

Technical advice on: The risk of New Zealand *Rickettsia*-like organism (NZ-RLO) and *Tenacibaculum maritimum* spread to the aquaculture industry and the environment

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Purpose of document

To support decision-making of the Chief Technical Officer and New Zealand *Rickettsia*-like Organism (NZ-RLO) and *Tenacibaculum maritimum* 2015 Response Governance by assessing the risk of NZ-RLO and *T. maritimum* spread to the aquaculture industry and the environment.

Background

- Between 2012 and 2015, elevated mortality rates of Chinook salmon (*Oncorhynchus tshawytscha*) were observed during summer at some New Zealand King Salmon (NZKS) farms in the Marlborough Sounds.
- During 2012 MPI tested tissue samples from dead fish but could not find a definitive cause of mortality. Following a 2015 mortality event, subsequent testing detected two bacteria species in the Marlborough Sounds that were new to New Zealand: a Rickettsiaceae species and *Tenacibaculum maritimum*.
- The Rickettsiaceae species detected in Chinook salmon was subsequently identified as a *Rickettsia*-like organism (RLO), and formally designated New Zealand *Rickettsia*-like organism (NZ-RLO). Two strains (NZ-RLO1 and NZ-RLO2) were found to co-occur in the Marlborough Sounds.
- Wider testing of (non-NZKS) salmon farms detected the presence of an additional NZ-RLO strain (NZ-RLO3) and *T. maritimum* in Canterbury (Akaroa Harbour), and *T. maritimum* at Stewart Island (Big Glory Bay).
- Geographic distribution and pathogenicity vary among the NZ-RLO strains and *T. maritimum*:
 - NZ-RLO1 and NZ-RLO2 occur in the Marlborough Sounds, and are associated with clinical disease in Chinook salmon.
 - NZ-RLO3 occurs in Akaroa Harbour and was detected in Chinook salmon that were not clinically diseased. This strain has not demonstrated pathogenic behaviour.
 - *Tenacibaculum maritimum* has a widespread New Zealand distribution, and was isolated not only from Chinook salmon with signs of clinical disease (i.e., skin ulcers), but also from apparently healthy salmon.
- Based on the 2015 mortality event and ensuing detection of a *Rickettsia*-like organism, the Ministry for Primary Industries (MPI) initiated a biosecurity response. Controls were imposed 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”).
- The Controlled Area Notice (CAN) has been in place since 2016. The intent of the CAN is to limit the spread of New Zealand Rickettsiaceae species (NZ-RLO) as Unwanted Organisms, and protect other marine salmon farming areas from an incursion of these species.

Summary of advice

- The risk of New Zealand *Rickettsia*-like organism (NZ-RLO) and *Tenacibaculum maritimum* spread to the aquaculture industry and the environment is a function of organism biology and pathogenicity, the geographic distribution of the bacteria, the pathways of potential spread/translocation, and biosecurity mitigation practices in place.
- Over the duration of the CAN, the biosecurity measures and practices that NZKS have adopted and implemented have resulted in demonstrable benefits to farming operations. As such, NZKS does not regard biosecurity management as something specific to MPI CAN requirements, but rather considers these practices to be fundamental to their farming operations.
- A fundamental consideration around assessing the risk of NZ-RLO spread is, in the absence of a CAN, whether or not NZKS will continue with the biosecurity practices that have been integrated into their BMP to manage the risk of spreading NZ-RLO1/NZ-RLO2.
- To address the possibility of a relaxation in NZKS biosecurity practices in a post-CAN situation, this risk assessment considers two scenarios:

Scenario 1: Continuation of the current NZKS biosecurity management practices used to comply with the CAN; and

Scenario 2: Cessation of key biosecurity management practices for NZ-RLO1/NZ-RLO2 containment, with:

- (a) limited connectivity between NZKS operations and other salmon farming areas in New Zealand; or
 - (b) increased connectivity between NZKS operations and other salmon farming areas in New Zealand.
- In Scenario 1, the risk of NZ-RLO spread to other salmon farming areas and the environment (including wild salmonid populations) is assessed as *very low* (environmental) / *low* (economic):

Likelihood of NZ-RLO establishment and spread	Consequence	
	Environmental	Economic
<i>Very low</i> (1 event in 20 to 100 years)	<i>Low</i> Establishment of NZ-RLO would have low biological consequences and would be amenable to control or eradication.	<i>Moderate</i> Establishment of disease may not be amenable to control or eradication; could harm economic performance at a regional level on an ongoing basis, which may or may not be irreversible.

- The available evidence suggests that the Chinook salmon summer mortality events in the Marlborough Sounds are driven by a geographically localised suite of biotic and abiotic factors (i.e., pathogens—including NZ-RLO1 & NZ-RLO2—and environmental conditions).
- The NZKS biosecurity management practices in place appear to effectively mitigate against the translocation of NZ-RLO1 and NZ-RLO2.

- In Scenario 2a and 2b, the risk of NZ-RLO spread to other salmon farming areas and the environment (including wild salmonid populations) is assessed as *low* (environmental) / *moderate* (economic):

Likelihood of NZ-RLO establishment and spread	Consequence	
	Environmental	Economic
<p><u>Scenario 2a</u> <i>Moderate</i> (1 to 2 events in 3 years)</p> <p><u>Scenario 2b</u> <i>High</i> (more than 2 events in 3 years)</p>	<p><i>Low</i></p> <p>Establishment of NZ-RLO would have low biological consequences and would be amenable to control or eradication.</p>	<p><i>Moderate</i></p> <p>Establishment of disease may not be amenable to control or eradication; could harm economic performance at a regional level on an ongoing basis, which may or may not be irreversible.</p>

- Scenario 2a: In the absence of fundamental biosecurity management practices (e.g., equipment decontamination, disease testing), the likelihood of NZ-RLO establishment and spread is moderate if NZKS maintains its limited operational connectivity with other salmon farming areas in New Zealand.
 - Scenario 2b: Increasing water temperatures and marine heatwaves may necessitate increased operational connectivity (e.g., disposal of mortalities, stock relocations) with sites beyond the original Controlled Area. In this context, and in the absence of key biosecurity management practices, the likelihood of NZ-RLO establishment and spread is high.
- The risk of *T. maritimum* spread outside of the Controlled Area is assessed as negligible, as this is a cosmopolitan species that is likely widespread in New Zealand. *Tenacibaculum maritimum* is an opportunistic pathogen, and as with NZ-RLO, tenacibaculosis outbreaks are driven by biotic and abiotic factors (i.e., pathogens—including NZ-RLO1 & NZ-RLO2—and environmental conditions).

Supporting information

The risk of New Zealand *Rickettsia*-like organism (NZ-RLO) and *Tenacibaculum maritimum* spread to the aquaculture industry and the environment is a function of organism biology and pathogenicity, the geographic distribution of the bacteria, the pathways of potential spread/translocation, and biosecurity mitigation practices in place:

1. New Zealand *Rickettsia*-like organism (NZ-RLO)

1.1. Organism background

1.1.1. *Rickettsia*-like organisms

“*Rickettsia*-like organism” (RLO) is an inclusive term for various bacteria that share some general characteristics: growth and reproduction within host cells (i.e., intracellular), 0.4–2.0 µm in size, aggregation within host cells (i.e., intracytoplasmic inclusions), and stain Gram-negative (Cano et al. 2018; Cruz-Flores & Caceres-Martinez 2020; Howells et al. 2021). RLOs are typically difficult to

isolate (usually requiring cell culture to grow) (Metselaar et al. 2010), and tools for species-specific identification are lacking (Howells et al. 2019).

The increasing availability and use of DNA sequencing tools/techniques has revealed that RLOs comprise a range of bacteria from different orders within the classes Alphaproteobacteria and Gammaproteobacteria (Cano et al. 2018; Cruz-Flores and Caceres-Martinez 2020). *Piscirickettsia salmonis*, the first RLO to be identified as the cause of a major fish disease, was originally considered to belong to class Alphaproteobacteria, order Rickettsiales, family Rickettsiaceae (Cvitanich et al. 1991). Further molecular characterisation showed that *P. salmonis* is not closely related to true *Rickettsia* species (Fryer et al. 1992), but rather is a member of class Gammaproteobacteria (Fryer & Hedrick 2003).

1.1.2. *Organism identity, biology and pathogenicity*

New Zealand *Rickettsia*-like organism (NZ-RLO) is a Gram-negative, facultative intracellular bacterium belonging to class Gammaproteobacteria (Brosnahan 2020). DNA sequencing of two genes (Internal Transcribed Spacer (ITS) rRNA region; 16s rRNA) identified three strains of NZ-RLO in Chinook salmon (*Oncorhynchus tshawytscha*): NZ-RLO1 and NZ-RLO2, which are associated with clinical disease, and NZ-RLO3, which occurs in apparently healthy salmon and does not demonstrate pathogenicity (Gias et al. 2018; Brosnahan et al. 2019a).

Comparisons of New Zealand strains to other published RLO sequences show that NZ-RLO1 has a high similarity to Tasmanian-RLO (a pathogen of farmed Atlantic salmon (*Salmo salar*) in Tasmania, Australia; Morrison et al. 2016), and NZ-RLO2 and NZ-RLO3 are highly similar to *P. salmonis* strains from Ireland (IRE-99C) and Chile (AL10015), respectively (Brosnahan et al. 2019a). Conclusive determination of NZ-RLO species-level identity will require whole-genome sequencing of each strain, and comparisons against whole-genome sequences of *P. salmonis* and Tasmanian-RLO (Brosnahan et al. 2017, 2019a).

The biology of NZ-RLO has not been characterised. Given the close relationship of NZ-RLO2 and NZ-RLO3 to *P. salmonis*, it is assumed that *P. salmonis* biology generally represents that of NZ-RLO (e.g., mechanism of infection, host range):

1.1.2.1. *Piscirickettsia salmonis*

The *Rickettsia*-like organism *Piscirickettsia salmonis* is the causal agent of salmonid rickettsial septicaemia (SRS), also known as piscirickettsiosis. *Piscirickettsia salmonis* was first isolated from diseased Coho salmon (*Oncorhynchus kisutch*) during a septicaemia outbreak among marine salmon farms in Chile in 1989 (Cvitanich et al. 1991; Almendras & Fuentealba 1997). This pathogen is a major infectious disease agent of sea-farmed salmon in Chile, North America and Europe (Brosnahan et al. 2019a; Xue et al. 2021).

Salmonid fishes appear to be particularly susceptible to *P. salmonis* (Department of Agriculture, Fisheries and Forestry (DAFF) 2013), including Atlantic salmon (*Salmo salar*), Chinook salmon (*O. tshawytscha*) and rainbow trout (*O. mykiss*) (McCarthy et al. 2005; Marcos-Lopez et al. 2017; Jones 2019). *Piscirickettsia*-like organisms have been reported from some non-salmonid fish such as tilapia (e.g., *Oreochromis niloticus*, *Sarotherodon melanotheron*) and blackspot grouper (*Epinephelus melanostigma*), however these reports lack definitive pathogen identification (Marcos-Lopez et al. 2017). *Piscirickettsia salmonis* has been confirmed infecting European sea bass (*Dicentrarchus labrax*) (McCarthy et al. 2005; Zrnčić et al. 2021) and white seabass (*Atractoscion nobilis*) (Arkush et

al. 2005), and *P. salmonis* genetic material has been detected in several wild marine fish species in Chile (Contreras-Lynch et al. 2015).

Piscirickettsia salmonis can survive outside of the host in sea water for up to 21 days, with the duration of extracellular survival decreasing as the temperature increases (Lannan & Fryer 1994). The bacterium cannot replicate outside of a host, but appears able to persist in the marine environment via an as-yet-unknown reservoir (or reservoirs) (e.g., as biofilms on biotic and/or abiotic surfaces) (Larenas et al. 2019; Levipan et al. 2020, 2022)

Details of the *P. salmonis* life cycle remain largely unknown (Rozas & Enriquez 2014). Transmission is horizontal through the water column, and infection may occur through ingestion, penetration of the gill epithelium, or directly through the skin surface (facilitated by scale damage) (Lannan & Fryer 1994; Smith et al. 1999; Rozas & Enriquez 2014; Bravo et al. 2020).

External clinical signs of piscirickettsiosis include darkening of body colouration, anorexia, lethargy, and skin lesions (Johnston 2008; Blackwell 2019 and references therein). Internally, clinically diseased fish have inflamed and necrotic lesions in the liver, kidney, spleen and intestine (Jones 2019).

The severity of piscirickettsiosis varies among *P. salmonis* strains, host species, and environmental conditions (House et al. 1999). Husbandry practices (e.g., increased stocking densities) and high water temperatures (> 15 °C) are key stressors associated with piscirickettsia outbreaks and associated mortality among farmed salmon (Carter 2005; Akbarzadeh et al. 2018; Jones 2019; Zillig et al. 2021; Valenzuela-Aviles et al. 2022). Some Chilean strains of *P. salmonis* are particularly virulent (e.g., EM-90-like), capable of causing > 90% mortality (Rozas & Enriquez 2014; Rozas-Serri et al. 2023).

Piscirickettsia salmonis can spread via hydrodynamic dispersal through the water column (to a distance of ~ 10 km; Rees et al. 2014), translocations of infected stock (including post-harvest carcasses and fresh or frozen fish product), and movements of aquaculture vessels and equipment (DAFF 2013; Blackwell 2019).

Based on these aspects of *P. salmonis* biology, it is assumed that NZ-RLO has similar characteristics:

- horizontal transmission through the water column;
- persistence in the environment via an as-yet-unknown reservoir;
- pathogenicity exacerbated by environmental stressors on salmon (e.g., increased stocking densities, elevated water temperatures); and
- spread occurs via local hydrodynamics, translocation of infected stock, and movements of aquaculture vessels and equipment.

1.1.2.2. NZ-RLO pathogenicity

Cumulative yearly mortality levels for New Zealand King Salmon (NZKS) operations are typically ~ 25% across all farms (Fischer & Appleby 2017). Since the beginning of the summer mortality events (2012), cumulative yearly mortality has risen to 30%, with a single site experiencing > 70% mortality during the summers of 2014-15 and 2021-2022 (Fischer & Appleby 2017; NZKS pers. comm.).

Within the Marlborough Sounds, the prevalence of NZ-RLO1 and NZ-RLO2 is strongly associated with elevated summer water temperatures (> 17 °C), which in turn are associated with salmon mortality events (Brosnahan et al. 2019a). NZ-RLO1 and NZ-RLO2 grow best at temperatures of 15–22 °C and 15–18 °C, respectively (Gias et al. 2018; Brosnahan 2020), and laboratory challenges demonstrate

that both strains can cause clinical disease in Chinook salmon (Brosnahan 2020). However, the role these strains play in summer mortality events remains unclear:

- Testing of Chinook salmon mortalities in early 2015 demonstrated a low prevalence of NZ-RLO, and there's insufficient evidence for NZ-RLO as a primary pathogen in summer mortality events (Brosnahan et al. 2017; Brosnahan 2020).
- Fish inoculated with NZ-RLO1 or NZ-RLO2 developed skin ulcers (21% and 25%, respectively; Brosnahan 2020)—this suggests that some skin ulcers of summer mortalities are likely caused by NZ-RLO, however another possibility is that a different disease agent causes the ulcers, which then increase salmon susceptibility to NZ-RLO1 and NZ-RLO2 (Brosnahan et al. 2019a). Alternatively, NZ-RLO may alter the skin defences and predispose fish to infection by other agents that cause skin ulcers (Brosnahan et al. 2019b).
- Challenge studies are required to understand the relationship among NZ-RLO1/NZ-RLO2, clinical signs of disease and mortalities—e.g., do higher seawater temperatures increase susceptibility to infection with NZ-RLO or have an adverse effect on fish infected with NZ-RLO (Brosnahan et al. 2019a; Brosnahan 2020).
- NZ-RLO1/NZ-RLO2 infections are more likely a multifactorial event, potentially including other infectious agents and environmental conditions (e.g., elevated water temperatures) that result in high summer mortalities (Brosnahan 2020).

Detections of NZ-RLO3 in Chinook salmon were associated with no signs of clinical disease (Brosnahan et al. 2019a). At the site where NZ-RLO3 is present (see section 1.3 below), water temperatures remain below 15 °C, which may be suboptimal for growth of this strain; alternatively, the cooler temperature may limit stress on fish immune system function, preventing NZ-RLO3 from entering, replicating or causing disease in salmon (Brosnahan 2020). The absence of disease in the presence of NZ-RLO3 may also indicate differences in pathogenicity of NZ-RLO strains (Brosnahan 2020), i.e., NZ-RLO3 may be non-pathogenic.

1.1.3. *Geographic distribution*

Between 2015 and 2017, targeted surveillance was done for NZ-RLO in New Zealand's three key salmon producing areas: Marlborough Sounds, Akaroa Harbour and Stewart Island. Detections of NZ-RLO were limited to the Marlborough Sounds (NZ-RLO1 & NZ-RLO2) and Akaroa Harbour (NZ-RLO3) (Brosnahan et al. 2019a). NZ-RLO was not detected in surveillance of wild Chinook salmon in New Zealand (Marlborough and Canterbury regions of South Island, April 2019–February 2020), nor was it detected in surveillance of NZKS's Takaka (February 2019) and Tentburn (January 2020) freshwater hatcheries (broodstock and smolt, respectively) (Tennakoon & Long 2021). In 2022, followup surveillance was done at salmon farms in Akaroa Harbour and Stewart Island, and all samples were negative for NZ-RLO1 and NZ-RLO2 (N. Long pers. comm.).

This evidence suggests that if NZ-RLO1 and/or NZ-RLO2 occur in wild salmon populations in New Zealand, it is at a very low prevalence (< 2%). To date, the geographic distribution of these pathogenic strains appears to be restricted to the Marlborough Sounds.

1.1.4. *Pathways of potential spread*

The pathways of NZ-RLO spread are assumed to be the same as *P. salmonis* (section 1.2.1):

- natural (hydrodynamic) dispersal through the water column (to a distance of ~ 10 km);

- translocations of infected stock (including post-harvest carcasses, and fresh or frozen fish product); and
- movements of aquaculture vessels and equipment.

If/when disease signs arise in salmon, this occurs 2-3 months post-transfer to seawater (New Zealand King Salmon pers. comm.). Given that NZ-RLO was not detected in surveillance of NZKS freshwater hatchery stock (section 1.3), this indicates that the bacteria persist in the marine environment (Brosnahan 2020). The environmental reservoir (or reservoirs) of NZ-RLO remains unknown, but may include native fish (Quintanilla et al. 2021), biofilms within the shells of bivalves (Larenas et al. 2019), and biofilms on aquaculture gear and equipment (Levipan et al. 2020).

1.2. Biosecurity mitigation practices in place

In April 2016, MPI used a *Declaration of Controlled Area and Notice of Movement Controls* (“Controlled Area Notice”; section 131 of the Biosecurity Act) to put legal controls on the movement of salmon, salmon products, and salmon farming-related equipment and materials from NZ-RLO affected areas within the Marlborough Sounds. The Controlled Area Notice (CAN) sets out two ‘Contained Zones’ (Outer Pelorus Sound and Queen Charlotte Sound; Figure 1a) and places restrictions on the movement of risk items out of the zones:

- All live or dead farmed Chinook salmon, including all developmental stages, and all products and by-products derived from live or dead farmed Chinook salmon.
- All equipment and materials used in the propagation and rearing of Chinook salmon for sale, including but not limited to: sea-cages, pontoons, nets, vessels, tools, cleaning equipment and fish food.
- Personal equipment used by persons engaged in the propagation and rearing of Chinook salmon for sale: wetsuits, other dive equipment, and footwear.

In 2019, the CAN was revised to include composting as a disposal option for dead farmed Chinook salmon.

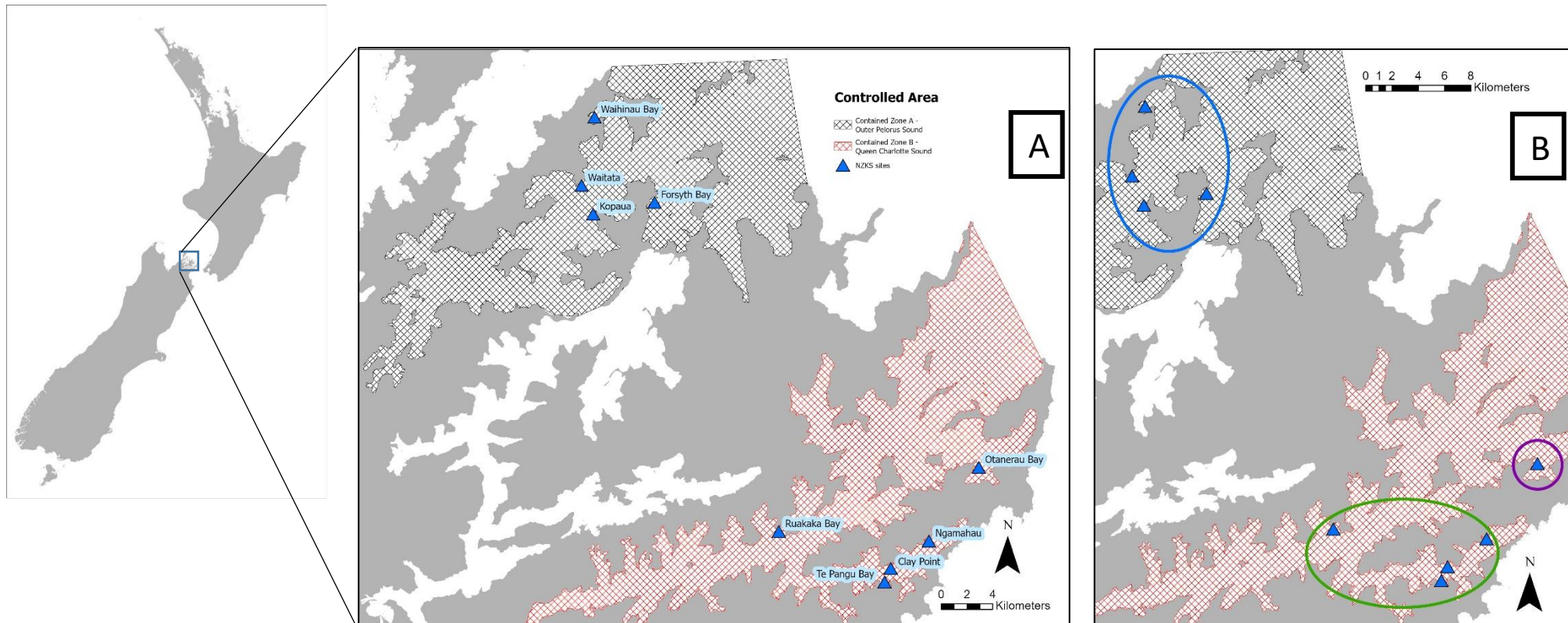


Figure 1. (A) Map of Marlborough Sounds Controlled Area for managing the spread of *Rickettsia*-like organism in farmed salmon; Contained Zones are Outer Pelorus Sound (Zone A, black crosshatch) and Queen Charlotte Sound (Zone B, red crosshatch), blue triangles denote New Zealand King Salmon (NZKS) farm locations. (B) NZKS Biosecurity Control Zones, designated by coloured circles: Pelorus Sound (blue), Tory Channel / Queen Charlotte Sound (green), Otanerau Bay (purple).

These risk items can be moved out of a contained zone provided that an MPI permit is obtained and that the permit conditions are fulfilled.

The intent of the CAN is to limit the spread of NZ-RLO1 and NZ-RLO2, and to protect other marine salmon farming areas from an incursion of these strains. The MPI permitting process is in place to ensure that any movement of risk items out of the CAN is done in a biosecure manner. Movement permits ostensibly apply to NZKS operations, and the fundamental permit conditions are that cleaning and decontamination (and/or containment) of risk items is done in accordance with the NZKS Biosecurity Management Plan (BMP).

The NZKS BMP includes specific biosecurity measures to minimise the likelihood of transferring aquatic pathogens or pests between CAN zones (Figure 1a) as well as between NZKS biosecurity control zones (Figure 1b; Waddington 2023a). Key pathways for which biosecurity measures are in place include:

- personal protective equipment (PPE) and personnel;
- equipment, vessels and transport vehicles;
- eggs and smolt transfer;
- marine-to-marine fish transfers across zones; and
- divers and diving equipment.

The NZKS BMP also details animal health and welfare procedures and operational practices that are aimed at general disease prevention, increasing fish resilience to disease, and avoiding adverse health outcomes. These include:

- daily health and welfare assessments (e.g., of fish behaviour, feed rate and response, mortality, clinical appearance, environmental conditions, and water quality);
- vaccination and health treatments;
- pathogen and disease awareness;
- mortality retrieval, classification and disposal;
- biosecurity audit;
- feed management;
- harvest management; and
- decontamination protocols for biosecurity risk items (e.g., vessels and aquaculture equipment).

The NZKS BMP also includes a veterinary health plan, which incorporates Standard Operating Procedures (SOPs) for disease management (e.g., reducing stock densities) and fish health and biosecurity checks. The health and biosecurity checks entail routine diagnostic testing of moribund fish, and pre-transfer testing before moving stock to a new location (freshwater-to-marine and marine-to-marine transfers). Pre-transfer sampling includes moribund fish plus a minimum of 30 randomly selected moribund, fresh dead, culled or random live fish (in that order of preference) for gross pathological examination, and any notable gross pathology prompts additional diagnostic sampling and testing (Waddington 2023b). Pre-transfer testing includes PCR screening for NZ-RLO1 and NZ-RLO2 (Waddington 2023b).

Specific practices that NZKS have put in place to improve fish welfare and reduce summer mortality include: shifting operations from Pelorus Sound to cooler water sites in the Tory Channel and Queen Charlotte Sound; reduced stocking densities; reducing unnecessary handling stress; and fallowing sites for a minimum of 30 days (N. Long pers. comm.).

In late 2022, NZKS began a stock vaccination programme using a bivalent autogenous vaccine (NZ-RLO1 and *Tenacibaculum maritimum*). All fish going out to sea are vaccinated, and remain in the smolt hatchery for 500 degree days post-vaccine for onset of immunity (Z. Waddington pers. comm.). Clinical trials of the vaccine showed 50–60% survival benefit against NZ-RLO1, and 6-month protective effect (Z. Waddington pers. comm.). NZKS has commissioned the development of a new bivalent autogenous vaccine (NZ-RLO1 and NZ-RLO2), which is expected to be available for use in late 2023 (Z. Waddington pers. comm.).

NZKS regards their BMP as a “living document” that undergoes repeated reviews and updates to ensure best biosecurity and health practices are maintained (Waddington 2023a; Z. Waddington pers. comm.). Over the duration of the CAN, the measures and practices that NZKS have adopted and implemented to improve biosecurity management have resulted in demonstrable benefits to farming operations (e.g., research on net cleaning regimes to identify key fish stressors, then taking steps to reduce their frequency and magnitude; Z. Waddington pers. comm.). Given these benefits, NZKS does not consider the BMP practices as something specific to MPI CAN permitting; rather, these have become a fundamental part of NZKS operations (Z. Waddington pers. comm.).

1.3. Risk assessment methodology

The general risk assessment methodology is based on the *Biosecurity New Zealand Risk Analysis Procedures – Version 1* (Biosecurity New Zealand 2006) and Chapter 2 of the Aquatic Animal Health Code (WOAH 2022). This risk assessment consists of five steps: release assessment, exposure assessment, likelihood of establishment and spread, consequence assessment and risk estimation. In each of the first two steps, a qualitative likelihood assessment (Table 1) is made based on the organism’s biology and the biosecurity mitigations in place. Likelihood of establishment and spread is based on the combined release/exposure likelihood (Table 2), and the corresponding consequences (Table 3) are then compared to determine the risk estimation (Table 4)

Table 1. Risk assessment likelihood categories (MPI 2022). For release assessment, “event” means the translocation of NZ-RLO -infected or contaminated material into an uninfected area; for exposure assessment, “event” means farmed salmon and/or wild aquatic animals being exposed to a dose of NZ-RLO1/NZ-RLO2 that leads to the establishment of the pathogen in a new location.

Likelihood category	Description
High	More than 2 events in 3 years
Moderate	1 to 2 events in 3 years
Low	1 event in 3 to 20 years
Very low	1 event in 20 to 100 years
Negligible	Less than 1 event in 100 years

Table 2. Matrix of combined release and exposure likelihoods for determining likelihood of establishment and spread (MPI 2022).

Likelihood Scores		Second Likelihood Score				
		Negligible	Very Low	Low	Moderate	High
First Likelihood Score	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	Very Low	Negligible	Negligible	Very Low	Very Low	Low
	Low	Negligible	Very Low	Very Low	Low	Moderate
	Moderate	Negligible	Very Low	Low	Moderate	Moderate
	High	Negligible	Low	Moderate	Moderate	High

Table 3. Definitions of terms used to describe establishment consequences of NZ-RLO1/NZ-RLO2. Based on Diggles (2016).

Consequence	Definition
Very High	Establishment of disease would cause substantial biological and economic harm at a regional or national level, and/or cause serious and irreversible environmental harm.
High	Establishment of disease would have serious biological consequences (high mortality or morbidity) and would not be amenable to control or eradication. Such diseases would significantly harm economic performance at a regional level and/or cause serious environmental harm which is most likely irreversible.
Moderate	Establishment of disease would cause significant biological consequences (significant mortality or morbidity) and may not be amenable to control or eradication. Such diseases could harm economic performance at a regional level on an ongoing basis and/or may cause significant environmental effects, which may or may not be irreversible.
Low	Establishment of disease would have moderate biological consequences and would normally be amenable to control or eradication. Such diseases may harm economic performance at a local level for some period and/or may cause some environmental effects, which would not be serious or irreversible.



Consequence	Definition
Very Low	Establishment of disease would have low biological consequences and would be amenable to control or eradication. Such diseases may harm economic performance at a local level for a short period and/or may cause some minor environmental effects, which would not be serious or irreversible.
Negligible	Establishment of disease would have no significant biological consequences and would require no management. The disease would not affect economic performance at any level and would not cause any detectable environmental effects.

Table 4. Likelihood/consequence matrix for overall risk estimation (MPI 2022).

Overall Risk Estimation		Consequences after Entry, Exposure and Establishment					
		Negligible	Very Low	Low	Moderate	High	Very High
Likelihood of Entry, Exposure and Establishment	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	Very low	Negligible	Negligible	Very Low	Low	Low	Moderate
	Low	Negligible	Very Low	Low	Moderate	Moderate	High
	Moderate	Negligible	Very Low	Low	Moderate	High	Very High
	High	Negligible	Very Low	Low	Moderate	High	Very High

A fundamental consideration around assessing the risk of NZ-RLO spread is, in the absence of a CAN, whether or not NZKS will continue with the biosecurity practices that have been integrated into their BMP to manage the risk of spreading NZ-RLO1/NZ-RLO2. NZKS have emphasised that the biosecurity practices that have been implemented since the initiation of the CAN have been beneficial to farming operations (see Section 1.2), however, if the CAN were removed, it’s possible that NZKS may make a business decision to discontinue some of these practices. Given the possibility of a relaxation in NZKS biosecurity practices in a post-CAN situation, each step of the risk assessment process considers two scenarios:

Scenario 1 -- continuation of the current NZKS biosecurity management practices used to comply with the CAN, and

Scenario 2 -- cessation of key biosecurity management practices for NZ-RLO1/NZ-RLO2 containment (i.e., equipment and salmon from the marine environment are moved to locations beyond the Marlborough Sounds without prior decontamination and disease testing, respectively.).

Another potential influence on NZ-RLO spread is New Zealand’s warming ocean climate:

- Increased water temperature is a risk factor for salmon susceptibility to disease, as well as NZ-RLO pathogenicity (Brosnahan et al. 2019b; Brosnahan 2020; see also section 1.1.2.2).
- Marine heatwaves have played a substantial role in New Zealand’s salmon summer mortality events (Broekhuizen et al. 2021), and the frequency, duration and severity of marine heatwaves are expected to increase (de Burgh-Day et al. 2022; Stevens et al. 2022; Montie et al. 2023).



Given these linkages, the release and exposure likelihood assessments consider how climate change may influence NZ-RLO spread.

1.4. Risk assessment

1.4.1. Release assessment

Translocation of infected or contaminated material presents a direct pathway for introduction and establishment of NZ-RLO into uninfected areas. The specific NZ-RLO pathways for translocation beyond the CAN are:

- all live or dead farmed Chinook salmon, and all products and byproducts derived from live or dead farmed Chinook salmon;
- all equipment and materials used in the propagation and rearing of Chinook salmon, including but not limited to: sea-cages, pontoons, nets, vessels, tools, cleaning equipment and fish food; and
- personal equipment used by persons engaged in the propagation and rearing of Chinook salmon: wetsuits, other dive equipment, and footwear.

Biosecurity risk mitigation practices are in place, as detailed in the NZKS BMP (see section 1.2), and the MPI NZ-RLO movement permit conditions explicitly call for risk items to be cleaned and decontaminated (and/or contained) in accordance with the NZKS BMP. On this basis, the NZKS BMP practices appear to effectively mitigate against the translocation of NZ-RLO1 and NZ-RLO2—these strains have continued to remain restricted to the Marlborough Sounds and have not spread to wild salmon stocks and other salmon farming areas in New Zealand.

Release assessment -- Scenario 1

In the absence of a Controlled Area and associated controls on risk items, and continuation of the current NZKS biosecurity management practices used to comply with the CAN, the likelihood of NZ-RLO1 and/or NZ-RLO2 release to other salmon farming areas and the environment (including wild salmonid populations) is assessed as *low*:

- The NZKS biosecurity management practices in place appear to effectively mitigate against the translocation of NZ-RLO1 and NZ-RLO2, and these practices have been embedded in NZKS operations (i.e., regardless of a CAN; Z. Waddington pers. comm.).
 - Increasing water temperatures and marine heatwaves may increase salmon susceptibility to NZ-RLO1 / NZ-RLO2 and the pathogenicity of these bacteria, however the fundamental biosecurity management practices used by NZKS (decontamination, disease testing) are robust against these types of changes.
- MPI records of CAN movement control permits demonstrate minimal operational connectivity between NZKS risk items and other salmon farming areas in New Zealand (see 1.4.2 below).

Release assessment -- Scenario 2

In the absence of a Controlled Area and associated controls on risk items, and cessation of the key NZKS biosecurity management practices used to comply with the CAN (i.e., equipment decontamination, salmon disease testing), the likelihood of NZ-RLO1 and/or NZ-RLO2 release to other salmon farming areas and the environment (including wild salmonid populations) is assessed as *moderate to high*:

- If NZKS maintains its limited operational connectivity with other salmon farming areas in New Zealand (see 1.4.2 below), the likelihood of NZ-RLO1 and/or NZ-RLO2 release is

moderate. It may be uncommon for risk items to be moved to other salmon farming areas, but without key safeguards (decontamination, testing), there remains a non-negligible likelihood of NZ-RLO1 / NZ-RLO2 release.

- Increasing water temperatures and marine heatwaves may increase operational connectivity with areas in New Zealand beyond the original Controlled Area (e.g., disposal of mortalities, stock relocations). Under increased operational connectivity with other salmon farming areas in New Zealand, the likelihood of NZ-RLO1 and/or NZ-RLO2 release is *high*.

1.4.2. Exposure assessment

The exposure assessment examines the likelihood that farmed salmon and/or wild aquatic animals in an uninfected population become exposed to an infective dose of NZ-RLO1 and/or NZ-RLO2, and the likelihood of the establishment of the pathogen.

Likelihood of exposure will depend on several factors: the capacity of NZ-RLO1 and NZ-RLO2 to survive in risk items being moved out of the Controlled Area, the availability of susceptible hosts in the receiving environment, degree of exposure to a risk item, and whether sufficient quantities of viable NZ-RLO1 and/or NZ-RLO2 are present to induce an infection following contact with a susceptible host.

Of ~ 150 movement permit applications since the beginning of 2020, there has been a single instance of a risk item moving from the CAN to another salmon farming area (a one-off movement of a feed barge, done under MPI-specified cleaning and decontamination conditions) (MPI 2023).

Exposure assessment -- Scenario 1

In the absence of a Controlled Area and associated controls on risk items, and continuation of the current NZKS biosecurity management practices used to comply with the CAN, the likelihood of exposure to an infective dose of NZ-RLO1 and/or NZ-RLO2 and subsequent establishment of the pathogen is assessed as *low*:

- There is minimal operational connectivity between NZKS risk items and other salmon farming areas in New Zealand, and the NZKS biosecurity management steps in place (e.g., cleaning, decontamination) minimize the likelihood of infective exposure to NZ-RLO1 and/or NZ-RLO2 and subsequent establishment of the pathogen in an uninfected population.
- Increasing water temperatures and marine heatwaves may necessitate increased operational connectivity (e.g., disposal of mortalities, stock relocations) with areas in New Zealand beyond the original Controlled Area. In this context, the fundamental biosecurity management practices used by NZKS (decontamination, disease testing) are effective at minimizing the likelihood of infective exposure to NZ-RLO1 and/or NZ-RLO2.

Exposure assessment -- Scenario 2

In the absence of a Controlled Area and associated controls on risk items, and cessation of the key NZKS biosecurity management practices used to comply with the CAN (i.e., equipment decontamination, salmon disease testing), the likelihood of exposure to an infective dose of NZ-RLO1 and/or NZ-RLO2 and subsequent establishment of the pathogen is assessed as *moderate to high*:

- If NZKS maintains its limited operational connectivity with other salmon farming areas in New Zealand, the likelihood of infective exposure to NZ-RLO1 and/or NZ-RLO2 is *moderate*. It may be uncommon for risk items to be moved to other salmon farming areas, but without

key safeguards (decontamination, testing), there remains a non-negligible likelihood of infective exposure to NZ-RLO1 and/or NZ-RLO2 and subsequent establishment of the pathogen in an uninfected population.

- Increasing water temperatures and marine heatwaves may necessitate increased operational connectivity (e.g., disposal of mortalities, stock relocations) with areas in New Zealand beyond the original Controlled Area. In this context, and in the absence of key biosecurity management practices (e.g., decontamination, testing), the likelihood of exposure to an infective dose of NZ-RLO1 and/or NZ-RLO2 and subsequent establishment of the pathogen is assessed as *high*.

1.4.3. Likelihood of establishment and spread

Likelihood of establishment and spread is determined by combining the likelihoods for the release and exposure assessment scenarios:

Scenario 1

Continuation of the current NZKS biosecurity management practices used to comply with the CAN -- combining the likelihoods for the release (low) and exposure (low) assessments, the likelihood of establishment and spread is very low.

Scenario 2

Cessation of key biosecurity management practices for NZ-RLO1/NZ-RLO2 containment -- combining the likelihoods for the release (moderate to high) and exposure (moderate to high) assessments, the likelihood of establishment and spread is moderate to high.

1.4.4. Consequence assessment

Diggles (2016) summarised key consequences of NZ-RLO release and exposure:

- When fish are infected with *Piscirickettsia*-like bacteria, mortality is observed almost exclusively in cultured fish that are stressed by other predisposing factors.
- There are very few documented instances of mortalities occurring in wild fish, and noticeable environmental effects are unlikely to occur.
- Persistence of infective NZ-RLO in populations of cultured fish has the potential to adversely affect the productivity and profitability of the salmon aquaculture industry.

Due to the ongoing summer mortalities associated with NZ-RLO1 and NZ-RLO2, the consequences of NZ-RLO1 and/or NZ-RLO2 establishing in other salmon farming areas and the environment (including wild salmonid populations) are assessed as *low* (environmental consequences) / *moderate* (economic consequences).

1.5. Risk estimation – NZ-RLO spread

Risk estimation is based on the combined likelihood of release and exposure, and the corresponding consequences.

Scenario 1

Continuation of the current NZKS biosecurity management practices used to comply with the CAN

- The likelihood of establishment and spread is assessed as *very low*.

- The consequences of NZ-RLO1 and/or NZ-RLO2 establishment beyond the Controlled Area are assessed as *low* (environmental consequences) / *moderate* (economic consequences).
- The risk (likelihood × consequence) of NZ-RLO spread is therefore assessed as *very low* (environmental) / *low* (economic).
 - The available evidence suggests that the Chinook salmon summer mortality events in the Marlborough Sounds are driven by a geographically localised suite of biotic and abiotic factors (i.e., pathogens—including NZ-RLO1 & NZ-RLO2—and environmental conditions).
 - The NZKS biosecurity management practices in place appear to effectively mitigate against the translocation of NZ-RLO1 and NZ-RLO2, and these practices have been embedded in NZKS operations (i.e., regardless of a CAN).

Scenario 2

Cessation of key biosecurity management practices for NZ-RLO1/NZ-RLO2 containment

- The likelihood of establishment and spread is assessed as *moderate to high*.
- The consequences of NZ-RLO1 and/or NZ-RLO2 establishment beyond the Controlled Area are assessed as *low* (environmental consequences) / *moderate* (economic consequences).
- The risk (likelihood × consequence) of NZ-RLO spread is therefore assessed as *low* (environmental) / *moderate* (economic).

The potential for NZ-RLO spread to the aquaculture industry and the environment is a function of organism biology and pathogenicity, the geographic distribution of the bacteria, the pathways of potential spread/translocation, and biosecurity mitigation practices in place:

- Laboratory challenge experiments demonstrate that strains NZ-RLO1 and NZ-RLO2 can cause clinical disease in Chinook salmon; detections of NZ-RLO3 in Chinook salmon were associated with no signs of clinical disease.
- NZ-RLO1 and NZ-RLO2 are likely to be involved in the summer mortality events occurring in Chinook salmon in the Marlborough Sounds, but possibly not as a primary pathogen.
- RLO/PLO outbreaks and associated mortality among farmed salmon are strongly influenced by environmental stressors and co-infection with other pathogens (House 1999; Jones 2019; Valenzuela-Aviles et al. 2022). Temperature can have a strong influence on the growth and proliferation of some pathogens, and can also affect the immune response of fish—particularly salmon (Carter 2005; Brosnahan 2022).
- Within the Marlborough Sounds, it is likely that higher seawater temperatures are a key stressor that affects the ability of salmon to cope with other stressors, including NZ-RLO1 and NZ-RLO2 (Brosnahan et al. 2017; Brosnahan 2020).
- Rather than NZ-RLO1 and NZ-RLO2 being primary pathogens, summer mortalities are more likely a multifactorial event that potentially includes NZ-RLO infection, other pathogens, and environmental stressors/conditions (Brosnahan 2020).

2. *Tenacibaculum maritimum*

2.1. Organism background

2.1.1. Organism biology and pathogenicity

Tenacibaculum maritimum (previously known as *Flexibacter marinus*, *Flexibacter maritimus*, and *Cytophaga marina*) is an aerobic, Gram-negative, gliding, filamentous bacterium (Suzuki et al. 2001). Members of the genus *Tenacibaculum* are widespread within the marine environment, however local species distributions remain largely unknown (Nowlan et al. 2020).

Tenacibaculum maritimum is the causative agent of tenacibaculosis in a wide range of farmed marine fish (Toranzo et al. 2005; Avendaño-Herrera et al. 2006a). Based on the wide variety of clinical signs, this disease was variously described as “black patch necrosis”, “bacterial stomatitis”, “gill rot”, “saltwater columnaris disease”, “marine flexibacteriosis”, “eroded mouth syndrome” and “gliding bacterial disease of sea fish” (Toranzo et al. 2005; Mitchell & Rodger 2011)

Isolation of *T. maritimum* for diagnostic purposes is hampered by its complex nutritional requirements and slow growth, which can lead to overgrowth by other bacteria (Avendaño-Herrera et al. 2006a; Apablaza et al. 2017; Jones & Madsen 2019; Le Breton 2020). Further, the number of phylogenetically and phenotypically similar species can impede identification, hence confirmation by molecular methods is recommended (Brosnahan et al. 2019c; Wade & Weber 2020).

Tenacibaculum maritimum has a broad host range of at least 30 marine fish species and has been reported from wild, cultured, and ornamental stock (Jones & Madsen 2019; Nowlan et al. 2020; Wade & Weber 2020). The true host range, covering anadromous and marine fish, is likely to be much larger (Avendaño-Herrera et al. 2006a; Jones & Madsen 2019). Non-fish species that host *T. maritimum* include green-lipped abalone (*Haliotis laevis*, Handlinger et al. 2005), jellyfish (*Phialella quadrata*, Ferguson et al. 2010; *P. noctiluca*, Delannoy et al. 2011; *Muggiaea atlantica*, Fringueli et al. 2012) and the sea louse *Lepeophtheirus salmonis* (Barker et al. 2009).

Tenacibaculosis in salmon

All major salmon-producing areas (e.g., Australia, Canada, Chile, Ireland, Norway, Scotland, Spain, USA) have reported instances of tenacibaculosis (Table 1; Olsen et al. 2019; Wade & Weber 2020), however it is not typically a notifiable disease thus diagnosis is often based on clinical signs and traditional techniques (Avendaño-Herrera et al. 2006a; Olsen et al. 2019). Therefore uncertainty may exist regarding the causative agent. Recently, *Tenacibaculum* species have been identified as causing similar disease signs to *T. maritimum*, including *T. dicentrarchi* (Avendaño-Herrera et al. 2016) and the putatively known *T. finnmarkense* (Småge et al. 2016a). The incidence of tenacibaculosis is likely underreported (Olsen et al. 2019).

Table 1. Instances of tenacibaculosis in salmonid aquaculture.

Common name	Species	Country	Reference*
Atlantic Salmon	<i>Salmo salar</i>	Spain	Pazos et al. (1993) in Ferguson et al. (2010)
		Australia	Soltani and Burke (1994); Handlinger et al. (1997); Powell et al. (2004)
		Canada (British Columbia)	Hicks (1989); Kent (1992); Ostland et al. (1999); Frisch et al. (2017)
		Chile	Apablaza et al. (2017)

Common name	Species	Country	Reference*
		Scotland	Ferguson et al. (2010)
		USA (Washington)	Frelier et al. (1994)
		Norway	PHARMAQ-Analytiq (2017)
		Ireland	Downes et al. (2018)
Chinook salmon	<i>Oncorhynchus tshawytscha</i>	USA (California)	Chen et al. (1995)
		New Zealand	Brosnahan et al. (2018)
Coho Salmon	<i>Oncorhynchus kisutch</i>	Canada	Nekouei et al. (2019)
Rainbow Trout	<i>Oncorhynchus mykiss</i>	Australia	Soltani et al. (1996); Handler et al. (1997)
		USA (Washington)	Frelier et al. (1994)
Sockeye Salmon	<i>Oncorhynchus nerka</i>	Canada	Nekouei et al. (2018)

* For full reference list, see Nowlan et al. (2020) and Wade & Weber (2020).

Tenacibaculosis has been previously reported in Chinook salmon on the Pacific Coast of the USA (Chen et al. 1995) and Canada (Wade & Weber 2020). However, this species does not appear to be as susceptible to infection as Atlantic salmon (Wade & Weber 2020).

Wild populations

As *Tenacibaculum maritimum* has a wide host range, infection of wild fish populations are expected to occur (Nowlan et al. 2020). However, there are few documented cases of tenacibaculosis of wild species.

In British Columbia, Canada, *T. maritimum* DNA was detected in 0.25% ($n = 5$ of 2006) of wild Fraser River Sockeye salmon (*Oncorhynchus nerka*) around or downstream of salmon farming regions. Transmission from farm activities was speculated as an influence (Nekouei et al. 2018).

Clinical signs

The clinical presentation of tenacibaculosis caused by *T. maritimum* may include atypical behaviour (e.g., lethargy, abnormal swimming, increased respiratory rate), haemorrhagic and necrotic lesions on the skin, frayed fins and tail rot, and necrosis of gills and eyes (Toranzo et al. 2005; Avendaño-Herrera et al. 2006a; Mitchell & Rodger 2011; Nowland et al. 2020).

While increased prevalence and severity of the disease has been reported at temperatures above 15 °C (Toranzo et al. 2005), infections have been known to occur under cooler conditions, which may reflect host and strain related differences (Boerlage et al. 2020). The majority of research conducted on *T. maritimum* has focused on warmer water strains (Frisch et al. 2018a).

Mode of transmission

Despite the significance of *T. maritimum* to global aquaculture, the mode of transmission and the route of infection represent major knowledge gaps (Delannoy et al. 2011; Mitchell & Rodger 2011; Levipan et al. 2019; Olsen et al. 2019; Wade & Weber 2020). Environmental reservoirs and natural vectors of *T. maritimum* infection also remain unknown (Olsen et al. 2019; Wade & Weber 2020). Observations of infection can vary depending on host, strain type, environmental conditions, and geographical location (Nowlan et al. 2020).



Transmission through sea water and direct transmission from host to host have been proposed (Mitchell & Rodger 2011), with mucosal surfaces of skin, gastrointestinal tract, and gills are generally regarded as the major routes of entry (Faílde et al. 2013; Mabrok et al. 2016).

Damage to the skin is a common precursor for tenacibaculosis (Frisch et al. 2018b; Olsen et al. 2019). In Ireland, stock impairment caused by jellyfish nematocysts is a common precursor to tenacibaculosis (Marcos-López et al. 2016; Olsen et al. 2019). *Tenacibaculum maritimum* has been detected in several jellyfish species (*Phialella quadrata*, Ferguson et al. 2010; *P. noctiluca*, Delannoy et al. 2011; *Muggiaea atlantica*, Fringuelli et al. 2012), which are suggested as a possible environmental reservoirs or transmission vectors to cultured stock, however this hypothesis remains to be tested (Wade & Weber 2020).

Environmental tolerance/survival

Knowledge gaps exist regarding the survival strategy of *T. maritimum* (Mitchell & Rodger 2011; Levipan et al. 2019). The source of tenacibaculosis in cultured fish is thought to be environmental, as *Tenacibaculum* spp. are ubiquitous in the marine environment (Olsen et al. 2019; Avendaño-Herrera et al. 2020). Survival of *T. maritimum* in natural (i.e., non-sterilised) seawater appears to be short (~ 5 days), though it may remain present in a viable but non-culturable state (Avendaño-Herrera et al. 2006b). The adhesive nature of the bacterium and its ability to form biofilms may play a role in both environmental persistence and pathogenicity (Magariños et al. 1995; Avendaño-Herrera et al. 2006a; van Gelderen et al. 2010; Mabrok et al. 2016; Levipan et al. 2019; Wade & Weber 2020). *Tenacibaculum maritimum* is strongly adhesive to the skin and mucus of fish, which do not contain inhibitory compounds to growth of this bacterium (Magariños et al. 1995). Levipan et al. (2019) demonstrated rapid biofilm formation on artificial structures suggesting aquaculture settings as transient reservoirs of the pathogen.

Tenacibaculum maritimum cultures can be grown at temperatures ranging from 8 °C to 35 °C, depending on the strain type and geographic location (Avendaño-Herrera et al. 2006a; Jones & Madsen 2019; Wade & Weber 2020).

2.1.2. *Geographic distribution*

Prior to the 2015 detection of *T. maritimum* during the MPI investigation of Chinook salmon summer mortalities, this pathogen had not been implicated in disease in New Zealand farmed salmon. Diggles et al. (2002) and Diggles (2019) state that *T. maritimum* is ubiquitous in New Zealand's marine environment and has been previously identified from several species including snapper (*Pagrus auratus*) and blue cod (*Parapercis colias*). *Tenacibaculum maritimum* is regarded as a cosmopolitan species (Avendaño-Herrera et al. 2006a) and is likely widespread in New Zealand (Brosnahan et al. 2019a).

2.1.3. *Pathways of potential spread*

Tenacibaculum maritimum is a widespread opportunistic marine pathogen (Avendaño-Herrera et al. 2006a). As such, the main drivers of tenacibaculosis outbreaks are environmental conditions/stressors rather than translocations via risk items (Avendaño-Herrera et al. 2006a; Mabrok et al. 2023).

2.1.4. *Biosecurity mitigation practices*

No biosecurity mitigation measures are specifically in place to manage *T. maritimum*, as the objective of the CAN is to limit the spread of *Rickettsia*-like organism. In the Marlborough Sounds

summer salmon mortalities, there is no evidence to demonstrate that *T. maritimum* is a primary pathogen (Brosnahan et al. 2019a).

2.2. Risk assessment methodology

This assessment uses the same methodology as per NZ-RLO – see section 1.3.

2.3. Risk assessment

2.3.1. Release assessment

The likelihood of *T. maritimum* release outside of the Controlled Area is assessed as negligible, as this is a cosmopolitan species that is likely widespread in New Zealand.

2.4. Risk of *Tenacibaculum maritimum* spread

The risk of *T. maritimum* spread outside of the Controlled Area is assessed as negligible. *Tenacibaculum maritimum* is a geographically widespread and opportunistic pathogen. As with NZ-RLO, tenacibaculosis outbreaks are driven by biotic and abiotic factors (i.e., pathogens—including NZ-RLO1 & NZ-RLO2—and environmental conditions).

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