regular basis; for the other farms sites, there is a 6 month overlap between a new year class being introduced to a site and the harvest of the oldest year class. This overlap means the disease cycles cannot be broken with hosts always present at a site, potentially acting as a reservoir for any pathogens present to be transferred to the next generation of fish. At present, there is insufficient farm space at these sites to separate year classes while also maintaining current production levels.

Fish feed is imported from Australia and Chile under an Import Health Standard (FISFOOIC.ALL (2 December 2011)). The feed is comprised of fish meal, fish oil, and meals and oils from other sources, and the necessary vitamins and minerals required for healthy salmon growth. Food is offered to the salmon throughout the day, using a camera system to monitor feeding. When satiety is reached, feeding is stopped by the operator. Feed quality has been an issue for NZKS in the past and the composition and supplier of feed has been changed a few times over recent years. Feed issues may compromise the health of the fish, leaving them susceptible to opportunistic infection by bacteria and viruses.

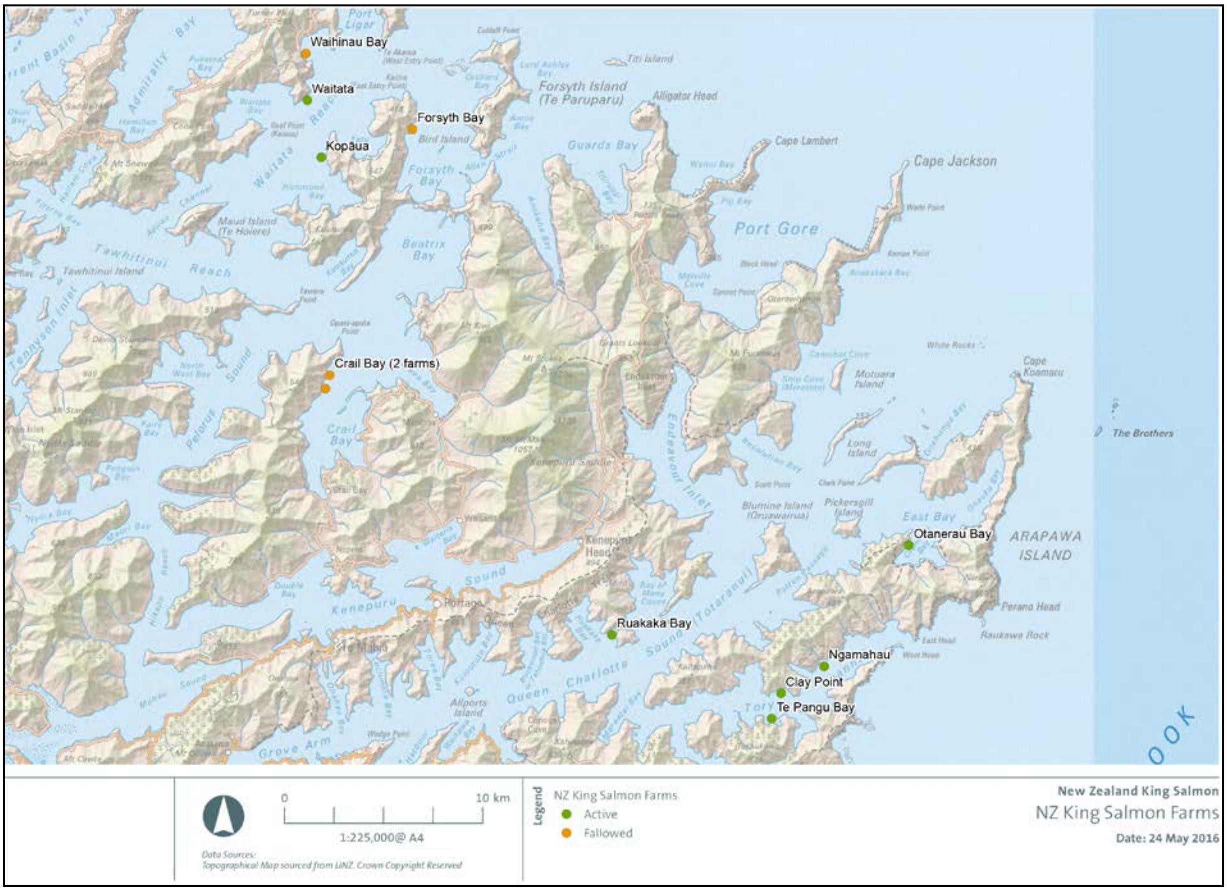

Figure 1: Location of New Zealand King Salmon farms in the Marlborough Sounds as of May 2016.

As with all farmed animals, mortality occurs throughout the farmed salmon lifecycle. NZKS expect a mortality rate of approximately 25%, with approximately 5% of the mortality being the result of recently (within 6 weeks) transferred fish, which is attributed to the stress of transport and failure of the fish to adapt to seawater. These 'runt' mortalities are considered normal in salmon production.

Regular (usually daily) mortality removal and categorisation is carried out by the site team. Carcasses are inspected on the surface and categorised on the basis of physical signs and recorded in *Fishtalk* software. An alert to NZKS management may be triggered by different than usual mortality categories, or an increase in numbers of mortalities, which may subsequently lead to an investigation by NZKS. The following classifications are used consistent with industry standards: early runts (fish that fail to adapt to seawater); late runts; 2 year old maturation; predation; salmon with external lesions; bloat (a feed related issue); operational issues; and fish which have no external sign of the mortality. Dead fish are transferred to mortality bins on the farms, which are then collected by vessels and taken to Havelock and Picton, and then sent to a rendering plant in Feilding or landfill in Marlborough. The number and type of mortality is recorded on the farm software system. This system is used by the aquaculture management team to take appropriate decisions as required.

Net cleaning occurs every 10-15 days using a machine; biofouling on solid farm structures gets scraped off at variable intervals. The biofouling material is released into the environment consistent with ANZECC in-water cleaning guidelines (ratified by MPI). The company has reported that the presence of certain biofouling organisms (e.g. anemones) is associated with an increased incidence of injuries (skin lesions) to the salmon, possibly caused by nematocysts. Investigations by NZKS are continuing to derive causality data.

5.2 Response Background

Historically mortality rates have remained up to about 25% across all farming sites in the Marlborough Sounds; however, in March 2012 NZKS reported a more significant mortality event (with cumulative losses ~30%) at its Waihinau Bay site where affected fish displayed signs of lethargy, reduced feed intake, and superficial skin lesions shortly prior to death.

Initial diagnostic investigations conducted by the Ministry for Primary Industries ruled out notifiable diseases including infectious salmon anaemia virus, viral haemorrhagic septicaemia virus, and infectious haematopoetic necrosis virus. On post-mortem examination, affected fish were found to have inflammation of the heart, liver, and muscle tissues, which was not consistent with other common infectious salmon diseases. There were also no obvious acute changes in environmental conditions (e.g. dissolved oxygen content, water temperatures, or weather events), management practices (e.g. feeding changes, grading events, or harvesting events), or predation that could explain the increase in mortality. No unusual mortality was reported at the other three production sites (Te Pangu Bay, Clay Point, and Ruakaka Bay) that also received smolt from the same land-based hatchery. Consequently, no definitive cause for the deaths was established at the time (MPI Technical Paper 2013/19, Norman *et al*. (2013)).

The Waihinau Bay site continued to experience unusually high mortality levels during the following two summers, with up to ~70% mortality in February 2015. In June 2015, an additional 10 moribund fish were screened for a wider range of infectious pathogens.

5.3 Diagnostic investigation into unusual mortalities

Following further high mortality events at Ruakaka and Waihinau in February 2015, NZKS sent samples to Chile for diagnostic testing. Preliminary results indicated the presence of *Piscirickettsia salmonis*, so NZKS reported the suspect presence of a disease agent in their salmon to MPI. NZKS provided samples to MPI's Animal Health Laboratory (AHL) where two new-to-New Zealand detections were confirmed in NZKS fish; a New Zealand *Rickettsia*-like organism (NZ-RLO1) and *Tenacibaculum maritimum*. This was the first time these two bacterial agents were tested for using PCR and the first time their presence was confirmed in New Zealand. *T. maritimum* is found globally and was previously known as *Flexibacter maritimus*. Of the two bacteria, the NZ-RLO1 is of particular interest because it is listed as an unwanted organism under the Biosecurity Act 1993 and is sufficiently different from *P. salmonis* that the standard test recommended for *P. salmonis* was usually negative or indicated a weak positive. A better test to detect the NZ-RLO was therefore developed at AHL.

Tissue samples retained by MPI from the 2012 mortality investigation were also re-tested using the new test and found to be positive for both organisms, which suggests a potential association with the earlier mortality events. Due to the lack of available tissue samples from periods prior to 2012, it was not possible to determine if the mortality events represented a new introduction of these organisms into the Marlborough Sounds region, if these organisms were already present and there was some other change in the production environment that may have resulted in the clinical expression of disease, or if the mortality events were unrelated to the presence of these organisms.

Further sampling and testing of NZKS farms revealed a second strain of NZ-RLO present in fish (NZ-RLO2). Subsequent surveillance sampling and testing at non-NZKS farms detected a third strain of NZ-RLO in farmed Chinook salmon from Akaroa Harbour (NZ-RLO3). These will be described later in further detail in 4.3.2. A summary of the samples and test results is provided in Table 1, below.

Table 1: Positive (+) and negative (-) detections of NZ-RLOs and *T. maritimum* at tested marine farms. Zone A and Zone B refer to the Controlled Area established in 2015.

Area	Zone	Site	NZ-RLO1	NZ-RLO2	NZ-RLO3	*T. maritimum*	# of Fish Samples
Marlborough	Zone A	Waihinau	(+)	(+)	(-)	(+)	25
		Waitata			(-)	(+)	8
		Forsyth			(-)		
		Kopāua			(-)		
Marlborough	Zone B	Clay Point	(+)		(-)		30
		Ruakaka	(+)	(+)	(-)	(+)	75
		Te Pangu			(-)	(-)	20
		Otanerau		(+)	(-)	(+)	5
		Ngamahau			(-)		1
Akaroa		Akaroa			(+)	(+)	303
Big Glory Bay		Sanford	(-)	(-)	(-)	(+)	310
Takaka			(-)	(-)	(-)		5
Ruakaka		Broodstock	(+)	(+)		(+)	7
Ngamahau		Broodstock	(-)			(+)	1
Te Pangu		Broodstock	(-)			(+)	4

5.3.1 Tenacibaculum maritimum

Tenacibaculum maritimum is a gram-negative filamentous bacterium that can cause ulcerative lesions on the body surface of affected fish (Figure 2). It is mainly associated with secondary infections in salmon, and can cause mortality. It has been isolated from many different species across the world including farmed Atlantic salmon in Australia, confirming its presence in the southern hemisphere. The pathogen is difficult to culture under laboratory conditions and diagnosis is therefore primarily based on the appearance of clinical signs. The most common lesions are pinpoint haemorrhages and shallow erosions appearing over sites of abrasion and trauma such as points of the fins brushing on the flanks. Salmon appear to be particularly susceptible to developing lesions on the eyes and gills compared with other fish species. Under experimental conditions, peak mortality occurs within one to two weeks of exposure and can range anywhere from 10% to 98% depending on the virulence of the strain as well as other environmental conditions.

Figure 2: Skin lesions on Chinook (king) salmon from affected farms in the Marlborough Sounds.

T. maritimum is a bacterium that grows optimally in water temperatures ranging from 15°C to 35°C. Mortality rates in susceptible fish species increase with higher water temperatures and lower water salinities, which favour pathogen replication. *T. maritimum* is only found in the marine environment, not in freshwater. Previous outbreaks in farmed salmonids have also been linked to extended periods of cloud-free days with water temperatures elevated above 20°C. However, outbreaks have also been reported during winter months in several production systems, which further emphasizes a multifactorial nature of the disease. Smaller fish appear to have an increased susceptibility to *T. maritimum* and are more likely to suffer severe disease. Little is currently known about the reservoirs for disease in the marine environment. *T. maritimum* is not an unwanted organism under the Biosecurity Act 1993 and may be widespread in New Zealand.

5.3.2 *Rickettsia*-like organisms

Numerous distinct *Rickettsia-like* organisms (RLOs) have been isolated from farmed salmonid species worldwide. They are increasingly being recognized as important pathogenic agents contributing to fish mortality. The potential host range of RLOs in New Zealand is currently unknown and the implications for species other than salmon in New Zealand are unknown. The most widely studied organism is *Piscirickettsia salmonis*, which has been linked to disease outbreaks in Chile, Scotland, Ireland, Norway and Canada. Testing by MPI shows that NZ-RLOs are genetically different from *P. salmonis*, although they are similar enough that random, general testing (i.e. testing not specific to RLO types) undertaken in foreign markets may result in a positive detection of *P. salmonis.* This could result in the closure of overseas markets for New Zealand salmon.

There is no evidence to suggest that *P. salmonis* is present anywhere in New Zealand.

Clinical signs in affected fish include lethargy, erratic swimming behaviour, patchy white skin lesions that progress to shallow ulcers, and pale gills secondary to anaemia. Post-mortem examinations often reveal white ring-like nodules on the liver and enlarged kidneys and spleen. The clinical signs of other RLOs appear to be similar. Mortality rates on farms affected with *P. salmonis* can exceed 90%, although more typically range from 50 to 70%.

The mortality rates associated with other RLOs under natural conditions are not widely reported in the published literature, but generally appear to be lower than for *P. salmonis*. The severity of outbreaks may be related to differences in environmental and management conditions. The optimal temperature for pathogen replication in laboratory culture is between 15°C and 18°C, whereas survival outside the fish is significantly increased at water temperatures below 10°C. Periods of rapidly fluctuating temperature have also been associated with outbreaks of *P. salmonis*. *P. salmonis* does not survive long in freshwater environments and transmission through eggs is not believed to be important; hence, it is unlikely for outbreaks to originate in land-based hatcheries. Similar to *T. maritimum*, little is currently known about the reservoirs for disease in the marine environment.

5.3.2.1 NZ-RLO1

The first New Zealand RLO which was isolated from Marlborough Sounds salmon appears to be closely related to a similar organism isolated from farmed Atlantic salmon in Tasmania (the Tasmanian RLO), and was named New Zealand *Rickettsia-like* organism (NZ-RLO1). The partial 16S rRNA and partial ITS genes of the two isolates (NZ-RLO1 and TAS-RLO) of the two organisms are identical (and were assumed to be the same strain).

This strain has now been detected in 3 sites in the Marlborough Sounds (Waihinau Bay, Clay Point and Ruakaka). Representative samples were sequenced from the PCR positive samples so the true prevalence of this strain in all of the samples tested is unknown.

NZ-RLO1 was detected in kidney, spleen and liver tissues as well as in skin lesions.

5.3.2.2 NZ-RLO2

A second RLO was isolated from affected fish in the Marlborough Sounds farms. This organism was positive for all three commercially available *P. salmonis* specific PCR tests, and sequencing of the partial 16S rRNA gene and partial ITS region was 99% and 100% identical to published strains of *P. salmonis* over the short sequence available from the PCR process. Accordingly, the organism was successfully cultured and sequenced and the full sequence was compared with NZ-RLO1. Subsequent analysis of the *rps* gene indicated that both NZ-RLO1 and NZ-RLO2 were genetically different from the Chilean strains of *P. salmonis*.

The second strain was also detected from Waihinau Bay but from a sampling event conducted in October 2015. This strain was detected from skin lesion tissue from 6 of 15 fish by real-time PCR and confirmed using ITS conventional PCR.

Sequencing of the ITS region was carried out and the results showed highest similarity (100%) to *P. salmonis* isolate IRE-99C (GenBank® accession No. AY498632).

Sequencing of the partial 16S rRNA gene was also carried out and the results showed highest similarity (99%) to *P. salmonis* isolate IRE-99D.

This strain has now been detected at 3 sites in the Marlborough Sounds (Waihinau Bay, Ruakaka and Otanerau). Representative samples only were sequenced from the PCR positive samples so the true prevalence of this strain in the samples tested is unknown.

NZ-RLO2 was also detected in kidney, spleen and liver tissues as well as in skin lesions.

5.3.2.3 NZ-RLO3

A third strain was detected from samples from Akaroa, which were sent in for surveillance as part of the response investigation. This strain was detected from kidney, spleen and liver tissue in 3 fish tested by real-time PCR. This result was confirmed using ITS conventional PCR and sequencing.

The NZ-RLO3 strain in Akaroa, which appears to be unique to Akaroa, was found at extremely low prevalence and in apparently asymptomatic fish. This, and the similarity of the NZ-RLO1 to the TAS-RLO from Australia, suggests that at least some of the NZ-RLO strains have been in Australasia long enough to exhibit regional differences. The significance of these findings is being further investigated.

To date this strain has only been detected in 3 of 292 fish from Akaroa, as confirmed by sequencing of the PCR positive samples.

5.3.3 Next generation sequencing

5.3.3.1 Phylogenetic analysis

Ribosomal MLST analysis showed that the Chilean strains of *P. salmonis* were clustered into 2 different groups, and the NZ-RLO1 and NZ-RLO2 were distinct from the Chilean isolates (Figure 3). The sequence of NZ-RLO1 was genetically more diverse compared to all other strains, whilst the NZ-RLO2 was closely related to one of the groups (EM90, LF89-ATCC-VR-1361-3, PM15972A1 and T-GIM). These results were reproducible when different alignment and tree reconstruction methods were used.

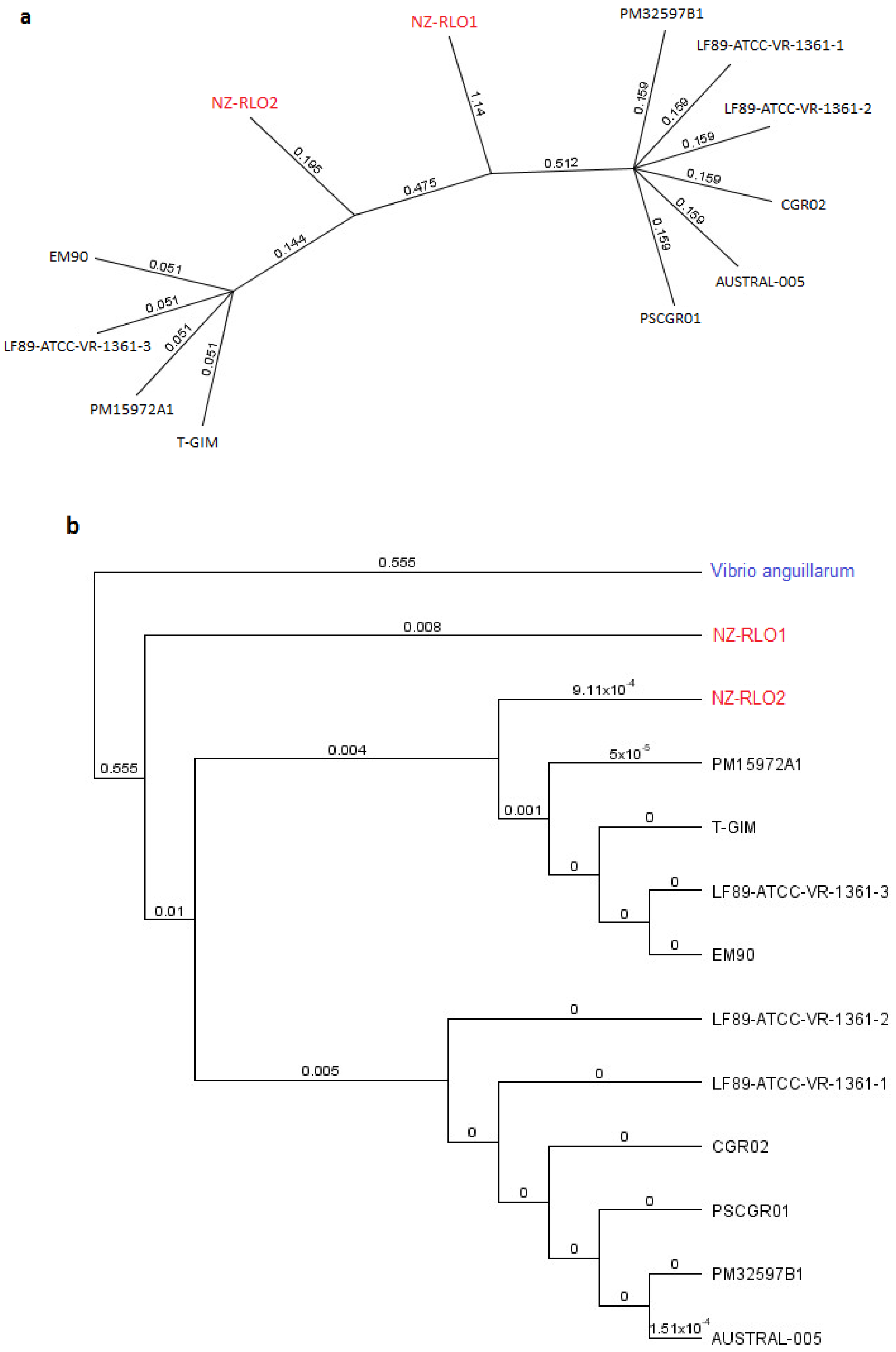

Figure 3. Unrooted (a) and rooted (b) trees of different Chilean strains of *P. salmonis* based on 52 *rps* genes constructed using ClonalFrame and PhyML, respectively. New Zealand strains in red font, outgroup in blue font. Branch labels indicate the amount of genetic change.

5.3.3.2 Whole genome comparison

A comparison of the whole *P. salmonis* genomes available on international genomes databases showed 2 different groups. NZ-RLO2 was closer to one *P. salmonis* group than the other. However, in all three analyses the NZ-RLO1 did not reach the cut-off value to suggest it was *P. salmonis*. The results for NZ-RLO2 were less conclusive, showing it had a close relationship to one of the two *P. salmonis* groups but the relationship was border-line for the other group. In the tetra analysis NZ-RLO2 did not reach the cut-off level for closeness to either group.

These analyses indicated that NZ-RLO1 and NZ-RLO2 are genetically different from the Chilean strains. The whole genome comparison supports the NZ-RLO2 belonging to the *Piscirickettsia* genus but further investigation is needed to clarify the relationship. The analysis was based on comparing the New Zealand genomes with a limited number (10) of published genomes from Chile, and this comparison is dependent on the dataset being used providing a good representation of the variation within the species. Four of the genomes are from the same strain, LF-89, originally from Chile in 1989. These four genomes showed a large degree of variation as they separated between the two groups. This could reflect accumulated changes as the isolate was cultured in the laboratory over time. Currently there is only one recognised species although the degree of variation found by previous researchers using 16S, ITR and 23S rDNA, suggests more than one species. More taxonomic investigation of the *Piscirickettsia* genus is needed to identify the level, phenotypic and genotypic variation and number of species involved.

The presence of an organism called Tasmanian RLO (TAS-RLO) has been reported in diseased Atlantic salmon in Tasmania, Australia. Though the TAS-RLO shares morphological characteristics with *P. salmonis*, it has a 19 base pair deletion in the rDNA ITS region when compared to *P. salmonis*. The first NZ- RLO which was isolated from Marlborough Sounds fish appears to be closely related to the TAS-RLO, and was named New Zealand *Rickettsia-like* organism (NZ-RLO). Comparing the partial 16S rRNA and partial ITS genes of the two isolates, NZ-RLO and TAS-RLO, the two are identical. The full genome of TAS-RLO is not publically available so comparison of the two isolates using Multi-locus sequence typing is not currently possible.

5.4 Multivariate Analysis of unusual mortalities in 2015

This analysis was undertaken by independent experts (Gates *et al.*, 2015) based on information supplied to MPI by NZKS.

NZKS maintains detailed computerised production records that offered a unique opportunity to explore the epidemiology of mortality events associated with the presence of NZ-RLO and *T. maritimum*. The main objectives of this analysis were therefore (1) to provide more detailed descriptive statistics on spatial and temporal trends in fish mortality rates across the NZKS production sites and (2) to investigate potential risk factors that could explain differences in fish mortality rates within and between production sites.

Periods of abnormally high mortality were observed at the Waihinau Bay and Forsyth Bay sites from 2012 to 2015 (Table 2). The largest peak occurred at the Waihinau Bay site in March 2015 with the daily mortality rates reaching over 320 deaths per 10,000 fish per day compared with rates of 100 to 150 deaths per 10,000 fish per day observed during the mortality events in previous years. The majority of fish on the Waihinau Bay site weighed between 2.2 kg and 3.0 kg (mean: 2.6 kg) during the peak mortality event in 2015, which was not significantly different than the average weights in 2014 (mean: 2.5kg, range: 2.1 kg to 3.2kg), but higher than the average weights in 2012 (mean: 1.6kg, range: 0.86 kg to 2.7 kg) during the same time period.

Table 2: Percentage of fish that died during the summer months (1st February to 1st May) across the six main New Zealand King Salmon production sites from 2010 to 2016. A marked reduction in mortalities was observed in 2016.

Farm	Year						
	2010	2011	2012	2013	2014	2015	2016
Waihinau Bay	6	15.9	21.8	n/a	32.5	66.5	Destocked
Forsyth Bay	Destocked	Destocked	Destocked	48.1	Destocked	Destocked	Destocked
Ruakaka Bay	3.3	5.4	7.5	7.6	6	20.2	16.7
Clay Point	3	3.7	5.3	14.4	7.4	7.3	5
Te Pangu Bay	8.6	9.8	11.8	13.3	12.9	8.9	2.7
Otanerau Bay	Destocked	Destocked	Destocked	Destocked	Destocked	Destocked	10.7

Despite the apparent ubiquitous presence of NZ-RLOs in the Marlborough Sounds region, only the Waihinau Bay and Forsyth Bay sites experienced periods of excessively high mortality in the summer months (February to May) from 2012 to 2015. During the largest mortality peak at the Waihinau Bay site in March 2015, daily mortality rates reached over 320 deaths per 10,000 fish per day causing the overall loss of almost 70% of fish on the site. This mortality event may have been unusually severe due to higher water temperatures (a greater number of days where the water temperatures exceeded 18°C) and an abrupt decline in feed intake approximately 4 to 6 weeks prior to the mortality peak that was not associated with any known dietary or management changes. These factors may have created an optimal environment for pathogen replication and/or predisposed fish to developing clinical disease through the immunosuppressive effects of thermal and nutritional stress. Apart from water temperature, Secchi depth, and the presence of *T. maritimum*, there were no other significant differences in environmental conditions or management practices between the six production sites that could explain the variation in mortality rates.

Although the organisms isolated from moribund fish have previously been associated with mortality, we cannot establish a direct causative relationship based on the historical data alone. This leads to several biologically plausible, but not mutually exclusive hypotheses:

1) The organisms may be acting synergistically and initial infection with *T. maritimum* may have increased susceptibility to NZ-RLOs by creating breaches in the skin barrier,

2) The organisms may be acting independently and only one may be responsible for the excessive mortality,

3) Thermal stress, nutritional stress, or stocking density may have predisposed fish to developing clinical disease following exposure to one or both organisms and/or,

4) The mortality was caused or enabled by other unmeasured environmental or management factors, perhaps unrelated to either organism.

The aetiology of mortality events associated with skin lesions in farmed salmon is complex and multifactorial. Assuming that both *T. maritimum* and NZ-RLOs are ubiquitous in the Marlborough Sounds region and may have been present for some time, the multivariate analysis findings suggest that high water temperatures coupled with an acute drop in feed intake may predispose fish to experiencing unusually high mortality rates.

6 Technical Advisory Group

The purpose of a Technical Advisory Group (TAG) is to offer independent and objective advice, and information provided is used by the response team to form options and make decisions. The advice is taken on board alongside the response team's views and the practicalities of implementing this advice. MPI does not always agree with, or accept this advice and the TAG do not make decisions on behalf of MPI. MPI makes decisions based, among other considerations, on the advice received from the TAG, from its own experts, and from those impacted/affected.

A Technical Advisory Group (TAG) was established in mid-2015 to provide independent, impartial advice to the Response Management Team on managing NZ-RLO and *T. maritimum* and on impacts on the aquaculture and fishing industries and the environment. In particular technical advice was required on treatment and farm management options. The TAG met in New Zealand and visited farms in the Marlborough Sounds followed by a workshop to discuss specific questions raised by the response team and provide recommendations on the management of the pathogens.

6.1 TAG Membership

The TAG was independent of MPI and consisted of renowned New Zealand and international experts in aquatic animal health, epidemiology, finfish welfare, salmon-specific diseases, the aquaculture industry, bacteriology, biosecurity, farmed-fish production management, OIE trade, and *Rickettsia-like* organisms.

6.2 TAG Recommendations

1) The pattern of mortalities has an epidemic curve that is suggestive of a transmissible pathogen. Thus there may be a third pathogen which has not yet been identified which could explain the unmeasured component. Further sampling and testing should be carried out to determine the other pathogen.
2) Testing of brood stock should occur to rule out vertical transmission.
3) Freshwater hatcheries should be tested to determine if the NZ-RLO is present in freshwater. The results should inform a decision on whether movement controls are posed on smolt transfers from freshwater hatcheries to the marine sites.
4) Testing of the other marine salmon farming operations (Akaroa and Sanford) is important to determine whether the pathogens are restricted to the Marlborough Sounds.

5) Further work is required to improve NZKS' Biosecurity Management Plan. NZKS should be encouraged to do this.
6) Year class separation and fallowing periods are key management practices to mitigate biosecurity issues. This should be made a business priority rather than maximising the size of fish at harvest or stocking numbers.
7) Fish should be sampled before they are transferred to a new location and tested, particularly if that new location has been laid to fallow. This could benefit both the response team's decision making and NZKS' operations.
8) Industry should take a lead on, and work with MPI, to develop a code of animal welfare.
9) A research regime should be initiated to gather information on the issue and make progress on possible treatment options.

7 MPI response to the detection of an unwanted organism

7.1 Legal directions and instruments

As a precautionary measure, MPI has enacted some legal controls on salmon farming activities in the Marlborough Sounds to help limit the spread of NZ-RLOs to other areas.

7.1.1 Requirement to provide information

On 12th October 2015 MPI issued a notice under section 43 of the Biosecurity Act 1993 to NZKS, Akaroa Salmon and Sanford Ltd, compelling them to provide information deemed necessary to ascertain the presence or distribution in New Zealand of an unwanted organism.

The information required included all data on daily mortality of each group of fish at each farm since 2009 and all planned fish movements into, out of, or between farming operations.

7.1.2 Notice of Direction July 2015

Given the potential significance of a RLO detection in New Zealand, on 19th October 2015 NZKS were served with a Notice of Direction under section 122 of the Biosecurity Act 1993 requiring them to apply all ‘status red’ biosecurity measures detailed in their Biosecurity Management Plan (dated 10th July 2015). This included the cleaning and disinfection of equipment and personal protective equipment (PPE), disposal of dead fish and restricting the movement of equipment and stock between farming operations. Dead salmon could only be taken out of the contained zones for human consumption or rendering.

7.1.3 Controlled Area Notice

On 20th April 2016 a Controlled Area Notice (CAN) was implemented under section 131 of the Biosecurity Act 1993 to limit the spread of New Zealand *Rickettsiaceae* species as unwanted organisms, protect other marine salmon farming areas from an incursion of these species, and to monitor associated risk pathways for the movement of *Rickettsiaceae* species to other parts of New Zealand.

The CAN specified two Contained Zones (A & B) (Appendix 1) and detailed movement restrictions on risk items such as salmon, salmon products and waste products, farm-related vessels, farming equipment, and Personal Protective Equipment.

7.1.4 Notice of Direction July 2016

On 18th July 2016 NZKS were served with a Notice of Direction under section 122 of the Biosecurity Act 1993 to cease the distribution of bait and berley products derived from farmed salmon taken within the Marlborough Sounds Contained Zones as specified in the CAN.

7.1.5 Notice of Direction November 2016

On 1st November 2016 the July 2016 Notice of Direction was revoked with a new version being served allowing the production and distribution of berley subject to treatment by heating or freezing.

8 Audits

MPI contracted AsureQuality to undertake audits on the legal controls and directions it imposed as part of the NZ-RLO and *T. maritimum* 2015 response.

8.1 Section 122 Audit December 2015

The first audit took place on 14th December 2015 with NZKS being audited against the s122 Notice of Direction requiring them to adhere to their own 'Status Red' Biosecurity Management Plan. The following locations were visited during the audit:

- NZKS Ngamahau farm
- NZKS Ruakaka farm
- NZKS Picton Service Base

8.2 Section 131 Audit June 2016

The second audit took place on 22nd and 23rd June 2016 with NZKS being audited against the s131 Controlled Area Notice which required them to apply for permits before moving specified items out of the two contained zones.

The following locations were visited during the audit:

- NZKS Waitata farm
- NZKS Waihinau Bay farm
- NZKS Ruakaka farm
- NZKS Te Pangu Bay farm
- NZKS offices, Picton
- O'Donnell Park Barging Ltd offices, Picton

8.3 Section 122 Audit November 2016

As of March 2017, NZKS were trialling berley treatment methods. Once production and distribution begins, an audit will be undertaken to ensure compliance with the s122 Notice of Direction requiring the treatment of berley and bait products prior to distribution. The treatment method development and trial work is being monitored by AsureQuality.

9 Berley production and distribution

9.1 NZKS berley production

Fish harvested for human consumption are processed at the NZKS processing facility in Nelson. NZKS reports that the head and frames are minced and frozen for berley at the facility. This is done in batches approximately every 2 weeks. When not producing berley, the by-product goes to Kakariki Proteins Ltd, Feilding for rendering. The viscera is not used for berley but sent to the rendering facility. The berley is sold to a distributor in Auckland who then on-sells to bait shops in Auckland and other North Island locations, although most is used in the Auckland area. Approximately 120 tonnes of berley are manufactured from this commodity each year. Retail value of berley is approximately $1.2 million per year compared to the alternate fish meal product value of approximately $280,000 per year.

Berley was identified by MPI as a risk pathway at the beginning of the response in May 2015. As a consequence, NZKS ceased distribution in the South Island but continued supplying their distributor in Auckland as they viewed distribution to the North Island as a low risk activity. In July 2016 NZKS made MPI aware of this and as a result, MPI served a Notice of Direction on NZKS to cease all berley distribution (see section 8.4, below).

9.2 Other salmon producers

At a salmon industry biosecurity workshop in July 2016, members of the New Zealand Salmon Farming Association (NZSFA) decided it was a good idea to halt berley production and distribution. It was agreed for each representative to go back to their respective companies to decide whether this would happen at the next NZSFA meeting in September 2016. All of the industry agreed to stop untreated berley sales and this was confirmed unanimously at the NZSFA meeting in September 2016.

Sanford Ltd has confirmed they bring fish from Big Glory Bay to Bluff for processing. Some of the frames and offcuts go to customers for human consumption and the rest go to Timaru for processing into fishmeal. The fish viscera gets packed into 15kg boxes and blast frozen at -25°C overnight, is stored at -23°C and used by cod fishers as bait. Sanford Ltd have provisions in their coastal permit to dump at certain locations at sea for their mortalities, but recently they have changed their practices and started to bring the fish to land in bins and send them to Greenhills (near Bluff) for composting, after a recommendation made by MPI.

Akaroa Salmon Ltd advised that they do not produce berley.

Kakariki Proteins Ltd advised that no berley is produced by or sold from their facility. All salmon waste received by them is rendered at a high temperature over a long period of time sufficient to inactivate any pathogens. The fish meal produced is used in pet food.

9.3 Risk assessment

Risk analysis on berley production and use was undertaken by MPI. The advice is based on information that was initially collated for a finfish risk assessment, also undertaken by MPI. The risk assessment identified that infectivity is concentrated in head, gills and viscera so berley manufactured from these will be a risk pathway for further spread. RLOs are identified as a risk in berley manufactured from infected farms. The risk assessment advised that limiting berley production to healthy fish and ensuring at least one freeze/thaw cycle would provide some risk mitigation, but the decision as to whether this is sufficient would be the responsibility of the Response Team, taking into account feasibility, economics and other risk factors.

9.4 MPI's response to the continued berley distribution

On 18th July 2016, a Notice of Direction was issued to NZKS under s122 of the Biosecurity Act 1993 with the purpose of preventing distribution of berley. The Chair of Governance and Chief Technical Officer agreed that MPI should prevent distribution of berley in the short term, though not prevent production.

In August 2016, NZKS contacted MPI to enquire whether MPI permits the sale of remaining berley stock to the distributor in Auckland. In November 2016 and following further risk assessment, a revised s122 Notice of Direction was issued directing NZKS to cease the distribution of berley unless it was treated via a freeze (<-70°C for 24 hours) or a heat treatment (60° for at least 44min).

10 Biosecurity practices in the salmon industry

10.1 Sector-wide issues

Mortalities that occur on a fish farm are a potential reservoir for pathogens and the timely and secure disposal of any dead stock is a key strategy for minimising the risk they present. Under the terms of their coastal permit Sanford Ltd are permitted to dispose of fish from their Big Glory Bay operation by dumping them at specified locations at sea (Sanford Ltd, pers. com; this information has not been verified by MPI). This method of disposal presents an obvious risk of disease transmission to wild fish stocks and other wildlife.

While Sanford has recently chosen to discontinue this practice, its authorisation as an approved method of disposal by a consenting authority under the Resource Management Act suggests a lack of awareness of the risk that such disposal presents. This indicates a need for national direction by MPI on how councils should consider and address biosecurity issues and suggests that Unitary Authorities may not be routinely considering biosecurity issues when making resource consent decisions for marine farming operations.

10.2 NZKS biosecurity practice – recommendations by the TAG

Recommendations made by the independent TAG for managing NZ-RLO and *T. maritimum* clearly signalled the need for NZKS to review and improve their biosecurity management planning and practices. The following issues were identified by the TAG:

- <u>The NZKS Biosecurity Management Plan is inadequate</u> and does not provide clear guidance on the management of biosecurity risks across the company's salmon farming operations. The TAG observed that the operational guidance provided by the plan is vague and the lack of detail on prescribed actions potentially makes the plan difficult to implement.

- <u>The requirements of the Biosecurity Management Plan are inconsistently applied by NZKS</u>, and it is uncertain to what extent the plan influences "day to day" management of their farms. The TAG's site visit to NZKS farm sites revealed several apparent breaches of the Biosecurity Management Plan, including:
  - Bins for the storage of mortalities were not properly covered or situated next to each salmon pen as required by the Biosecurity Management Plan;

    - Dive staff moving between farm sites, using the same dive suits at each site, and although the Biosecurity Management Plan calls for the disinfection of all gear being moved between sites there was no indication that this was a regular practice;
    - The visitor management procedures in the Biosecurity Management Plan call for all visitors to farm sites to declare particular information on arrival at the farm (e.g. recent visits to other fish farms), but NZKS present during the TAG visit did not ask for this information.

- Management of NZKS farming operations reflected a low awareness of biosecurity risks. The TAG noted several instances of poor procedures for managing potential biosecurity risks, including:
    - Failure to quickly remove dead salmon from the pens and place them into secure storage for later removal and disposal;
    - Movement of the boat collecting mortalities between farm sites, with storage bins of dead fish being collected along the way and moved between farms.

- The NZKS production cycle is not consistent with international best practice for the prevention of disease. Because new stock is transferred onto farms before the harvest of stock that have attained marketable size, the new stock will be exposed to any disease agent that is present amongst the older stock in adjacent pens. Separation of different year-classes of salmon and the regular “fallowing” (destocked for a period of time) of farm sites are key strategies employed internationally to mitigate disease risks in salmon farming operations.

Potential solutions for industry to operate as per international best practice include providing more farm space, or reducing production to utilise existing farm space. Regardless of these potential solutions, improving biosecurity practices will be critical to minimising the risk exposure and spread of pests and diseases, and enable the sector to protect itself.

11 Animal welfare of farmed fish

The Animal Welfare Act 1999 is the primary legislation for animal welfare in New Zealand. This Act applies to farmed finfish, including fish caught from a wild state and held in captivity, and fish bred in captivity for farming. The Act treats commercial and recreational fishing as hunting and killing of animals in a wild state, and therefore separately from the care of farmed animals.

The Act places obligations on owners and persons in charge of animals, and also lays out offences for the lack of care of or conduct towards animals. A 'person in charge' may have responsibility for the fish temporarily or on a more permanent basis, depending on their role. The owner of farmed fish may place the fish in the care of others who become the person(s) in charge, but this does not derogate from the owner's responsibility to ensure that the requirements of the Act are met.

Owners and persons in charge must meet the physical, health, and behavioural needs of farmed fish in accordance with available technology, good practice and scientific knowledge. Farmed fish need to be provided with proper and sufficient food, space, adequate shelter, appropriate handling, and the opportunity to display normal patterns of behaviour and protection from, and rapid diagnosis of significant injury or disease, as appropriate to the species, environment and circumstances of the animal.

Owners and persons in charge of fish must ensure treatment that alleviates unnecessary or unreasonable pain or distress, and must kill animals in a way that does not cause unreasonable or unnecessary pain or distress.

11.1 NZKS Animal Welfare Plans and Policies

The increasing awareness and importance of animal welfare highlighted the importance of managing the welfare of farmed fish appropriately. Hence, NZKS need to be sufficiently prepared for any large mortality event or large numbers of sick or moribund fish in their sea cages. Appropriate animal welfare management should occur at all times, not only when there is a mortality or disease event. NZKS had a written Animal Ethics Policy in place prior to this response and sits on the NMIT animal ethics committee.

As part of this response NZKS has further updated their policies to include written management plans in the event of high mortality events (e.g. standard operating procedures for emergency harvests), and has also updated their Animal Ethics Policy. NZKS also brought these issues up with the industry body (NZ Salmon Farmers Association) to ensure a potential industry-wide issue was being managed. An industry-wide Biosecurity Policy has been developed and is currently in draft form. An industry-wide Welfare Policy is expected to be developed during 2017.

12 Kenepuru & Central Sounds Residents Association (KCSRA)

The Kenepuru & Central Sounds Residents Association Inc. (KCSRA) is a “watchdog for residents and property owners” and they “co-ordinate dealings with local and central government and to promote and act in the best interests of residents, ratepayers and persons associated with the Kenepuru and Central Sounds' area” (KCSRA website).

The KCSRA first showed an interest in unusual mortalities of salmon on the Waihinau Bay farm in 2012 and submitted an Official Information Act (OIA) request to gather more information on the mortalities. As some of the information was withheld to avoid prejudicing the entrusting of information to the Government of New Zealand on a basis of confidence, KCSRA complained to the Ombudsman and an interim report on the salmon mortality response was produced and posted on MPI’s website.

MPI staff met with KCSRA representatives to discuss the NZ-RLO & *T. maritimum* 2015 response, explain the response system, and form an official relationship through the response Liaison channel. This has certainly improved the communication between KCSRA and the biosecurity response team.

In June 2016, KCSRA published a paper on their website on the salmon issue. MPI was given the opportunity to provide comments and some were incorporated into the final version.

13 Conclusion

At this stage of the response MPI has not been able to ascertain the definitive cause of the mortality events at Waihinau Bay. MPI has isolated three strains of RLO and further testing is required to determine whether these could be the cause of mortality.

Possible further work that would benefit this response, as well as improve on-farm biosecurity and animal welfare might include:

- Testing of all NZKS farms in the Marlborough Sounds to build a complete picture of the distribution of the pathogens.
- Testing of the wild and recreational fisheries to determine presence or absence of pathogens outside of marine salmon farms.
- Further exploration of the possible relationship between NZ-RLOs and elevated mortalities through:
    - Challenge testing of salmon in a PC2 lab,
    - Development of a vaccine suitable for industry use, subject to results of challenge testing.
- For NZKS and the rest of New Zealand's salmon industry to adopt international best management practices for minimising biosecurity risk.
- MPI working with the NZSFA to develop a Code of Practice for Animal Welfare.
- Collaboration with Tasmania to compare the complete gene sequences between TAS-RLO and NZ- RLOs.
- Conducting further analysis on mortality data supplied by NZKS, given knowledge on the presence of NZ-RLO1 and NZ-RLO2 as obtained during the response.

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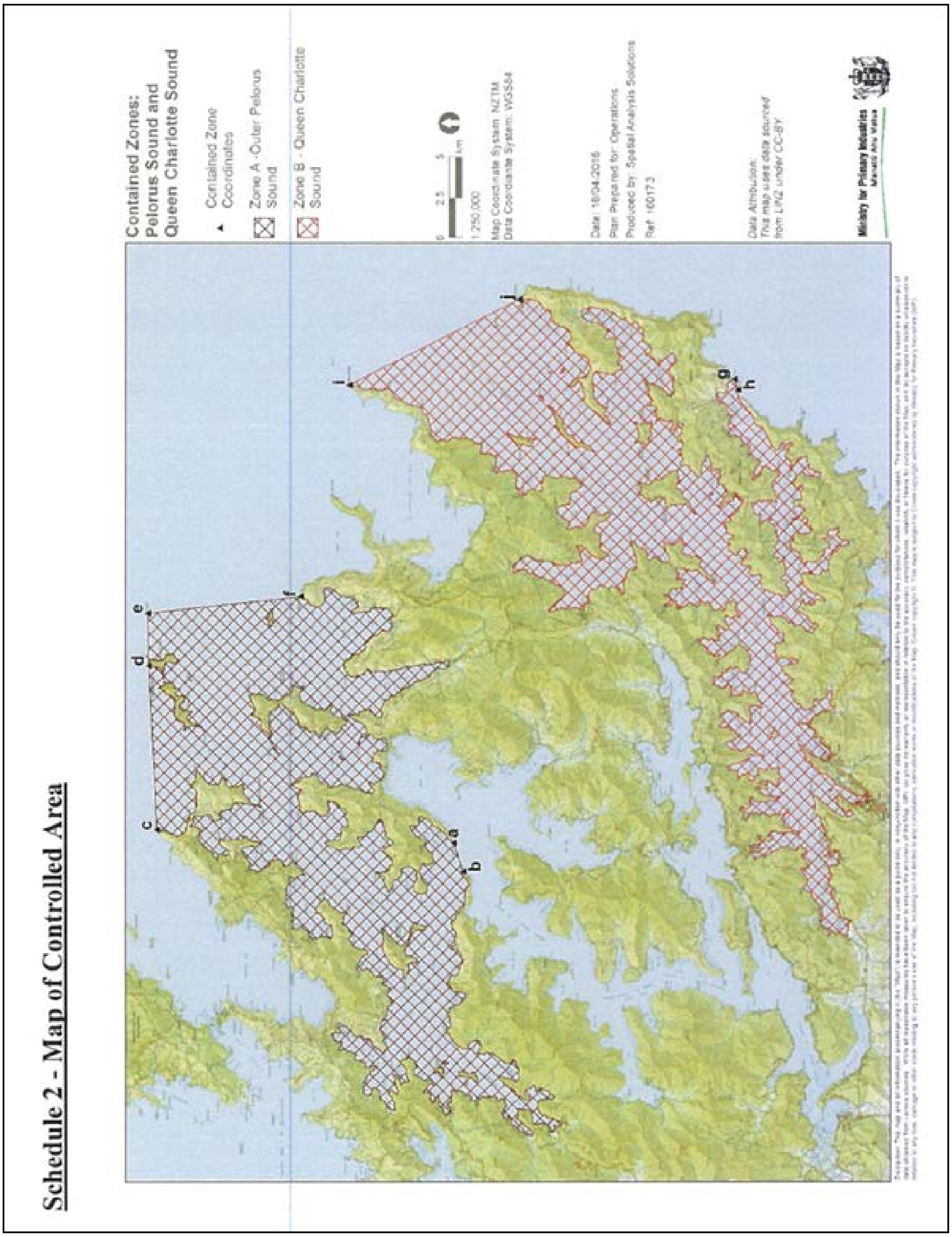
15 Appendices

Appendix 1 – Controlled Area Notice Contained Zones



Map of Contained Zones A & B imposed pursuant to s131 of the Biosecurity Act 1993.