UPDATED DISEASE RISK ASSESSMENT REPORT – RELOCATION OF SALMON FARMS IN MARLBOROUGH SOUNDS, NEW ZEALAND

DigsFish Services Report: DF 16-01
7 September 2016

UPDATED DISEASE RISK ASSESSMENT REPORT – RELOCATION OF SALMON FARMS IN MARLBOROUGH SOUNDS, NEW ZEALAND

Prepared by:

Ben Diggles PhD

Prepared for:

Ministry for Primary Industries
Private Bag 14, Nelson
New Zealand

DigsFish Services Pty. Ltd.
32 Bowsprit Cres, Banksia Beach
Bribie Island, QLD 4507
AUSTRALIA
Ph/fax +61 7 3408 8443
Mob 0403773592
ben@digsfish.com
www.digsfish.com

Contents

Abbreviations and Acronyms

AGD	Amoebic Gill Disease
ALOP	Appropriate level of protection
IHN	Infectious Haematopoietic Necrosis
IPN	Infectious Pancreatic Necrosis
IPNV	Infectious Pancreatic Necrosis Virus
ISA	Infectious Salmon Anaemia
ISAV	Infectious Salmon Anaemia Virus
OIE	World Organisation for Animal Health (formerly Office International des Epizooties).
PLB	*Piscirickettsia*-like bacteria
RA	Risk Analysis
SD	Sleeping Disease
SPD	Salmon Pancreas Disease
TAB	Tasmanian aquabirnavirus
$TCID_{50}$	Tissue culture infectious dose 50% endpoint
VHS	Viral Haemorrhagic Septicaemia
VHSV	Viral Haemorrhagic Septicaemia Virus

Version control

First draft:	1 June 2016
Draft comments received:	1 August 2016
Draft Final Report:	8 August 2016
Final report:	7 September 2016

Disclaimer:

DigsFish Services have taken all reasonable care and diligence to ensure the information contained in this report is accurate at the time of publication. However this report is offered as general advice only, we do not warrant the accuracy of the information contained within and accept no liability for any loss or damage that may result from reliance on this information.

Executive Summary

This report was undertaken to update a previous Environmental Assessment Report (Diggles 2011) and assess potential changes to disease risks associated with a proposal to relocate several salmon farms in the Marlborough Sounds, New Zealand to 9 new high water flow sites. A review of the disease status of chinook salmon (*Oncorhynchus tshawytscha*) in New Zealand since 2011 revealed few changes to the hazards identified previously in Diggles (2011), identifying 21 infectious agents and 13 non-infectious diseases of cultured salmon in New Zealand. An outbreak of disease in salmon cultured at Waihinau Bay in early 2012 was originally thought to be solely related to suboptimal environmental conditions at that site (MPI 2013). However, subsequent testing has shown diseased fish at that location were also infected with an emerging rickettsia-like agent (NZ-RLO) and the endemic opportunist bacterium *Tenacibaculum maritimum*. These bacterial disease outbreaks at the low flow site in Waihinau Bay provide examples of the increased risk of disease emergence in fish cultured at suboptimal sites.

The current risk assessment found that clinical infection with *Piscirickettsia*-like bacteria in seacaged chinook salmon was likely to pose an increased risk of disease transfer to wild fishes, unless additional risk mitigation measures were implemented. However, it also remains recognised that an unquantifiable risk remained that biosecurity leaks could allow exotic diseases to be introduced, and/or new endemic diseases could emerge in salmon aquaculture in New Zealand at some time in the future. Because of this, it was important that biosecurity risks were managed using worlds best practice, notably including establishment of independent farm management areas separated by ideal buffer zones (Diggles 2011).

The proposal to move several salmon farms from low flow sites to more suitable sites with higher water flow would improve the environmental conditions to which cultured salmon are exposed. This would reduce both the risk of outbreaks of non-infectious diseases, and mitigate significant risk factors for emergence of infectious diseases like the NZ-RLO at suboptimal sites. The current proposal would therefore allow the salmon farming industry in the Marlborough Sounds to improve its existing biosecurity practices and move incrementally towards establishing worlds best practice biosecurity management arrangements. This is because the proposal would allow establishment of two independent farm management areas separated by a significant buffer zone.

The farms in the Tory Channel (Clay Point, Te Pangu, and Ngamahau and potential sites 42, 47, 82 and 156 at Tio Point) could be managed as one farm management area (Tory Channel Management Area). The farms proposed for Pelorus Sound (Waitata, Richmond and potential sites 34, 106, 122, 124 and 125) could be managed as a second farm management area (Outer Pelorus Sound Management Area). As the density of individual farms in each management area remains relatively low by world standards, the number of farms within each management area is not a major concern (especially if any additional sites are used to initiate regular farm fallowing), provided water quality remains optimal, on-farm stocking densities remain optimal, and biosecurity practices are maintained.

Based on conclusions from this risk analysis, I encourage the deletion of 6 sub optimal sites and relocation of salmon farms to the proposed high flow locations, to reduce risks of outbreaks of NZ-RLO and other infectious and non-infectious diseases, and to allow establishment of large on-water buffer zones that will allow independent management of the two farm areas.

Objectives of this document

The Ministry for Primary Industries has been working with the Marlborough District Council and New Zealand King Salmon (NZKS) to implement the Best Management Practice Guidelines for Salmon Farms in the Marlborough Sounds (Benthic Standards Working Group 2014). This includes the potential relocation of some existing farms to more suitable higher water flow locations to ensure the guidelines can be met.

Six existing salmon farm sites are proposed for relocation with the aim of meeting the recently developed best management practice guidelines as well as achieving improved environmental, social and economic outcomes for the Marlborough Sounds. A total of 9 potential relocation sites have been identified for further Assessment of Environmental Affects (AEE) (Figure 1). One component of the AEE process is to provide a disease risk assessment report on relocating the existing farms to the 9 proposed farm relocation sites.

The objective of this document was therefore to review and update a previous disease risk assessment (Diggles 2011) developed as an Environmental Assessment Report for the NZKS EPA Board of Enquiry in 2011 to include:

- Disease risk information for the 9 potential relocation sites
- New industry biosecurity protocols
- Consultation with relevant New Zealand fish disease experts.

This report presents the outcomes arising from this process.

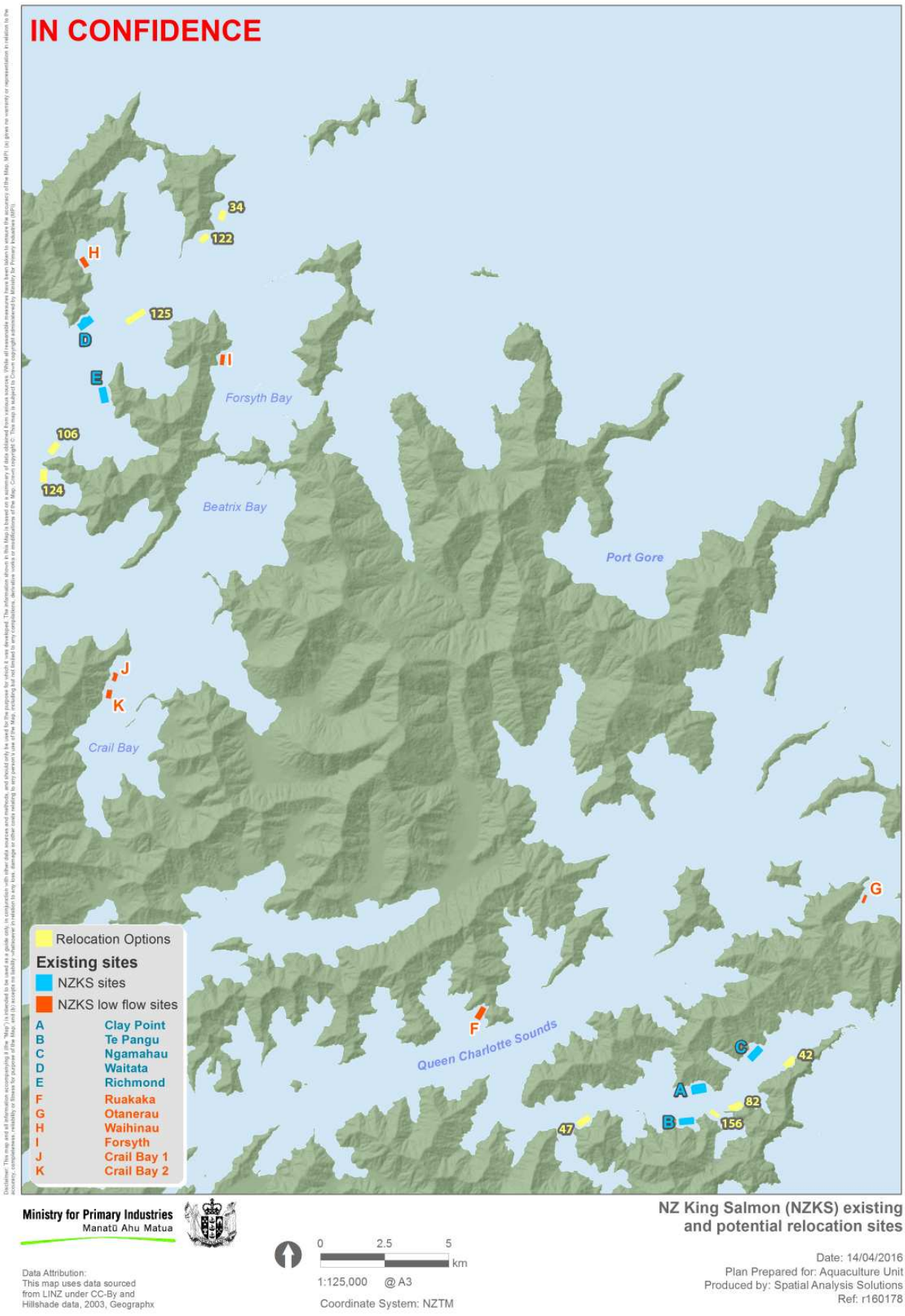

Figure 1. Map showing location of existing NZKS sites and the 9 potential relocation sites (yellow).

1.0 Introduction

The aquaculture industry is of national significance to New Zealand, providing jobs, wealth through export income and food security for New Zealanders. Fortunately, by virtue of its geographic isolation, New Zealand is in a unique position to further develop a sustainable salmon farming industry that is free from many of the problems that have emerged in salmon farming elsewhere. The geographic isolation of the country, world leading biosecurity arrangements and the absence of native salmonids means that New Zealand is free from many important diseases of salmonids (and other aquatic animals) at this time (Boustead 1989, Diggles et al. 2002). Furthermore, chinook salmon *Oncorhynchus tshawytscha* (also known as quinnat or king salmon) were introduced into New Zealand by acclimiatization societies as ova only between 1875 and 1907 (McDowall 1978, 1994), virtually eliminating the risk of introduction of many diseases that have emerged in northern hemisphere salmon in recent years and spread with salmonid farming (Costello 2006, Kibenge et al. 2009, Snow 2011). The exclusive use of *O. tshawytscha* by NZ King Salmon appears a wise choice as this species appears innately resistant to many of the disease agents that have been problematic in salmon culture overseas (Boustead 1989, Johnson and Albright 1992a, Rolland and Winton 2003, Gottesfeld et al. 2009). Because of these and other reasons, NZ King Salmon has become a successful company that in 2011 produced over 7500 tonnes of salmon per year (around 50% of the world production for this species), from a seacage surface area of around 5 hectares within the Marlborough Sounds.

However, there is no room for complacency when implementing a successful salmonid aquaculture industry (Wilson et al. 2009). New diseases continue to emerge in aquaculture (Murray and Peeler 2005, Asche et al. 2010, Thrush et al. 2011, Snow 2011) and the dynamics of infectious diseases are often related to the density of host populations (Grenfell and Dobson 1995, Krkosek 2010). Furthermore, even world leading biosecurity policy arrangements are not perfect, as demonstrated by biosecurity leaks that have resulted in the introduction and establishment of several aquatic pests and diseases in New Zealand waters such as the seaweed *Undaria pinnatifida* in 1987 (see Stuart 2004), swimming crab *Charybdis japonica* in 2000 (see Smith et al. 2003), the diatom *Didymosphenia geminate* in 2004 (see Kilroy et al. 2009) and *Bonamia ostreae* (see Lane et al. 2016). Because of this, it is important to ensure that the salmon industry in New Zealand is well managed in order to firstly avoid disease problems, and in a worse case scenario, to be able to effectively manage any new problems that may emerge (Munro et al. 2003, Murray and Peeler 2005, Gustafson et al. 2005, 2007, Marine Harvest 2008, Kibenge et al. 2009, Mardones et al. 2009).

This environmental assessment report has been undertaken to assess the disease risks associated with a proposal to relocate several salmon farm sites in the Marlborough Sounds. This report will update a previous report (Diggles 2011) and will start by briefly reviewing the diseases in seacage farming of salmon in an international context, to identify the various types of significant diseases that have occurred overseas, summarise their environmental impacts (if any), and identify the best practice management measures currently used for their avoidance and control. To describe the existing environment, the known diseases of salmon in New Zealand will be reviewed and their current environmental impacts (if any) will be assessed. An assessment of environmental effects that may result from the proposed movement of the salmon farm sites will then be undertaken. This assessment will

include a qualitative risk analysis of the likelihood of any changes to the existing disease status of chinook salmon, native fishes and other aquatic animals within the Marlborough Sounds and assess the consequences of disease spread (should it occur). Recommendations will then be made to help ensure that the outcomes from the proposed relocations, if they go ahead, will be consistent with moving towards current worlds best practice for seacage aquaculture farm management, in order to minimise risks to the environment and industry development that may be presented by disease agents of salmon.

2.0 Review of Disease Agents in Global Salmon Seacage Culture

Seacage culture of salmon can be associated with a range of infectious disease agents, including microparasites such as viruses, bacteria and protozoa, metazoan macroparasites such as monogeneans and crustaceans, as well as several non-infectious diseases (Kent and Poppe 1998, Kent 2000). The potential for diseases of seacage aquacultured salmon to impact the marine environment has attracted much scientific study. It is also a controversial issue in some parts of the world, hence it is important that decision making in this area is based on the best available scientific data. In this section the major types of diseases in seacage farming of salmon in the international context are briefly reviewed. This has been done firstly to identify the significant diseases that have occurred overseas, to summarise their environmental impacts (if any), and identify the best practice management measures currently used for their avoidance and control.

2.1 Viral diseases

Overview

Several viral diseases have caused problems in seacage aquaculture of salmonids in the northern hemisphere, including Infectious Haematopoietic Necrosis (IHN), birnaviruses including Infectious Pancreatic Necrosis (IPN), Infectious Salmon Anaemia (ISA), Viral Haemorrhagic Septicaemia (VHS), Salmon Pancreas Disease (SPD) and Sleeping Disease (SD), Viral Erythrocytic Necrosis, Erythrocytic Inclusion Body Syndrome, and Salmonid Herpes Virus 2 Infections (including *Oncorhynchus masou* virus) (see Kent and Poppe 1998, Dale et al. 2009). In the southern hemisphere, viral diseases in cultured salmon have been caused by ISA (in Chile), other orthomyxoviruses such as Pilchard orthomyxovirus (POMV) and other viruses including Tasmanian salmonid reovirus (TSRV) (Zainathan 2012, DPIPWE 2015). Of these, the viral diseases which have caused the most significant problems in seacaged salmonids are IPN and ISA (Murray et al. 2005), though SPD and SD are emerging diseases of seacage cultured Atlantic salmon and rainbow trout, respectively, due to an alphavirus (Graham et al. 2010). VHS was once known only from salmonids cultured in freshwater, but is now known to occur naturally in wild marine and freshwater non-salmonid fish and has also emerged and caused disease in seawater farmed rainbow trout in Norway (Dale et al. 2009). Various strains of birnaviruses have been described from at least 65 species of fish in 20 families (McAllister 1993), and also from bivalve molluscs and crustaceans (Reno 1999). Isolates of IPNV-like birnaviruses from returning chinook salmon in New Zealand were identified as belonging to IPNV Genogroup 5 (see Davies et al. 2010) and appear non pathogenic to salmonids (Diggles et al. 2002, McColl et al. 2010). VHSV has been recorded

from at least 82 species of marine and freshwater fish (including chinook salmon) at water temperatures of 18°C or less, but VHSV has not been recorded from the southern hemisphere at this time (Diggles and Landos 2010). ISA has been reported in Europe, Canada, USA, Faroe Islands and Chile, but not New Zealand or Australia, and it appears that chinook salmon are resistant to ISAV (Rolland and Winton 2003). New information from western Canada suggests that there may be evidence of genetic material similar to benign ISA-like variants in less than 2% (2 non negatives from 102 fish examined) of healthy chinook salmon sampled from the wild (Kibenge et al. 2016). Even so, there is debate about the meaning of these "non-negative" results as the presence of ISAV has never been confirmed in the Pacific North America region despite over 36,000 Pacific salmon (including O. *tshawytscha*) being sampled since July 2010 using cell culture methods and over 400 O. *tshawytscha* sampled using molecular tests (Amos et al. 2014), suggesting the results of Kibenge et al. (2016) may be an artefact of a decoupling of modern vs traditional diagnostic methods (Burge et al. 2016). Chinook salmon are also known to be susceptible to IHNV, and Viral Erythrocytic Necrosis (Kent and Poppe 1998), but these disease agents have not been recorded from the southern hemisphere. Of the remaining viruses infecting salmon in the southern hemisphere, the Pilchard orthomyxovirus (POMV) has caused mortalities in Atlantic salmon in Tasmania (where it is vectored by wild pilchards), while Tasmanian salmonid reovirus (TSRV) has occasionally been associated with mortalities of salmon exposed to *Piscirickettsia*-like bacteria or certain adverse environmental conditions (Zainathan 2012, DPIPWE 2015).

Environmental impacts

Viruses originating from cultured salmonids appear to have minimal impact on wild populations of finfish. Wallace et al. (2008) found a significantly higher prevalence of IPNV (0.32%) in wild marine fish caught at a distance less than 5 km from aquaculture sites, than from wild marine fish caught at a distance greater than 5 km from fish farms (0.03%). This suggests that fish farms may act as a localized source of "backspill" IPNV infection to local wild fish, rather than wild reservoirs of infection posing a high risk to farmed fish (Wallace et al. 2008). However, Wallace et al. (2008) also reported that IPNV is endemic in wild marine fish, (particularly flatfish) at low prevalences (overall prevalence in 30627 fish was 0.15%), with maximum prevalence in flatfish being 12.5% in flounder (*Platichthys flesus*), while for roundfish maximum prevalence of IPNV was 1.1% in saithe (*Pollachius virens*). Indeed, it appears that IPNV and IPNV-like aquabirnaviruses and other viruses such as VHSV are naturally widespread and persistent in the marine environment in many parts of the world (Skall et al. 2005a, 2005b, Wallace et al. 2008, Davies et al. 2010). No clinical signs of IPN disease have been observed in any of the wild fish sampled from Scotland, Australia or NZ, however outbreaks of VHS due to various different genotypes of VHSV have been recorded in wild fishes in both freshwater and marine areas of the northern hemisphere (Lumsden et al. 2007, Bain et al. 2008). These outbreaks of VHS in wild fishes have not been associated with aquaculture activities, but have been variously associated with stressors due to spawning, pollution or other environmental factors (Elston and Myers 2009), including introduction of virus into naïve populations of fish via natural or anthropogenic movements of live fishes or ballast water (Bain et al. 2010, Diggles and Landos 2010). Disease outbreaks of other viruses such as IHNV have been associated with immunosuppression of wild salmon due to reduced water quality (Clifford et al. 2005). SPD and SD have been reported from some wild species of flatfish which act as reservoirs for the disease (Snow et al. 2010, Bruno 2014), but the presence of the virus has not

been associated with disease or any detectable changes in population status of infected flatfish (Bruno et al. 2014, Jones et al. 2015), nor has the SPD alphavirus been found in wild salmonids (Snow 2011, Jones et al. 2015). An orthomyxovirus that was first reported from farmed Atlantic salmon in Tasmania in 2006 was recently confirmed to be identical to pilchard orthomyxovirus (POMV) that was originally identified from wild pilchards (*Sardinops sagax-neopilchardus*) in South Australia in 1998 (SCAHH 2015). Outbreaks of disease due to POMV in cultured salmon have occurred in Tasmania since 2012 and are associated with sub-clinically infected wild pilchards schooling around salmon sea cages (SCAHH 2015).

Given their apparent resistance to other orthomyxoviruses such as ISA, it is not known whether chinook salmon are susceptible to POMV, and at this time there is no evidence that POMV occurs in pilchards in New Zealand. In 1995, widespread mortalities of pilchards infected by the first outbreak of pilchard herpesvirus (PHV) were observed in New Zealand, after PHV disease spread throughout populations of pilchards in Australia (Whittington et al. 2008). It appears the herpesvirus was introduced into New Zealand in 1995 via infected frozen pilchards used as bait 4 weeks after a shipment of infected pilchards was received from Bremer Bay, Western Australia (Hine 1995, Fletcher et al. 1997, Crockford 2007, P.M Hine, personal communication). However in 1998-99 a second epizootic due to PHV in Australia did not reach New Zealand, probably due to immediate implementation of a temporary ban on movements of frozen pilchards from Australia to New Zealand during the entire course of the second event (P.M. Hine and B.K Diggles, personal observation). Taking these factors into consideration, it is considered unlikely that POMV infected pilchards from Australia would be able to naturally reach New Zealand and precipitate a disease outbreak in cultured salmon without human intervention.

Best practice management

Because salmon farming is done in seacages in regions where wild fishes occur, it is impossible to fully control the presence of viral disease agents in the rearing environment. There are no effective treatments for viral diseases and prevention is the key form of management. Screening of broodstock for key viruses and vaccination of seedstock can be highly effective in controlling the spread of viral diseases (Nylund et al. 2007, Munro et al. 2010). Maximising water quality and reducing or eliminating exposure to pollutants such as pesticides can also assist in maximizing immune competence of cultured fish, which can reduce the prevalence and severity of viral diseases (Clifford et al. 2005). Good husbandry that includes prevention of fish escapes, frequent removal of sick or dead fish, optimising fish nutrition, control of potential vectors, and implementation of effective on farm biosecurity controls (e.g. disinfection) at critical areas such as personnel entry points are also useful preventative measures. Biosecurity strategies that have also been used at an industry planning level to minimize the risk of outbreaks of viral diseases such as ISA have included use of independent farm management areas where production from several farming sites can be co-ordinated and synchronised using single year classes of fish, and where integrated disease management procedures that include site fallowing can be implemented if necessary (Munro et al. 2003, Chang et al. 2007, Marine Harvest 2008, Brooks 2009, Jones et al. 2015). Separation of the farm management areas by buffer zones of sufficient distance to reduce the risk of horizontal disease transmission via movements of water and wild fishes is also useful to avoid and/or control outbreaks of viral disease (Scheel et al. 2007). When best practice disease

management methods such as those mentioned above are utilized, risks of viral pathogen transmission to wild fish populations are effectively mitigated (Jones et al. 2015).

2.2 Bacterial diseases

Overview

All fish have a "normal" bacterial flora that changes seasonally (Bisset 1948) and which is moved with the fish whenever the host is translocated. There are also facultative bacterial pathogens such as those in the *Flavobacterium*/*Cytophaga*/*Tenacibaculum* group (including *Tenacibaculum maritimum*) and *Vibrio* sp. groups that are considered to be ubiquitous in aquatic environments (Austin and Austin 2007), including in New Zealand (Diggles et al. 2002), but certain strains of which can cause disease and mortalities in a wide range of aquatic animals that are stressed, injured and/or exposed to adverse environmental conditions. However there are also specific bacterial pathogens that are not considered to be ubiquitous and which are limited in their distribution (Toranzo et al. 2005). The latter include typical strains of *Aeromonas salmonicida*, which can cause Furunculosis (*A. salmonicida* subsp salmonicida), and atypical strains of *A. salmonicida* which can cause other diseases such as goldfish ulcer disease. Both typical and atypical strains of *A. salmonicida* have been translocated to new areas with movements of live, dead and frozen fish (Ostland et al. 1987, Whittington et al. 1987). Atypical strains of *A.salmonicida*, the cause of goldfish ulcer disease, occur in Australia and Atlantic salmon *(Salmo salar)* in that country were shown to be extremely vulnerable to infection (Whittington and Cullis 1988), but fortunately typical strains of *A. salmonicida* have not been recorded from New Zealand to date (Diggles et al. 2002, McIntyre et al. 2010). However, in the spring of 2011 wild lampreys (Kanakana, *Geotria australis*) were reported with haemorrhagic external lesions in several river systems in Southland. Testing by MAF Biosecurity using molecular probes confirmed that an uncharacterized, unculturable atypical strain of *A. salmonicida* was associated with the lesions but was not acting as a primary pathogen (MAF Biosecurity 2011, Brian Jones, personal communication 29 May 2016). The bacterium *Yersinia ruckeri* occurs in freshwater and is the causative agent of enteric redmouth disease (ERM), which was first described in rainbow trout from the Hagerman Valley, in Idaho, USA in the 1950s (Rucker 1966). ERM was reported for the first time in Europe in the 1980's, with the bacterium possibly being introduced through movements of live baitfish from the USA (Michel et al. 1986, Davies 1990). A different strain of *Y. ruckeri* occurs in New Zealand, where it occasionally infects juvenile chinook salmon reared in freshwater causing the milder disease yersinosis (Diggles et al. 2002). Bacterial Kidney Disease caused by *Renibacterium salmoninarum* causes chronic mortality in seacage cultured chinook and coho salmon in British Columbia (Kent and Poppe 1998). *Piscirickettsia, Francisella*, and other rickettsia-like organisms (RLOs) have been problematic in the culture of salmon in several regions of the world (Corbeil et al. 2005, Colquhoun and Duodu 2011), including chinook salmon in British Columbia (Kent and Poppe 1998), Atlantic salmon in Australia (Corbeil et al. 2005, Corbeil and Crane 2009), and most recently in chinook salmon in New Zealand (MPI 2015). The onset of piscirickettsial disease in salmonids usually occurs after transfer of fish from freshwater to seawater net pens and wild marine fish are likely candidates as reservoirs for *Piscirickettsia*-like bacteria (PLB) (Mauel and Miller 2002, Colquhoun and Duodu 2011).

Environmental impacts

Bacteria originating from cultured salmonids generally have minimal impact on wild populations of finfish or other aquatic animals. Optimisation of the rearing environment and elimination of stressors such as low oxygen or overstocking, and incorporation of routine fish health management procedures (see below) are usually sufficient to prevent most bacterial infections from reaching intensities that could promote "backspill" infection of wild fish stocks. Consequently, there is little evidence that most types of bacteria harboured by cultured fish can cause clinical disease in wild fish, however spread of furunculosis from cultured salmonids in Norway into naïve populations of wild salmonids has been observed (Johnsen and Jensen 1994), with potentially detrimental impacts on wild salmonids being noted by those authors. On the other hand, it is well established that wild fish act as reservoirs of infection of aquacultured fish with many bacterial disease agents (Kent and Poppe 1998), and the vast majority of detections of significant bacteria such as *A. salmonicida* in wild fishes are from asymptomatic carrier fish (Nomura et al. 1993).

Best practice management

Best practice management of bacterial diseases include many of the management methods used to minimize spread of viral disease agents. As bacterial disease agents are usually opportunistic pathogens, good husbandry that reduces/eliminates physical handling of fish to avoid damage to skin and fins, optimising fish nutrition, maximising water quality to maximize immune competence (including maintaining dissolved oxygen levels above 6 mg/L, reducing temperature and salinity fluctuations and avoiding temperature extremes and exposure to pollutants (Ellard 2015)), and prompt removal of moribund or dead fish can markedly reduce the prevalence and severity of bacterial diseases (Kent and Poppe 1998). Many bacteria are susceptible to antibiotic treatment which is usually administered in-feed (Kent and Poppe 1998), however resistance to antimicrobials can develop, and vaccination is being increasingly used to reduce or eliminate bacterial diseases in seacage growout of salmonids and other marine fishes (Håstein et al. 2005).

2.3 Fungal diseases

Overview

Several types of fungi are considered to be ubiquitous opportunistic saprobes which can overwhelm the innate immune system and infect aquatic animals that are injured, stressed or immunocompromised by exposure to suboptimal conditions, such as pollutants or rapid drops in water temperature (Roberts 2001). Examples include oomycete water moulds such as *Saprolegnia* which are well known opportunistic invaders of compromised salmonids or their eggs in freshwater areas (Roberts 2001), and *Exophiala* spp. which have caused disease in salmonids cultured in seacages (Kent and Poppe 1998). However there are also other fungus-like pathogens of salmonids that are not considered to be ubiquitous in their distribution, such as *Ichthyophonus hoferi* which is a fungus-like protistan that has caused disease in salmonids reared in seawater (Kent and Poppe 1998). *Icthyophonus hoferi* has low

host specificity, infecting at least 70 species of fish (Zubchenko and Karaseva 2002), including brown trout, rainbow trout and Atlantic salmon in Tasmania (Slocombe 1980, Ellard 2015). The closely related rosette agent is considered to be an obligate intracellular parasite of chinook salmon that was identified as *Spaerothecum destruens* by Arkush et al. (2003), and which has been placed with *Ichthyophonus* in the Ichthyosporea within the clade Mesomycetozoa (Sina et al. 2005, Gozlan et al. 2009). The fungus-like rosette agent has never been recorded in the southern hemisphere.

Environmental impacts

There is no evidence that fungi harboured by cultured salmonids can cause increased disease in wild fish. Optimisation of the rearing environment and reduction of stressors are usually sufficient to prevent most fungal infections from progressing. On the other hand, it is well established that wild fish can act as reservoirs of infection of aquacultured fish with many fungal disease agents (Kent and Poppe 1998), and indeed, *Ichtyophonus hoferi* is well known to naturally cause disease, morbidity and even mass mortality in wild fishes (Zubchenko and Karaseva 2002), as well as in cultured fishes fed *I. hoferi* infected wild fishes (Slocombe 1980).

Best practice management

Best practice management of fungal diseases include many of the management methods used to minimize spread of viral and bacterial disease agents. As many fungal disease agents are ubiquitous opportunistic pathogens that usually secondarily invade damaged tissues, good husbandry that reduces/eliminates physical handling of fish to avoid damage to skin and fins, optimising fish nutrition, maximising water quality, including maintaining dissolved oxygen levels above 6 mg/L, reducing temperature and salinity fluctuations and avoiding temperature extremes and exposure to pollutants to maximize immune competence can reduce the prevalence and severity of fungal diseases. Use of formulated pellet diets and avoidance of natural feeds may be useful methods of avoiding infection with *Ichthyophonus hoferi* and *Spaerothecum destruens*, which may be transmitted orally through consumption of infected fish (Slocombe 1980, Kent and Poppe 1998). Use of hydrogen peroxide or iodophores can reduce water mould infections of eggs, however treatment of growout fish with other fungicidal drugs is usually problematic due to the potential for residues that conflict with strict food safety requirements (Kent and Poppe 1998).

2.4 Protozoal diseases

Overview

A variety of diseases caused by protozoan agents have been recorded from salmonids cultured in seacages, including infections by amoebae, microsporidians, flagellates and ciliates (Kent and Poppe 1998). Cultured salmonids in many parts of the world are adversely affected by amoebic gill disease (AGD), which is caused by infection of the gills with free living amoebae, predominantly *Neoparamoeba perurans* (see Young et al. 2007, Young et al. 2008). *Neoparamoeba perurans* has been

recorded in chinook salmon in New Zealand in the absence of disease (Young et al. 2008), and chinook salmon appear relatively resistant to this disease agent (Munday et al. 2001). Microsporidians are obligate, intracellular parasites that infect arthropods, fish, and mammals (Lom and Dykova 1992). In fish, microsporidian infections can be widespread in various tissues or concentrated into cysts that are often grossly visible. The lifecycle is usually direct, but can include an intermediate host (Vossbrinck et al. 1998). The microsporidian *Loma salmonae* infects the gills and other vascularized tissues of wild and hatchery-reared salmonids in fresh water throughout the Pacific Northwest (Kent et al. 1995). Severe gill infections have been reported in cultured rainbow trout (*Oncorhynchus mykiss*) and kokanee salmon (*O. nerka*), while systemic infections by *L. salmonae* have also been reported in cultured chinook salmon. The susceptibility of various salmonid species to *Loma* was investigated by Ramsay et al. (2002), and chinook salmon was shown to be the most susceptible species. Although the gill is the primary site of infection, parasites and associated lesions can occur elsewhere, including the heart, spleen, kidney, and pseudobranch. *Loma salmonae* is considered to be a freshwater parasite, but infections can persist after fish are transferred to seawater (Kent and Poppe 1998). This parasite has not been recorded in the southern hemisphere. The flagellates *Ichthyobodo*, *Hexamita* and *Cryptobia* have caused mortality in a range of species of seacaged salmonids in the northern hemisphere (Kent and Poppe 1998). *Hexamita salmonis* is normally a parasite of the intestinal tract of salmon in freshwater, but it persisted after transfer of fish to marine sites and caused severe disease and up to 50% mortalities in seacage cultured chinook salmon in British Columbia (Kent and Poppe 1998). *Ichthyobodo necator* and *Cryptobia salmositica* are other flagellate parasites of freshwater fishes that can persist on salmonids after transfer into seawater, causing disease in chinook salmon (Kent and Poppe 1998). The ciliate *Ichthyophthirius multifiliis* is a ubiquitous parasite that is responsible for white spot disease in freshwater fish (Matthews 2005). *Ichthyophthirius multifiliis* can infect salmonids during the freshwater stages of the production cycle, but the parasite cannot complete its lifecycle in seawater. The only ciliates that have been recognised to cause disease in seacaged salmon are *Trichodina* spp., which can occur on the skin and gills (Kent and Poppe 1998).

Environmental impacts

There is no evidence that protozoans harboured by cultured salmonids can cause increased disease in wild fish. Amoebae responsible for AGD are ubiquitous and free living in the environment (Bridle et al. 2010) and only proliferate on the gills and cause disease in fish cultured under certain situations (Kent et al. 1988). Although common in wild salmon, *L. salmonae* is not usually considered a severe pathogen in wild salmon (Kent et al. 1998, Kent 2000). In contrast, wild fish are recognized as reservoirs of infection of many parasitic protozoans, including flagellates and ciliates that only become problematic in aquacultured fish held at high densities.

Best practice management

Best practice management of protozoan diseases include many of the management methods used to minimize spread of viral, bacterial and fungal disease agents. Good husbandry that reduces/eliminates physical handling of fish to avoid damage to skin and fins, optimising fish nutrition, maximising water quality, including maintaining dissolved oxygen levels above 6 mg/L, reducing temperature and salinity

fluctuations and avoiding temperature extremes and exposure to pollutants to maximize immune competence can reduce the prevalence of protozoan diseases. Proliferation of protozoan parasites is encouraged by high stocking densities (Crosbie et al. 2010), hence use of moderate stocking densities is recommended. Protozoan infections can be reduced by bathing seacaged fish in freshwater, hydrogen peroxide or formalin baths, though this is a laborious process that increases production costs.

2.5 Metazoan diseases

Overview

Salmonids in seacages can be infected by a wide range of metazoan disease agents, including myxosporeans, copepods, monogeneans, digeneans, cestodes, and nematodes (Kent and Poppe 1998, Kent 2000). Some metazoan parasites have complicated multi-host lifecycles (Rohde 1984), while others (particularly ectoparasitic monogeneans and crustaceans) have direct lifecycles which can be readily completed when fish are confined at high densities in seacages. Several species of myxosporean parasites have been recorded in seacage cultured salmonids, including *Kudoa thyrsites*, *Chloromyxum truttae*, *Myxobolus* spp. and *Parvicapsula* sp. (see Kent and Poppe 1998). The lifecycle of many myxosporeans requires invertebrate intermediate hosts (Markiw and Wolf 1983, Wolf and Markiw 1984), though it appears that some myxosporeans can be transmitted directly (Diamant 1997, Swearer and Robertson. 1999, Yasuda 2002). Species such as *K. thyrsites* which causes muscle liquefaction, appear ubiquitous (Moran and Kent 1999, Moran et al. 1999a, Whipps et al. 2003). While *Kudoa* spp. have been recorded from New Zealand fishes (Hine et al. 2000), *K. thyrsites* has not been officially recorded (Hine et al. 2000), though known hosts (barracouta, *Thyrsites atun*) are present in New Zealand and indeed juvenile *T. atun* have been observed in seacages with cultured salmon (B. Diggles, personal observations). Attempts to transfer *K. thyrsites* infection by feeding spores to Atlantic salmon failed to transmit infection, however Atlantic salmon held in seacages in marine waters where *K. thyrsites* was enzootic became infected within 2 weeks (Moran *et al.* 1999b). This suggests that fish in seacages may become infected indirectly through contact with infective stages (actinospores) released by intermediate hosts, directly by eating presporogonic stages excreted by other infected fishes (or via cannibalism), or even by obtaining presporogonic stages via blood transferred by blood feeding vectors such as copepods or leeches (Moran et al. 1999b).

Crustacean ectoparasites of fish invade the fins, gills, skin and other body cavities (Kabata 1984). Their lifecycles are direct with fish being infected by planktonic copepodid larval stages that hatch from eggs deposited by adult copepods (Kabata 1984). Several types of crustaceans have been recorded from salmonids cultured in seacages. These include members of the families Caligidae (Sealice), Ergasilidae, Penellidae, isopods and branchiurans (Kent and Poppe 1998). Wild salmonids and other marine fish are the usual reservoirs for crustacean parasites that affect cultured salmonids (Brooks 2009, Gottesfeld et al. 2009, Penston et al. 2011, Molinet et al. 2011). Of the various groups of crustacean ectoparasites, sealice infections are responsible for the majority of problems in seacage culture of salmonids in the Northern hemisphere and Chile (Krkosek et al. 2005, Costello 2006, 2009, Todd 2007, Molinet et al. 2011), while deaths due to consumption of free living isopods by seacaged fish has been recorded in salmon culture in New Zealand (Boustead 1989).

Monogeneans are ectoparasitic helminths with direct lifecycles that are occasionally seen in cultured salmonids in the northern hemisphere, but are seldom problematic (Kent and Poppe 1998). Digeneans, cestodes and nematodes are endoparasitic helminths that live in the gastrointestinal tract of fishes and other vertebrates. The digenean lifecycle requires a molluscan first intermediate host with plankton eating fishes as final hosts, or second intermediate hosts in some lifecycles where final hosts include larger fishes, birds and mammals. The cestode lifecycle generally requires crustaceans (e.g. copepods) as the first intermediate host with plankton eating fishes as final hosts, or second intermediate hosts in some lifecycles where final hosts include larger fishes, sharks, birds or mammals (Rohde 1984, Noga 2010). The lifecycle of nematodes requires crustaceans (e.g. copepods) as the first intermediate host with plankton eating fishes as final hosts, or second intermediate hosts in some lifecycles where final hosts include larger fishes, birds or mammals (Rohde 1984). Salmonids in seacages worldwide can be infected by several species of digeneans, cestodes and nematodes if the fish consume natural prey items such as molluscan or crustacean intermediate hosts (Kent and Poppe 1998), but these helminth infections naturally occur in wild fishes and seldom, if ever, cause disease.

Environmental impacts

The environmental impacts of the majority of metazoan disease agents of cultured salmonids are negligible, however there is evidence in some regions of the world where intensive salmon farming occurs in seacages and salmonids are native fishes that occur naturally in the wild, that farmed salmon can act as reservoirs of sea lice (mainly *Lepeophtheirus salmonis*, but also other species including *Caligus elongatus*) which can result in increased "spillback" infection of wild salmonids that must swim past seacage sites during their migrations (Krkosek et al. 2005, Costello 2006, 2009, Todd 2007, Jones et al. 2015). The additional infection pressure exerted by salmon farms can increase sea lice burdens on wild fish, potentially resulting in increased morbidity or even mortality in juveniles leaving salmon rivers or early river entry in adult fish returning to rivers to spawn (Krkosek et al. 2005, Wells et al. 2007, Todd 2007, Costello 2009). Experimental treatment of wild salmon to remove sealice increased salmon survival by odds ratios of 1.14 – 1.17 in Irish and Norwegian studies, respectively, although meta-analyses by other authors conclude sealice treatments improve wild salmon survival even more (Jones et al. 2015). The ongoing scientific debate regarding the quantitative effect of sea lice infection on wild salmonids emphasises the challenges associated with attempting to quantify the incremental impact of these parasites within wild fish populations already experiencing >95% natural mortality (Jones et al. 2015).

Best practice management

Best practice management of metazoan diseases include many of the management methods used to minimize spread of viral, bacterial, fungal and protozoan disease agents. Good husbandry that optimises fish nutrition, and ensuring the best possible water quality will maximize immune competence and potentially reduce the prevalence of some metazoan disease agents. Proliferation of metazoan parasites is encouraged by high stocking densities, hence use of moderate stocking densities is indicated. Biosecurity strategies that have also been used at an industry planning level to minimize the risk of sealice outbreaks include use of independent farm management areas where production from

several farming sites can be co-ordinated and synchronised, and where integrated disease management procedures that can include site fallowing can be implemented if necessary (Chang et al. 2007, Brooks 2009). Separation of the farm management areas by buffer zones of sufficient distance to reduce the risk of horizontal disease transmission via water movements is also useful to avoid and/or control outbreaks of disease caused by sealice (Brooks 2009). Sealice have been controlled in salmon cultured in the northern hemisphere through oral administration of drugs such as emamectin benzoate (SLICE) and, more recently, by exposure to warm freshwater baths[1]. There are no drugs commercially available to control myxosporean infections, however regular cleaning of nets may help remove a range of invertebrates that are potential intermediate hosts for myxosporeans such as *K. thyrsites*. Most helminth infections can be reduced by oral treatment with anthelmintics, while many types of ectoparasitic metazoans can also be managed by bathing seacaged fish in freshwater, formalin or hydrogen peroxide baths, though this is a laborious process that increases production costs.

3.0 Description of the existing environment -Disease Agents in New Zealand Salmon

A comprehensive review of the literature relating to the diseases and parasites of salmon (*Oncorhynchus tshawytscha, O. nerka*) in New Zealand was conducted, including key references such as Boustead (1982, 1989), Anderson (1995, 1996, 1998), Hine et al. (2000), Diggles et al. (2002), McIntyre et al. (2010) and the references cited therein. Information was also obtained by searching multiple electronic databases including Cambridge Scientific Abstracts, Scirius, Scopus and Web of Knowledge with keywords salmon and New Zealand. Unpublished data relating to the infectious and non-infectious diseases of chinook salmon encountered by NZ King Salmon veterinarians was also included. A list of the known diseases and parasites of wild and cultured salmon in New Zealand is contained in Table 1. The list contains 22 infectious disease agents of wild and cultured salmon, including 1 virus (aquatic birnavirus), 5 bacterial diseases, 1 fungal disease, 3 protozoan disease agents and 12 metazoan disease agents (Table 1). The list also contains 12 non-infectious diseases that have been reported mainly from cultured salmon (Table 1).

[1] http://www.steinsvik.no/en/products/e/seaculture/fish-health/thermolicer

Table 1. List of the known infectious and non-infectious diseases and parasites recorded from wild and cultured salmon (*Oncorhynchus* spp.) in New Zealand.

Disease	Under official control	Occurs in cultured salmon in NZ	May cause significant disease in wild marine fish	May cause significant disease in seacaged fish
INFECTIOUS AGENTS				
VIRUSES				
Aquatic Birnavirus (IPNV Genogroup 5)	Yes	No	No	Yes
BACTERIA				
Flexibacter spp./ *Tenacibaculum* spp.	No	Yes	No	Yes
Bacterial gill disease	No	Yes	No	No
Piscirickettsia-like bacteria (NZ-RLO)	Yes	Yes	No	Yes
Vibrio spp.	No	Yes	No	Yes
Yersinia ruckeri (Yersinosis)	No	Yes	No	No
FUNGI				
Saprolegnia spp.	No	Yes	No	No
PROTOZOA				
Chilodonella sp.	No	Yes	No	No
Ichthyophthirius multifiliis	No	Yes	No	No
Neoparamoeba perurans / Cochliopodida sp.	No	Yes	No	No
METAZOA				
Digenea				
Derogenes varicus	No	No	No	No
Lecithocladium seriolellae	No	No	No	No
Parahemiurus sp.	No	No	No	No
Tubovesicula angusticauda	No	No	No	No
Cestoda				
Hepatoxylin trichiuri	No	No	No	No
Phyllobothrium sp.	No	No	No	No
Nematoda				
Heduris spinigera	No	No	No	No
Hysterothylacium sp.	No	Yes	No	No
Crustacea				
Caligus spp.	No	Yes	No	Yes
Cirolana sp.	No	Yes	No	No
Paeonodes nemaformis	No	No	No	No
Myxozoa				
Myxobolus cerebralis	Yes	Yes	No	No
NON-INFECTIOUS AGENTS				
Algal blooms	No	Yes	Yes	Yes
Cardiomyopathy	No	Yes	No	No
Gas Bubble Disease	No	Yes	No	No
Gastric Dilation and Air Sacculitis (GDAS)	No	Yes	No	Yes
Isopod invasion	No	Yes	No	Yes
Jellyfish strike	No	Yes	No	Yes
Neoplasia	No	Yes	No	No
Nephrocalcinosis	No	Yes	No	No
Pinhead syndrome, Runting	No	Yes	No	No
Seal predation	No	Yes	No	No
Skin lesions/sunburn	No	Yes	No	No
Spinal deformity	No	Yes	No	No

4.0 Assessment of Environmental Effects

After defining the known diseases of salmon in New Zealand (Table 1), the next step in the assessment of environmental effects is to identify those diseases that represent potential hazards to the environment. For the remainder of this risk assessment, the commodity being considered will be chinook salmon (*O. tshawytscha*) reared in sea cages in the Marlborough Sounds.

4.1 Hazard Identification

To determine which diseases are likely to represent hazards to the environment, the criteria for consideration during the hazard identification process were as follows:

For each disease agent in the initial list, the following questions were considered:

1. Is the disease agent infectious ?, and;

2. Whether chinook salmon cultured in seacages could potentially be infected by the disease agent.

For any disease agent, if the answers to both questions 1 and 2 was 'yes', it was classified as a potential hazard (Figure 1). For all potential hazards, any of those considered likely to cause detrimental impacts to the environment based on one or more of the following criteria were classed as diseases of concern that required detailed risk assessment. The criteria used included whether:

- If the disease agent is "under official control", by its listing in New Zealand's national list of reportable diseases of aquatic animals (Table 2); and/or
- it would be expected to cause a distinct pathological effect in an infected population; and/or
- it would be expected to cause economic harm (e.g. increased mortality, reduced growth rates, decreased product quality, loss of market access, increased costs); and/or
- it would be expected to cause damage to the environment and/or endemic species (defined as either native species that occur naturally in New Zealand's waters, or species that were introduced into New Zealand and are now considered to be acclimatised).

The process used for decision making in relation to the hazard identification process is summarised in Figure 1. Non-infectious diseases and infectious disease agents that are not considered likely to cause a distinct pathological effect in affected populations, and/or economic harm, and/or damage to the environment were considered to represent a negligible risk, and were excluded from further assessment. The reasons why these other infectious disease agents were excluded from detailed risk assessment are elaborated upon in more detail in the following section.

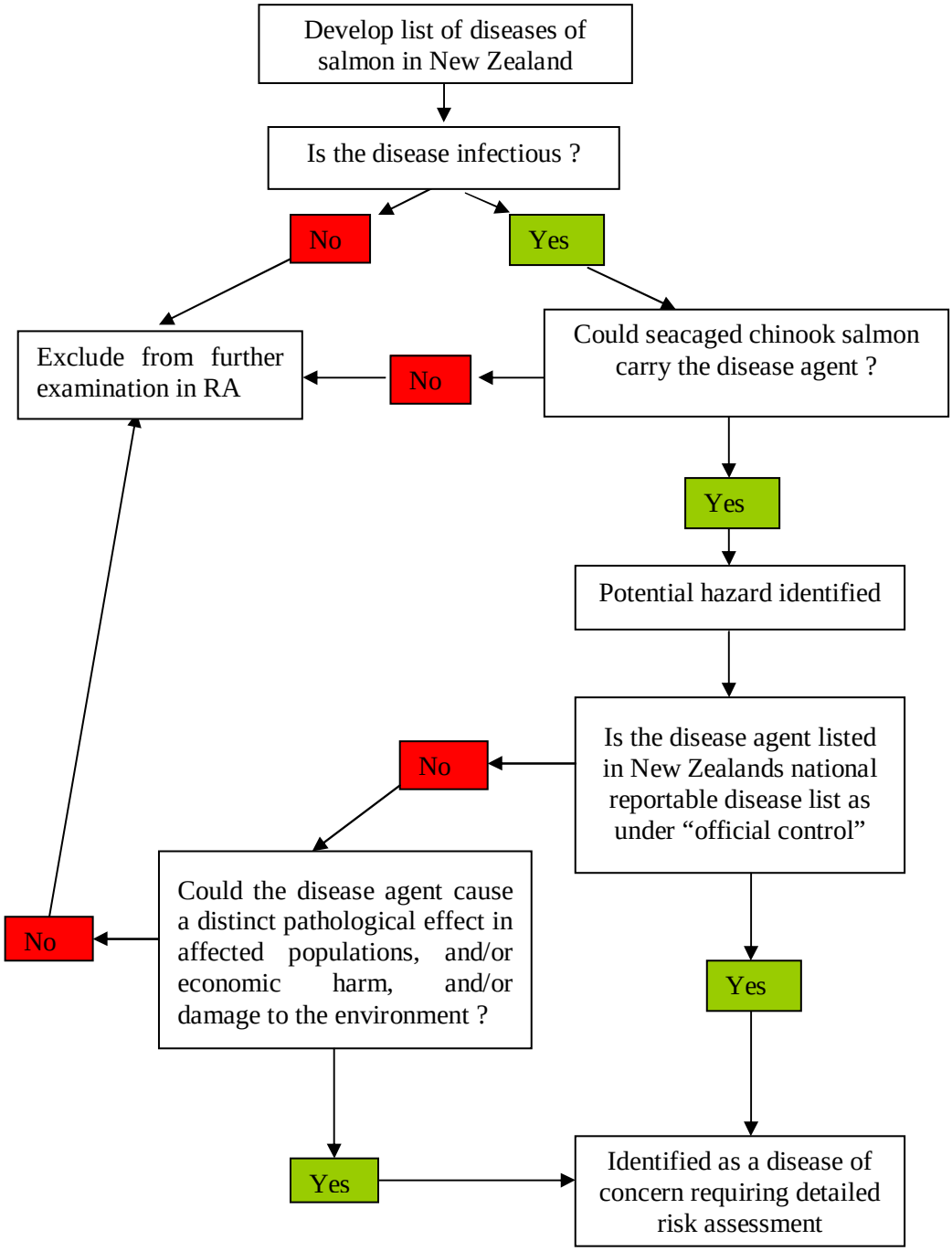

Figure 2. Flow chart showing the decision making process used to identify diseases of concern in the hazard identification step.

Table 2. New Zealand's national list of reportable diseases of finfish (ie. diseases under official control).

New Zealand's National List of Reportable Diseases of Finfish (as of Aug 2016)	**Listed in the OIE *Aquatic Animal Health Code (2016)***	**Exotic to New Zealand**	**Found in salmon in New Zealand**
1. Bacterial kidney disease (*Renibacterium salmoninarum*)		✓	
2. Enteric redmouth disease (*Yersinia ruckeri* – Hagerman strain)		✓	
3. Epizootic haematopoietic necrosis – EHN virus	✓	✓	
4. Epizootic ulcerative syndrome (*Aphanomyces invadans*)	✓	✓	
5. Furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*)		✓	
6. Gyrodactylosis (*Gyrodactylus salaris*)	✓	✓	
7. Infectious haematopoietic necrosis – IHN virus	✓	✓	
8. Infectious pancreatic necrosis (exotic strains) – IPN virus			✓*
9. Infectious salmon anaemia – ISA virus	✓	✓	
10. Koi herpesvirus disease – KHV	✓	✓	
11. *Oncorhynchus masou* virus	✓	✓	
12. Red sea bream iridoviral disease	✓	✓	
13. Spring viraemia of carp – SVC virus	✓	✓	
14. Viral haemorrhagic septicaemia – VHS virus	✓	✓	
15. Whirling disease (*Myxobolus cerebralis*)			✓

* A birnavirus within IPNV Genogroup 5 has been found in returning sea run salmon in New Zealand (Davies et al. 2010).

4.2 Elimination of insignificant diseases

4.2.1 Non-infectious diseases

As a general rule, all of the non-infectious diseases of salmon do not pose a threat to the natural environment, as by definition they are non-infectious and cannot be transmitted to other marine fishes or other aquatic animals. However, one exception to this rule is algal blooms, which represent a risk to not only cultured salmon, but also other aquatic animals and the wider environment (Chang et al. 1990, 2001). Increased risk of algal blooms can sometimes be linked to increased nutrient loads from seacage aquaculture in regions where flushing of nutrients is insufficient (Buschmann et al. 2006, San-Diego et al. 2008), however a range of other environmental conditions are also usually required before conditions are suitable for algal blooms to occur (Diggles et al. 2002). Evaluation of the potential environmental effects in relation to nutrient loading due to the proposed planning changes are outside the scope of this document, and are covered elsewhere in the planning documents (such as in the NIWA report on Modelled water column effects on potential salmon farm relocation sites in Pelorus Sound). The reasons why some other infectious disease agents were excluded from detailed risk assessment are elaborated upon below.

4.2.2 Bacteria

Bacterial gill disease, coldwater disease including *Flexibacter* spp./*Tenacibaculum* spp.

Flavobacteria including members of the genera *Flexibacter*, *Tenacibaculum*, *Flavobacterium*, and *Cytophaga* are ubiquitous in aquatic environments (Austin and Austin 2007, Pulkkinen et al. 2010), but some strains are facultative pathogens that can cause disease (for example, columnaris, bacterial gill disease, fin rot, gill rot) and mortalities in freshwater and marine fish that are stressed, injured and/or exposed to adverse environmental conditions (Mitchell and Rodger 2011). Freshwater genera include *Flavobacterium columnare* (agent of columnaris disease), *F. psychrophilum* (agent of cold water disease) and *F. branchiophilium* (agent of bacterial gill disease), while the marine equivalent is *Tenacibaculum* (formerly *Flexibacter*) *maritimus* (see Diggles et al. 2002). The freshwater flavobacteria found on salmon in New Zealand (Boustead 1989), including *F. psychrophilum* which has been isolated from trout (B. Jones, personal communication, 29 May 2016), do not grow at marine salinities and hence they do not affect marine fish. On the other hand, *Tenacibaculum maritimus* can cause disease in marine fish, but the bacterium is already ubiquitous in the New Zealand marine environment and has been previously identified from several species including snapper (*Pagrus auratus*) and blue cod (*Parapercis colias*) (Diggles et al. 2002, B.K. Diggles, personal obs.) as well as more recently in cultured salmon (MPI 2015). Good husbandry methods such as conservative stocking densities, avoidance of temperature extremes, avoiding damage to fish during handling, maintenance of high water quality and prompt removal of dead fish from tanks and cages can significantly limit the proliferation of these bacteria in cultured fish (Boustead 1989, Diggles et al. 2002, Pulkkinen et al. 2010). *Tenacibaculum maritimus* occurs naturally on wild fish and other aquatic animals in the absence of disease, and have also been found in large numbers on jellyfish, which may act as vectors for *T. maritimus* infections of seacage cultured salmon if the salmon are damaged by contact with jellyfish tentacles (Ferguson et al. 2010). Because these disease agents are already ubiquitous in the marine environment and only cause disease in cultured fish held in stressful conditions at high densities, these bacteria are unlikely to pose a threat to wild fishes or other aquatic animals in the Marlborough Sounds, and thus they do not need to be considered further.

***Vibrio* spp.**

Bacteria of the genus *Vibrio* are ubiquitous in marine environments (Egidus 1987, Austin and Austin 2007), and several species within the genus are facultative pathogens that can cause disease and mortalities in marine fish that are stressed, injured and/or exposed to adverse environmental conditions (Austin and Austin 2007). At least two species of *Vibrio* have been recorded from salmon in New Zealand, including *Vibrio anguillarum* and *V. ordalii* (see Boustead 1989, Diggles et al. 2002), but *Vibrio* spp. including *V. parahaemolyticus* recently isolated from snapper (B. Jones, personal communication, 29 May 2016) are ubiquitous and can infect damaged fish anywhere in the New Zealand marine environment (Diggles et al. 2002). Good husbandry methods such as conservative stocking densities, avoidance of damage to fish during handling, maintenance of high water quality and prompt removal of dead fish from tanks and cages can significantly limit the proliferation of these bacteria in cultured fish (Boustead 1989, Diggles et al. 2002). Because these disease agents are already ubiquitous in the marine environment and only cause disease in cultured fish that are damaged or held in suboptimal conditions, these bacteria are unlikely to pose a threat to wild fishes or other aquatic animals in the Marlborough Sounds, and thus they do not need to be considered further.

***Yersinia ruckeri* (Yersinosis)**

The bacterium *Yersinia ruckeri* is a member of the family *Enterobacteriaceae*, and has a worldwide distribution (Carson and Wilson 2009). This bacterium has been associated with disease in cultured freshwater fishes, mainly salmonids, but also eels, goldfish, carp and others (Tobback et al. 2007, Carson and Wilson 2009). In New Zealand, *Y. ruckeri* has been isolated in salmon from freshwater hatcheries on the east coast of the South Island (Anderson et al. 1994, Anderson 1995, Diggles et al. 2002), but given its ubiquitous distribution worldwide, it is likely that the bacterium is enzootic in the New Zealand environment (Diggles et al. 2002), though it has only been detected at salmonid hatcheries (Anderson et al. 1994). Infection with *Y. ruckeri* results in bacterial septicaemia and disease is most commonly detected due to exophthalmos and blood spots in the eye (Anderson et al. 1994). The severity of the disease is dependant upon the virulence of the variant of the bacterium involved and environmental conditions, being most problematic at higher water temperatures and high stocking densities (Tobback et al. 2007). Acute infections in trout with the 'Hagerman' strain are referred to as enteric red mouth (ERM), however in New Zealand the 'Hagerman' strain is considered exotic (Carson and Wilson 2009), and a milder form of the disease that occurs in salmon is termed yersiniosis.

Yersinia ruckeri is considered an opportunistic pathogen that rarely causes disease in healthy unstressed fish. Disease outbreaks associated with *Y. ruckeri* in cultured salmon occur almost exclusively in freshwater hatcheries when they are injured or held in high densities under poor conditions (Anderson et al. 1994, Anderson 1997, Carson and Wilson 2009), though smolts previously exposed to the bacterium in freshwater may become diseased if they become stressed after transfer to saltwater (Sparboe et al. 1986). The risk of outbreaks of marine yersinosis in cultured salmon is greatly reduced through maximising water quality during the freshwater hatchery phase and vaccination prior to seawater transfer (Ellard 2015). The survival of the bacterium is greatly reduced in seawater (Thorsen et al. 1992), and adhesion of the bacterium to fish is inhibited at higher salinities, preventing entry (Altinok 2004), hence the disease does not affect obligate marine fish (Tobback et al. 2007). Because of this, *Y. ruckeri* is unlikely to pose a threat to wild fishes or other aquatic animals in the Marlborough Sounds, and thus this disease agent does not need to be considered further.

4.2.3 Fungi

***Saprolegnia* spp.**

Water moulds (Class Oomycetes) of the genus *Saprolegnia* (Family Saprolegniales) are ubiquitous in freshwater environments worldwide (Noga 2010). *Saprolegnia* spp. are common opportunistic saprophytes which are associated with disease in freshwater only when the host fish is compromised or stressed (Roberts 2001). These fungi can infect all species of freshwater finfish in New Zealand, including salmon, trout, eels, and native species, as well as their eggs (Hine and Boustead 1974). Good husbandry methods such as avoidance of damage to eggs or fish during handling, maintenance of high water quality and avoidance of extremes in water temperature can significantly limit the proliferation of these fungi in cultured fish (Noga 2010). However, these fungi do not tolerate salt and they cannot survive in seawater (Noga 2010). Because of this, freshwater moulds and fungi are unlikely to pose a

threat to wild fishes or other aquatic animals in the Marlborough Sounds, and thus they do not need to be considered further.

4.2.4 Protozoa

Chilodonella* spp. and *Ichthyophthirius multifiliis

Ciliate protozoans of the genera *Chilodonella* and *Ichthyophthirius multifiliis* infect a wide range of species of freshwater fishes worldwide, including both wild and captive freshwater fishes in New Zealand (Diggles et al. 2002). These parasites can infect salmon in New Zealand (Boustead 1989), and because their direct lifecycle includes multiplication by binary fission (*Chilodonella* spp.) or within the benthic encysted tomont stage (*I. multifiliis*), heavy infections can quickly lead to epizootics when fish are held at high densities and are left untreated. However, these protozoa do not tolerate salt (Selosse and Rowland 1990, Roberts 2001) and they cannot survive in seawater. Because of this, they are unlikely to pose a threat to wild fishes or other aquatic animals in the Marlborough Sounds, and thus they do not need to be considered further.

4.2.5 Metazoa

Digeneans

Digenean trematodes are endoparasitic helminths which have been recorded from a wide range of marine and freshwater fish species throughout New Zealand (Hine et al. 2000). Their indirect lifecycle requires a molluscan first intermediate host with plankton eating fishes as final hosts, or second intermediate hosts in some lifecycles where final hosts include larger fishes, birds and mammals. Under most circumstances, the multi host lifecycles of these parasites reduce the risk of their translocation, because additional hosts need to occur in the receiving environment in order to complete the life cycle. Four species of digeneans have been recorded from wild salmon in New Zealand (Table 1). All of these are parasites of endemic marine fishes (Hine et al. 2000) which have low host specificity and switched hosts to the introduced salmon during the oceanic stages of their lifecycle (Margolis and Boyce 1990). Infected salmon become infected with these parasites through consumption of intermediate hosts or natural exposure to infective stages in natural food items (Margolis and Boyce 1990). Because cultured salmon are fed artificial feeds, they are not regularly exposed to infective stages of digenean parasites via the diet, and hence they do not tend to pick up large numbers of these parasites during their time in seacages, though they can occasionally become infected by preying on natural prey items which may stray into the seacages. Because of these reasons, they are unlikely to pose a threat to wild fishes or other aquatic animals in the Marlborough Sounds, hence these disease agents do not need to be considered further.

Nematodes and Cestodes

Nematodes and cestodes are endoparasitic helminths that live in the gastrointestinal tract of a wide variety of fishes in New Zealand (Hine et al. 2000). Their lifecycle generally requires crustaceans as the first intermediate host with plankton eating fishes as final hosts, or second intermediate hosts in some lifecycles where final hosts include larger fishes, sharks, birds or mammals (Rohde 1984, Noga 2010). Under most circumstances, the multi host lifecycles of these parasites reduce the risk of their translocation, because additional hosts need to occur in the receiving environment in order to complete the life cycle. Two species of cestodes and two species of nematodes have been recorded from wild salmon in New Zealand (Table 1). All of these are parasites of endemic marine fishes (Hine et al. 2000) which have low host specificity and switched hosts to the introduced salmon during the oceanic stages of their lifecycle (Margolis and Boyce 1990). The salmon become infected with these parasites through consumption of intermediate hosts or natural exposure to infective stages in natural food items (Boustead 1989, Margolis and Boyce 1990). Because cultured salmon are fed artificial feeds, they are not regularly exposed to infective stages of helminth parasites via the diet, and hence they do not tend to pick up large numbers of these parasites during their time in seacages, though they can occasionally become infected by preying on natural prey items which may stray into the seacages. Because of these reasons, they are unlikely to pose a threat to wild fishes or other aquatic animals in the Marlborough Sounds, and hence these disease agents do not need to be considered further.

Crustaceans

Parasitic crustaceans, mainly isopods and copepods, live on the body surfaces, gills and in the musculature of a wide variety of marine and freshwater fishes (Hine et al. 2000). Their lifecycles are direct with fish being infected by planktonic copepodid larval stages that hatch from eggs deposited by adult copepods (Kabata 1984). In New Zealand, three species of crustaceans have been recorded from salmon. One of these is *Paenodes nemaformis*, a copepod that usually infects brown trout in freshwater, but there is also a single record of it infecting chinook salmon from Queenstown, also in freshwater (Boustead 1989). A species of isopod, *Cirolana* spp., was found in the mouth of returning sea run chinook salmon (Boustead 1982). Other free living isopods can sometimes be ingested by salmon in seacages, survive being swallowed and damage the stomach and internal organs, causing death (Boustead 1989). *Paenodes nemaformis* is a parasite of freshwater fishes only (Hewitt 1978), and does not occur on salmon in seacages. *Cirolana* sp. appear to be an example of opportunistic host switching in wild fishes, and salmon in seacages do not tend to pick up these parasites (Boustead 1989). Because of these reasons, these two parasites do not need to be considered further.

The third species of crustacean parasite that has been observed on salmon in New Zealand is *Caligus longicaudatus*, an ectoparasitic copepod that was found in small numbers in sockeye salmon (*Oncorhynchus nerka*) reared in seacages in New Zealand (Boustead 1989), while chinook salmon in nearby seacages were not affected (Boustead 1989). Members of the genus *Caligus* are known as "sealice", and there are several species of *Caligus* that occur on marine fish in New Zealand waters (Hewitt 1963, Jones 1988, Hine et al. 2000). One species, namely *Caligus elongatus*, is a host generalist which has been problematic in salmonid aquaculture in the northern hemisphere, and could threaten a wide range of hosts in sea cage culture (Todd 2007) as it has been found on at least 60 host species (Jones 1988), though some of these may be misidentifications of *Caligus chiastos* (see Hayward

et al. 2009). *Caligus elongatus* has been found in the South Island of NZ in the Heathcote Estuary, Christchurch on flounder *Rhombosolea* spp. (Jones 1988, Hine et al. 2000). Another species, namely *C. epidemicus*, is found on fishes in the North Island. It is another host generalist that has been associated with disease outbreaks on wild and cultured fishes in various locations (Hewitt 1971, Ho et al. 2004). Because of these reasons, Caligid copepods will be subjected to detailed risk assessment.

4.3 The diseases of concern requiring detailed risk assessment

After excluding the non-infectious diseases and the insignificant infectious diseases listed in Table 1 for the reasons outlined above, the diseases of salmon in New Zealand that will require detailed risk assessment are listed below in Table 3.

Table 3. List of the diseases of concern that will be subjected to detailed risk assessment.

Disease	**Under official control**	**Occurs in cultured salmon in NZ**	**May cause significant disease in wild marine fish**	**May cause significant disease in seacage cultured fish in NZ**
VIRUSES				
Aquatic Birnavirus	Yes	No	No	Yes
BACTERIA				
Piscirickettsia-like bacteria (NZ-RLO)	Yes	Yes	No	Yes
PROTOZOA				
Amoebic gill disease *(Neoparamoeba perurans / Cochliopodida* sp.)	No	Yes	No	No
METAZOA				
Crustacea				
Sea lice (*Caligus* spp.)	No	Yes	No	Yes
Myxozoa				
Whirling Disease (*Myxobolus cerebralis*)	Yes	Yes	No	No

5.0 Detailed Risk Assessment

5.1 Infection with Aquatic Birnavirus

5.1.1 Aetiologic agent: Non-enveloped viruses with a double-stranded RNA genome of the genus *Aquabirnavirus* within the Family Birnaviridae.

5.1.2 OIE List: No **Reportable disease in New Zealand:** Yes

5.1.3 New Zealand's status: An aquatic birnavirus strain (IPNV Genogroup 5) has been reported from returning chinook salmon in the South Island and cultured turbot in Wellington Harbour (Tisdall and Phipps 1987, B.K. Diggles, unpublished data, Davies et al. 2010).

5.1.4 Epidemiology

Aquatic birnaviruses have been isolated from a large number of marine and freshwater aquatic animals (McAllister 1993), to the extent that these viruses are considered to be ubiquitous in aquatic environments worldwide (Reno 1999). Various strains of birnavirus have been described from at least 65 species of fish in 20 families (McAllister 1993), and also from bivalve molluscs and crustaceans (Reno 1999). The type species for the genus *Aquabirnavirus* is Infectious Pancreatic Necrosis Virus (IPNV), which causes infectious pancreatic necrosis (IPN), a significant disease of salmonids (Wolf et al. 1960). The genus includes both virulent and avirulent viruses with the term 'infectious pancreatic necrosis' (IPN) virus being reserved for those isolates that are pathogenic for species within the Family Salmonidae (McColl et al. 2009). IPN disease has not been formally recorded in New Zealand, however an aquatic birnavirus was isolated from apparently healthy sea run chinook salmon (*O. tshawytscha*) returning up the Rakaia River (Tisdall and Phipps 1987), and the Hakataramea River (Anderson 1998), but this virus has never been associated with disease in these fishes. More recently, a birnavirus was found to be associated with a suspicious outbreak of bacterial disease in juvenile turbot (*Colistium nudipinnis*) in New Zealand (Diggles et al. 2000), with the virus being isolated from surviving fish several years after the epizootic (B.K. Diggles unpublished data, Davies et al. 2010, who incorrectly identified the host as *Psetta maxima*). New Zealand virus isolates were identified as belonging to IPNV Genogroup 5 (see Davies et al. 2010) and appear non pathogenic to salmonids (Diggles et al. 2002, McColl et al. 2010). However, it is possible that members of this genogroup may be pathogenic in non-salmonid hosts (such as flatfish, which are common carriers of the virus (Wallace et al. 2008)) or even to salmonids under different environmental or husbandry conditions (such as small juvenile fish in hatcheries) (Davies et al. 2010). The isolate from turbot showed a high level of sequence identity (97-99%) to birnavirus isolates from wild marine fish in Tasmania, suggesting that the Australian and New Zealand isolates originate from the same source, presumably wild marine species inhabiting the Southern Ocean. The isolate from returning chinook salmon was also very similar to the birnavirus isolates from wild marine fish in Tasmania (94-98% identity) (Crane et al. 2000, Davies et al. 2010).

In Japan aquatic birnaviruses cause some of the most important diseases of juvenile yellowtail (*Seriola quinqueradiata*), kingfish (*S. lalandi aureovittata*) and amberjack (*S. dumerili*) (see Isshiki and Kusuda 1987, Isshiki et al. 2001, Nakajima et al. 1998, Muroga 2001). This suggests that cultured kingfish in New Zealand may also be susceptible to aquatic birnaviruses. Aquatic birnaviruses are known to cause disease almost exclusively in juvenile fish (Novoa et al. 1993, Reno 1999), with yellowtail less than 10 grams being particularly susceptible in Japan, with moribund juveniles typically exhibiting anaemic gills, haemorrhaging in the liver, severe ascites, and pancreatic necrosis (Nakajima et al. 1998). Water-born birnaviruses accumulated by bivalves, crustaceans and birds can remain viable when excreted (Mortensen et al. 1992), and can be subsequently used to infect fish experimentally (Mortensen 1993), although viral replication does not appear to occur in other hosts, hence the main method of translocation remains live fish and eggs (Reno 1999).

5.1.5 Release assessment

Birnaviruses are isolated only rarely from marine fish in New Zealand, however wild marine fish must be considered a reservoir of infection. A comprehensive survey of wild marine fish for aquabirnavirus has not been undertaken in New Zealand, though surveys of thousands of cultured and returning chinook salmon over many years have shown the virus to be rare (Anderson 1995, 1996, 1998, McIntyre et al. 2010). Nevertheless, aquatic birnaviruses are known to occur in the marine waters adjacent to the South Island of New Zealand at low prevalences, and indeed they are considered likely to be present throughout the Southern Ocean (Davies et al. 2010), though the required surveys have not been conducted to determine the range of host species or prevalence of infection.

Infection is direct via horizontal exposure to viral particles in the water, or by vertical transmission from infected gametes (McAllister 1993). Juvenile fish that survive infection can be lifelong carriers which shed the virus via the urine, faeces and sexual products (Reno 1999), however large juveniles and adults exposed to the virus for the first time may be refractory to infection or can spontaneously recover (Novoa et al. 1993). Aquabirnaviruses are very persistent in the environment, with minimal loss of infectivity after 10 weeks in filtered seawater at 4 and 10°C, and they are also very resistant to a broad range of disinfectants (Bovo et al. 2005). Aquatic birnavirus is not inactivated by passage through the bird digestive system and as such, the disease agent can also be spread naturally via mechanical vectors such as sea birds (Reno 1999).

The release of birnavirus into the environment from seacaged salmon requires the following pathway to occur. A chinook salmon that has become naturally and subclinically infected with birnavirus through contact with seawater is selected for use as broodstock. Viable aquabirnavirus must persist in the sexual fluids and survive surface treatments of the eggs (e.g. hydrogen peroxide and/or iodophor treatment), then subsequently persist in the larvae after fertilized eggs hatch. Larval and juvenile salmon reared in freshwater must then survive without being detected during routine surveillance of clinically healthy fishes for viruses, without outbreaks of clinical disease (because every disease outbreak is routinely investigated), and survive the stress of saltwater acclimation as smolts. Only after all of these conditions are met, would viable aquabirnavirus be present in cultured salmon and potentially be able to be released into the environment. Taking into account the extremely low prevalence of the disease

agent in returning salmon, and the fact that the vast majority of broodstock chinook salmon used by NZ King Salmon are held over in freshwater for their entire lives, the likelihood estimations for the release of salmon infected by aquatic birnavirus into seacages in the Marlborough Sounds is considered to be **Extremely Low**.

5.1.6 Exposure assessment

Marine teleosts and invertebrates in New Zealand are already at risk of exposure to the local strain of aquatic birnavirus, which probably occurs in turbot (*C. nudipinnis*) and other species of wild fishes, which act as a reservoir of infection for returning salmon to be exposed to the virus. Several species of wild fish and molluscs in New Zealand are therefore likely to be susceptible to infection by aquatic birnaviruses carried by infected salmon in seacages, but infection would occur only if sufficient quantities of virus (i.e. an infective dose) was introduced into an area where susceptible hosts were present. Susceptible fish and bivalves can become infected with aquatic birnavirus via horizontal transmission through the water (immersion) and also by *per-os* exposure (Reno 1999). The infectious dose of birnavirus by the immersion pathway varies according to the strain of virus used and the species challenged (McAllister and Owens 1995), ranging from more than 10^3 $TCID_{50}$/mL for arctic char (McAllister et al. 2000), to as low as < $10^{-1}TCID_{50}$/mL for Atlantic salmon post smolts exposed to pathogenic strains of IPNV (Urquhart et al. 2008). The infectious dose of birnavirus by *per-os* exposure to infected feed also varies, with Mortensen (1993) requiring a dose of 10^6 $TCID_{50}$/g of IPNV obtained from scallops before successful transmission to brown trout was obtained. However Wechsler et al. (1987) reported successful transmission of IPNV to striped bass fed brook trout infected with between 2 x 10^2 and 2 x 10^5 $TCID_{50}$/g IPNV.

Clinically diseased fish infected with birnavirus can have very high viral titres in their internal organs (10^7 - 10^9 $TCID_{50}$/g,) as well as high viral shedding rates (Reno 1999, Sommer et al. 2004, Urquhart et al. 2008), however no fish with clinical disease caused by birnavirus infection have ever been formally recorded in New Zealand (Diggles et al. 2002, Davies et al. 2010). The levels of birnavirus in sub-clinically infected fish can still be relatively high (10^2 - 10^6 $TCID_{50}$/g , see McAllister et al. 2000), but are usually lower and often around the limits of detection using cell culture techniques (c. 10^1 - 10^2 $TCID_{50}$/g, Wechsler et al. 1987, Roberts 2001). Even given that susceptible fish can be infected by immersion at low infective doses (Urquhart et al. 2008), the likelihood that an infectious dose can be transmitted horizontally into a natural water body via its spread from seacaged salmon that are sub-clinically infected with aquatic birnavirus appears unlikely, however the likelihood would increase if the salmon became clinically diseased. Indeed, prevalence of IPNV was increased slightly above background levels (from 0.15% prevalence to 0.58% prevalence) in wild fishes within 5 km of salmon farms in Scotland that contained fish clinically diseased with IPN (Wallace et al. 2008). However, the birnavirus isolates recorded to date in New Zealand are not pathogenic to salmon (Diggles et al. 2002, P.M. Hine, personal communication) hence the risk of seacaged salmon becoming diseased appears extremely low. Nevertheless, given that there is a direct pathway for virus particles shed by seacaged salmon to enter the marine environment and infect wild fishes that may be attracted to seacages (Dempster et al. 2009, Uglem et al. 2009), and acknowledging the range of susceptible hosts may be

broad, the risk of exposure and establishment is non-negligible, and the likelihood of exposure and establishment of aquatic birnavirus in wild fish and mollusc populations is considered to be **Very Low**.

5.1.7 Consequence assessment

When fish become infected with aquatic birnavirus, mortality is mainly restricted to larval and early juvenile stages, and disease does not necessarily occur in larger fish. Indeed, many fish experimentally infected with aquatic birnavirus can naturally resolve the infection provided they are healthy and remain unstressed (Reno 1999). However, others fish remain carriers for life, and risk spreading birnavirus to their progeny vertically via infected gametes. Given that aquabirnavirus is already present in some parts of New Zealand's environment, and these viruses only tend to occur at subclinical levels in juvenile and adult fish in the wild (Anderson 1995, 1996, 1998, Wallace et al. 2008, McIntyre et al. 2010), the consequences of localized slight increases in prevalence of birnaviruses in wild fish within 5 km of affected salmon farms (Wallace et al. 2008) appear related mainly to possible increased mortality of larval and early juvenile stages, which although never documented in wild fishes, if it occurs it could have some impact on wild fish at the population level. These viruses may also increase costs of production in marine finfish aquaculture hatcheries due to use of infected wild caught broodstock. These viruses are no longer listed by the OIE and hence their presence is unlikely to have adverse impacts on national or international trade, but aquabirnaviruses remain reportable in New Zealand. Considering all of these factors, establishment of the disease in cultured salmon would have mild biological consequences, which would be amenable to control, and would not cause any noticeable environmental effects. It is therefore estimated that the consequences of introduction of birnavirus strains into New Zealand's environment via salmon in sea cages in the Marlborough Sounds would likely be **Very Low**.

5.1.8 Risk estimation

The unrestricted risk associated with aquatic birnavirus is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Tables 6, 7). The unrestricted risk estimate for aquatic birnavirus does not exceed the ALOP, suggesting that additional risk management for this disease agent is not required.

Risk estimate for infection with Aquatic Birnavirus

Commodity type	**Sea caged salmon**
Combined likelihood of release and exposure	Extremely Low
Consequences of establishment and spread	Very Low
Risk estimation	Negligible Risk

5.2 Infection with *Piscirickettsia*-like bacteria (NZ-RLO)

5.2.1 Aetiologic agent: Gram negative, obligate interacellular gamma proteobacteria of the genus *Piscirickettsia* within the Family Piscirickettsiaceae.

5.2.2 OIE List: No **Reportable disease in New Zealand:** Yes

5.2.3 New Zealand's status: A rickettsia-like bacterium closely related to *Piscirickettsia* (the NZ-RLO) has been isolated from chinook salmon cultured in the outer Pelorus Sound and Queen Charlotte Sound (MPI 2015, 2016).

5.2.4 Epidemiology

Members of the genus *Piscirickettsia* are obligate intracellular bacterial disease agents which cause rickettsial septicaemias in fishes. *Piscirickettsia salmonis* was the first member of the group to be described following its involvement in outbreaks of piscirickettsiosis disease in coho salmon (*Oncorhynchus kisutch*) cultured in seawater net pens in Chile in the late 1980's (Fryer et al. 1990, 1992). Mortalities of up to 90% (more usually 20-30%) were recorded in affected farms eventually resulting in a shift to farming more *Piscirickettsia*-resistant species such as Atlantic salmon (Mauel and Miller 2002, Fryer and Hedrick 2003). Since then *P. salmonis* has been isolated from several species of salmonids throughout the northern hemisphere including Atlantic salmon (*Salmo salar*), chinook salmon (*O. tshawytscha*), rainbow trout (*O. mykiss*), Pink salmon (*O. gorbuscha*), cherry salmon (*O. masou*) as well as white sea bass (*Atactoscion noblis*) and European seabass (*Dicentrarchus labrax*), whilst closely related *P. salmonis*-like bacteria have also been isolated from grouper (*Epinephelus melanostigma*) and tilapia (*Oreochromis* sp., *Tilapia* sp., *Sarotherodon* sp.), amongst others (Mauel and Miller 2002, DAFF 2013). In the southern hemisphere, besides infections of salmonids in Chile, a *P. salmonis*-like organism (Tas-RLO) was reported from Atlantic salmon in Tasmania (Corbeil et al. 2005, Corbeil and Crane 2009), and most recently a similar organism (NZ-RLO) has been identified from chinook salmon cultured in the Marlborough Sounds since 2012 (MPI 2015).

The onset of piscirickettsiosis in cultured salmon usually follows the transfer of fish from freshwater hatcheries to marine sites where they are exposed to the bacterium via marine reservoir hosts or vectors such as ectoparasites (Kent and Poppe 1998). Horizontal transmission of the disease by cohabitation occurs readily in saltwater via the skin, gills or intestine (Corbeil and Crane 2009), however, there is evidence that the disease can also occur in brackish water or even in freshwater where horizontal transmission is limited by reduced survival of the disease agent, but the disease may nevertheless be vertically transmitted to juveniles from infected adult fish returning from the sea (Kent and Poppe 1998). Vertical transmission has been demonstrated under experimental conditions and *P. salmonis* has been detected in milt, eggs and coelomic fluid from infected broodstock (DAFF 2013). Larenas et al. (2003) estimated that 10% of eggs and fry originating from one or more infected broodstock were infected with *P. salmonis*. *Piscirickettsia salmonis* can adhere to the surface of eggs, can occur within the yolk of unfertilised eggs, and is capable of penetrating the ovum (Larenas et al. 2003). This has

implications for the biosecurity of hatcheries, because the surface disinfection of fertilised eggs may not inactivate all *P. salmonis* bacteria (DAFF 2013).

Fish of all ages are susceptible to infection and outbreaks of disease usually follow periods of stress from various husbandry related factors including osmotic shock during smoltification, high water temperatures or rapid fluctuations in temperature, exposure to algal blooms, and co-infection with other disease agents (particularly viruses, see Zainathan 2012, DAFF 2013), all of which can increase susceptibility to infection (Fryer and Hedrick 2003). In Tasmania, salmon that tested positive for Tas-RLO without the presence of aquatic reovirus did not display signs of clinical disease and did not have increased mortality (DAFF 2013).

Gross signs of clinical piscirickettsiosis include darkening in colour, lethargy, swimming at the surface and inappetance. Erratic swimming and exophthalmos may occur in some fish where the bacteria can be isolated from the brain, while skin lesions which progress to shallow ulcers may also be present (Fryer and Hedrick 2003). Internally, the liver and spleen may be enlarged and exhibit multifocal, grossly visible pale nodular lesions and ascites fluid may be present. The optimal temperature for growth of *P. salmonis* in vitro is 15–18 °C which corresponds with water temperatures reported during most disease outbreaks in salmonid culture, while growth is retarded above 20°C and below 10°C and ceases above 25°C (Fryer and Hedrick 2003). In contrast, outbreaks of piscirickettsiosis in non salmonids such as tilapias in Hawaii can occur at water temperatures as high as 26°C (Mauel et al. 2003). As for other bacterial diseases, use of best practice husbandry methods to control known risk factors can reduce the liklihood of *Piscirickettsia* outbreaks, including maximizing water quality, using broodstock that have never been exposed to seawater, rearing fish at lower densities, allowing farms in a given region to fallow, controlling ectoparasites that may act as vectors, and avoiding horizontal transmission between year classes by holding single year classes of fish at any given site (Fryer and Hedrick 2003).

5.2.5 Release assessment

The NZ-RLO is known to occur in cultured chinook salmon in the outer Pelorus Sound and Queen Charlotte Sound regions of the Marlborough Sounds. A survey of cultured salmon elsewhere in New Zealand has shown that disease associated with the NZ-RLO does not occur outside the Marlborough Sounds and is most prominent in salmon farmed at the low flow site at Waihinau Bay in Pelorus Sound (B. Jones, personal communication 27 May 2016, MPI 2016), suggesting that environmental conditions at that particular site are more permissive for infection to occur and disease to emerge than at other sites, especially during summer when salmon may be stressed by high water temperatures.

Infection is direct via horizontal exposure to bacteria in the water, or by vertical transmission from infected gametes. Given that this disease is currently restricted to some salmon held at sub-optimal seacage farm sites, this suggests that the onset of piscirickettsiosis in cultured salmon in New Zealand occurs due to horizontal transmission after the transfer of fish from freshwater hatcheries to certain marine sites where the fish are exposed to the bacterium via marine reservoir hosts (or vectors) under certain environmental conditions that permit establishment of infection. Once an index case occurs,

horizontal transmission of the disease from a clinically infected fish to other fish cohabiting the seacage can be expected via increased shedding of the bacterium via lesions, bile, faeces or urine and uptake in new hosts via skin, gills or intestine (Corbeil and Crane 2009, DAFF 2013). Hence once established on a farm and in the absence of disease mitigation efforts, the buildup of infectious stages is likely to result in increased shedding of the bacterium into the water column, increasing the risk of "backspill" infection of wild non-salmonid hosts. Taking into account that these disease agents are known to occur in the New Zealand environment and have been observed in diseased cultured chinook salmon, the likelihood estimation for salmon infected by *Piscirickettsia*-like bacteria occurring in seacages in the Marlborough Sounds is considered to be **High**.

5.2.6 Exposure assessment

Marine teleosts and invertebrates in New Zealand are already at risk of exposure to the local strain of *Piscirickettsia*-like bacteria, including as yet unidentified species of wild fishes or invertebrates which probably act as a reservoir of infection and/or vectors for cultured salmon. It is assumed susceptible hosts occur in the New Zealand environment, but "backspill" infection of these would occur only if sufficient quantities of bacteria (i.e. an infective dose) were introduced into an area where susceptible hosts were present close to salmon cages.

The virulence and infectious dose of *Piscirickettsia*-like bacteria by horizontal transmission varies according to the strain of bacteria and the species challenged. House et al. (1999) determined that coho salmon injected with $10^{2.6}$ $TCID_{50}$ of a less virulent Norwegian strain of *P. salmonis* had no increase in mortality rate compared to controls, but a similar dose of a virulent strain from Chile resulted in mortalities exceeding 50%. In contrast, minimum infective dose via the immersion route appears to be much higher. For example, Birbeck et al. (2004) studied a *P. salmonis* strain with an LD50 by injection into Atlantic salmon of < 200 $TCID_{50}$, which caused only 10% mortality when Atlantic salmon were exposed by immersion to 10^5 $TCID_{50}$/ml for 1 hour at 14°C.

Assuming New Zealand strains of *Piscirickettsia*-like bacteria are highly virulent (until proven otherwise), given that susceptible fish can be infected by immersion in virulent *P. salmonis* only at moderately high infective doses (Birbeck et al. 2004), it appears unlikely that an infectious dose can be transmitted horizontally into a natural water body via its spread from seacaged salmon that are sub-clinically infected with *Piscirickettsia*-like bacteria. However, the likelihood of this occurring would increase if the salmon became clinically diseased. Given that there is a direct pathway for bacteria shed by seacaged salmon to enter the marine environment and infect wild fishes that may be attracted to seacages (Dempster et al. 2009, Uglem et al. 2009), and acknowledging that susceptible hosts are likely to be present in the vicinity of sea cages, the risk of exposure and establishment is non-negligible, and the likelihood of exposure and establishment of *Piscirickettsia*-like bacteria in wild fish populations is considered to be **Low** if salmon are subclinically infected, and **Moderate** if they are clinically diseased.

5.2.7 Consequence assessment

When fish are infected with *Piscirickettsia*-like bacteria, mortality is observed almost exclusively in cultured fish that are stressed by other predisposing factors, and there are very few documented instances of mortalities occurring in wild fish. One possible exception to this was during an outbreak of disease due to a *Piscirickettsia*-like bacteria in tilapia in Hawaii, where diseased wild tilapia were observed and suspected to be the origin of the infection, but no other species were affected (Mauel et al. 2003). To date the NZ-RLO has been associated with chronic mortalities in cultured populations of chinook salmon in at least one low flow farming site in New Zealand, however, if the risk of exposure to the NZ-RLO is left unmitigated, its persistence in populations of cultured fish has the potential to adversely affect the productivity and profitability of the salmon culture industry in Marlborough Sounds. These bacteria are no longer listed by the OIE and hence their presence is unlikely to have adverse impacts on national or international trade, but the NZ-RLO remains reportable in New Zealand and is currently subject to a containment notice (MPI 2016). Considering all of these factors, establishment of the disease in cultured salmon is likely to have mild biological consequences, which would be amenable to control, and would be unlikely to cause any noticeable environmental effects. It is therefore estimated that the consequences of spillback introduction of *Piscirickettsia*-like bacteria into New Zealand's environment via salmon in sea cages in the Marlborough Sounds would likely be **Low**.

5.2.8 Risk estimation

The unrestricted risk associated with *Piscirickettsia*-like bacteria is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Tables 6, 7). The unrestricted risk estimate for *Piscirickettsia*-like bacteria does not exceed the ALOP for subclinically diseased salmon, but does exceed the ALOP if salmon are clinically diseased, suggesting that additional risk management for this disease agent is required under such circumstances.

Risk estimate for infection with *Piscirickettsia*-like bacteria (NZ-RLO)

Commodity type	Sub-clinically diseased seacaged salmon	Clinically diseased seacaged salmon
Combined likelihood of release and exposure	Low	Moderate
Consequences of establishment and spread	Low	Low
Risk estimation	Very Low Risk	Low Risk

5.3 Amoebic/Nodular Gill Disease

5.3.1 Aetiologic agent: Amoebae including *Neoparamoeba perurans* and *Cochliopodia* spp.

5.3.2 OIE List: No **Reportable disease in New Zealand:** No

5.3.3 New Zealand's status: Amoebae such as *N. perurans* and *Cochliopodia* –like species are known to occur on cultured salmon in marine and freshwater environments, respectively.

5.3.4 Epidemiology

Amoebic gill disease (AGD) is an economically important disease of salmon cultured in seacages in several regions of the world (Munday et al. 2001, Young et al. 2008, Mitchell and Rodger 2011). *Neoparamoeba pemaquidensis*, a free-living marine amoeba, was for some time regarded as the only aetiological agent of AGD as it had been consistently isolated from diseased fish (Kent et al. 1988, Dykova et al. 2000). However, attempts to experimentally transmit AGD using cultured *N. pemaquidensis* failed to cause disease in Atlantic salmon (Morrison et al. 2005). The true agent responsible for AGD was subsequently found to be a new species of amoebae (Young et al. 2007), now known as *Neoparamoeba perurans*. Since that time, *N. perurans* has been confirmed to be the predominant causative agent of AGD in Atlantic salmon, rainbow trout, chinook salmon and turbot from regions as diverse as Tasmania, Ireland, Spain, Norway, North America, Scotland, and New Zealand (Young et al. 2008, Steinum et al. 2008). Molecular studies have confirmed that *N. perurans* is a free-living amoeba that occurs naturally in the rearing environment around seacages containing cultured salmonids (Bridle et al. 2010).

Bermingham and Mulcahy (2007) suggested that other amoebae may also be involved in AGD in addition to *Neoparamoeba* spp., including the genera *Platyamoeba*, *Flabellula* and *Vexillifera* which have all been recorded on the gills of Atlantic salmon with AGD from both Ireland and Tasmania. Although present in New Zealand, AGD is not considered a significant problem because chinook salmon (*Oncorhynchus tshawytscha*) appear to be resistant to this disease, being often infected but rarely experiencing significant mortality in marine seacages (Munday et al. 2001, Tubbs et al. 2010). However, nodular gill disease caused by other genera of freshwater amoebae, including agents that resemble *Cochliopodia* spp., can be associated with disease and mortalities in juvenile chinook salmon held at high densities in freshwater raceways (Tubbs et al. 2010). These freshwater amoebae cannot survive the transfer to seawater (Lom and Dykova 1992), hence they are unlikely to survive the transfer of smolts into seacages.

Amoebae can be found on salmon gills at temperatures of around 10°C in both marine (Mitchell and Rodger 2011) and freshwater sites (Tubbs et al. 2010), however in Tasmania clinical disease is most commonly reported between temperatures of 12 – 20°C and salinities approaching 35 ppt (Munday et al. 2001). Projected increases in global water temperatures are thought to be a risk factor likely to increase the incidence of AGD in years to come (Bridle et al. 2010). Seacaged salmon are worst affected during

their first year at sea, and in serious AGD outbreaks, up to 50% mortality can occur if there is no treatment (Mitchell and Rodger 2011). The disease appears of less clinical significance in countries other than Tasmania, but occasionally AGD can cause substantial morbidity or mortality, especially when associated with pre-existing disease or unusual environmental conditions (Mitchell and Rodger 2011). Affected fish have multifocal gill lesions characterised by hyperplasia, proliferation of mucous cells and necrosis (Kent et al. 1988, Roubal et al. 1989, Munday et al. 1990, 1993, 2001).

5.3.5 Release assessment

Because *N. perurans* is a free-living amoeba, it occurs naturally in the marine environment, including in areas around seacages containing cultured salmon (Bridle et al. 2010). It appears that these parasites are opportunistic pathogens that cause disease only in salmonids cultured at high density under adverse environmental conditions (Kent and Poppe 1998, Mitchell and Rodger 2011). Studies of wild fishes near seacage farms containing infected salmon have found that they are not a significant reservoir of infection, and indeed none of 325 wild fish of 12 different species sampled from around seacages in Tasmania were infected by *Neoparamoeba* spp. (see Douglas-Helders et al. 2002). However it remains to be seen whether wild fish are identified as a reservoir of infection once sensitive molecular diagnostic methods optimised for *N. perurans* are used (Bridle et al. 2010). Infection is direct via horizontal exposure to amoebae in the water (Munday et al. 2001). Taking into account that these disease agents are known to occur in the New Zealand environment and are sometimes observed in cultured chinook salmon, the likelihood estimation for salmon infected by amoebae occurring in seacages in the Marlborough Sounds is considered to be **High**.

5.3.6 Exposure assessment

Marine teleosts in New Zealand are already at risk of exposure to free living amoebae such as *N. perurans*, which occurs naturally in the environment. It appears that these parasites are mainly opportunistic pathogens that cause disease only in salmonids cultured at high density under adverse environmental conditions (Kent and Poppe 1998, Mitchell and Rodger 2011). Wild fish are therefore unlikely to be susceptible to infection unless they are also exposed to high numbers of amoebae under adverse environmental conditions at high stocking densities. Susceptible fish can become infected with amoebae via horizontal transmission through the water. Salmon with clinical AGD can be infected by high numbers of amoebae, and amoebae can survive and multiply on the gills of dead fish up to at least 30 h post-mortem (Douglas-Helders et al. 2000). However, chinook salmon appear resistant to AGD (Munday et al. 2001), and thus AGD outbreaks seldom cause mortality in seacages in New Zealand (Tubbs et al. 2010), while routine husbandry practices such as early identification and removal of runts and dead fish from seacages limits proliferation of the amoebae and the chances of them spreading to wild fish. Nevertheless, given that there is a direct pathway for amoebae from seacaged salmon to re-enter the marine environment and infect wild fishes that may be attracted to the vicinity of seacages (Dempster et al. 2009, Uglem et al. 2009), and acknowledging that susceptible hosts may occur in the wild, the risk of exposure and establishment is non-negligible, and the likelihood of additional backspill exposure of wild fish populations to amoebae is considered to be **Low.**

5.3.7 Consequence assessment

Although *N. perurans* is present in New Zealand, AGD is not considered a significant problem because chinook salmon are resistant to infection. Wild fishes are not a significant reservoir of infection, and indeed they do not seem to become clinically infected by amoebae even in areas around seacages that contain clinically diseased salmon (Douglas-Helders et al. 2002). Given that free living amoebae are already present in the New Zealand marine environment, they do not cause disease in wild fish, and their presence does not adversely impact national or international trade, the consequences of introduction of amoebae into the environment of the Marlborough Sounds with live salmon cultured in seacages are likely to be **negligible,** and no further analysis is required.

5.4 Sea lice

5.4.1 Aetiologic agent: Ectoparasitic crustaceans within the Family Caligidae.

5.4.2 OIE List: No **Reportable disease in New Zealand:** No

5.4.3 New Zealand's status: Several different species of the genera *Lepeophtheirus* and *Caligus* (Family Caligidae) occur on a wide variety of wild fishes throughout New Zealand (Hewitt 1963, Jones 1988, Hine et al. 2000).

5.4.4 Epidemiology

Ectoparasitic copepods are parasitic crustaceans that live on the body surfaces, gills and fins of marine and freshwater fishes. Their lifecycles are direct with fish being infected by planktonic larval stages that hatch from eggs deposited by adult copepods (Kabata 1984). In New Zealand, a large number of marine fishes harbour copepod ectoparasites from the Family Caligidae (sea lice) (see Hewitt 1963, Jones 1988, Hine et al. 2000). These copepods encounter their host as copepodid larvae then attach to the host fish via the specialized chalimus larvae (Kabata 1984), which is sedentary until such time as the copepod moults to the pre adult and adult stages, which are mobile and can be found attached to gills, skin or fins (MacKenzie et al. 1998). Chalimus larvae can cause localized pathological changes at their attachment sites (Roubal 1994, MacKenzie et al. 1998), while high numbers of mobile pre-adult and adult caligids (particularly members of the genera *Lepeophtheirus* and *Caligus*) on cultured fish damage the skin of the fish as they feed on host mucus and blood, resulting in morbidity and in some cases, death (Grimnes and Jakobsen 1996, Kent and Poppe 1998). The numbers of sea lice that can be tolerated by the host fish varies with host size, with 1 motile *Lepeopthirius salmonis* per 0.75–1.6 g body weight being tolerated (Grimnes and Jakobsen 1996, Krkosek et al. 2005). This means that small fish such as juvenile coho and pink salmon around 40 mm long may only be able to tolerate 1 adult sea louse, or less (Krkosek et al. 2005). Sea lice have been responsible for disease and significant mortalities in the culture of salmonids in several overseas countries (Pike and Wadsworth 1999). In cases where cultured fish become heavily infected, they become stressed, and death commonly occurs, ultimately due to osmoregulatory failure or secondary bacterial infection (Grimnes and Jakobsen 1996, MacKenzie et al. 1998, Pike and Wadsworth 1999).

In regions of the world where salmonids are native fishes that occur naturally in the wild, there is some evidence indicating that in areas where intensive salmon farming occurs in seacages, farmed salmon can act as reservoirs of sea lice (mainly *Lepeophtheirus salmonis*, but also other species including *Caligus elongatus*) which can result in increased infection of wild salmonids that must swim past seacage sites during their migrations (Krkosek et al. 2005, Costello 2006, 2009, Todd 2007). Even though wild salmonids and other marine fish are also reservoirs for sealice (Brooks 2009, Gottesfeld et al. 2009, Penston et al. 2011), the additional infection pressure exerted by salmon farms may increase sea lice burdens on wild fish, possibly resulting in increased morbidity or even mortality in juveniles leaving salmon rivers (Krkosek et al. 2005, Costello 2009) or early river entry in adult fish returning to rivers to

spawn (Wells et al. 2007, Todd 2007). Experimental treatment of wild salmon to remove sealice increased salmon survival by odds ratios of 1.14 – 1.17 in Irish and Norwegian studies, respectively, although meta-analyses by other authors conclude sealice treatments improve wild salmon survival even more (Jones et al. 2015). The ongoing scientific debate regarding the quantitative effect of sea lice infection on wild salmonids emphasises the challenges associated with attempting to quantify the incremental impact of these parasites within wild fish populations already experiencing >95% natural mortality (Jones et al. 2015).

5.4.5 Release assessment

Parasitic copepods occur on a range of marine and freshwater fishes throughout New Zealand (Hine et al. 2000). Different species of copepods exist on various hosts in different parts of the country, and the identity and distribution of many species is probably not known at this time. *Caligus elongatus* is a host generalist which has been problematic in salmonid aquaculture in the northern hemisphere, and could threaten a wide range of hosts in sea cage culture (Todd 2007) as it has been found on over 80 different hosts (Kabata 1979, Todd 2007, Oines and Heuch 2007). *Caligus elongatus* has been found in the South Island of NZ in the Heathcote Estuary, Christchurch on flounder *Rhombosolea* spp. (Jones 1988, Hine et al. 2000), but has not been reported on cultured salmon in New Zealand to date. *Caligus longicaudatus* was found on sockeye salmon (*O. nerka*) reared in seacages in New Zealand (Jones 1988), but chinook salmon in nearby seacages were not affected (Boustead 1989). Indeed, chinook salmon are relatively resistant to sea lice (*Lepeophtheirus salmonis*) infection compared to Atlantic salmon, but are not as resistant as coho salmon (Johnson and Albright 1992a). Host resistance to sealice infection is due to both innate genetic factors as well as immunological competence (Johnson and Albright 1992b, MacKinnon 1998, Glover et al. 2001). Another notable species of *Caligus* that is present in New Zealand is *Caligus epidemicus*, which has been recorded on flounder (*Rhombosolea leporina*) in northern New Zealand (Hine et al. 2000). *Caligus epidemicus* has caused mortality in wild fishes (Hewitt 1971), and is an important disease agent in aquaculture of several fish species. For example, one yellowfin bream (*Acanthopagrus australis*) held in experimental seacages was infected by over 6000 *C. epidemicus* (see Roubal 1994). Similarly, a single surgeonfish in the Philippines was recorded to have been infected by 5000 *C. epidemicus* (see Ho et al. 2004), while in Taiwan, heavy infections by *C. epidemicus* resulted in mass mortalities of cultured Tilapia (Lin et al. 1996). *Caligus epidemicus* is known to infect a broad range of hosts (see Hewitt 1971, Byrnes 1987, Roubal 1994, Hallett and Roubal 1995, Venmathi Maran et al. 2009), but to date it has not been recorded from the South Island of New Zealand (Hine et al. 2000).

The lack of evidence of sea lice infection in chinook salmon in New Zealand after many years of culturing these fish at high densities demonstrates that chinook salmon are resistant to infection by endemic species of sea lice. However, two species of sea lice that have been recorded in flatfish in New Zealand, namely *C. elongatus* and *C. epidemicus*, are known to have low host specificity, and this may mean that chinook salmon could be susceptible to infection by these parasites in the future at some stage if they were to become exposed to them and host switching occurred. The water temperatures in the Marlborough Sounds (annual range 10 – 19°C) may be too cold for *C. epidemicus* at this time, as this species is usually found in tropical and warm temperate regions. On the other hand, *Caligus elongatus*

has already been recorded from the South Island (Jones 1988). However, it appears that this parasite has not been problematic in culture of chinook salmon elsewhere (Jackson et al. 2000), and it is not generally found on wild chinook salmon in the northern hemisphere either (Gottesfeld et al. 2009), which reinforces the empirical evidence that chinook salmon in New Zealand do not appear susceptible to *C. elongatus* at this time. However, host switching by caligids onto new hosts is known to occur (Molinet et al. 2011), and one possible mechanism that could encourage this process is increased use of artificial lighting to delay onset of maturation of seacaged salmon (Unwin et al. 2005). The copepodid infective stage of caligid copepods is photopositive (Heuch et al. 1995, Genna et al. 2005), hence use of artificial lighting tends to attract them and increase the number of encounters between copepodids and caged salmon (Hevroy et al. 2003), potentially increasing the risk of host switching. Taking into account that sea lice have not been observed in cultured chinook salmon at this time, but acknowledging that sea lice species known to be problematic in seacage aquaculture elsewhere are known to occur in marine waters of the South Island, and increased intensity of sea cage salmon farming in the Marlborough Sounds could trigger host switching due to increased host density (Krkosek 2010) and/or increased use of artificial lighting, the likelihood estimation for salmon becoming infected by sea lice in seacages in the Marlborough Sounds is non-negligible and is considered to be **Low**.

5.4.6 Exposure assessment

Marine teleosts throughout New Zealand are already at risk of exposure to endemic caligid parasites. They naturally infect wild fishes and a few species of copepods cause disease, usually in circumstances where environmental conditions are favourable for their multiplication on the host. Infection and establishment in wild fish would occur only if sufficient quantities of infective copepodid stages (i.e. an infective dose) were introduced into an area where susceptible hosts were present. However, copepod infections can become established if susceptible hosts are exposed even to only one viable copepodid larvae (B.K. Diggles, personal observations), although in the natural environment several factors will influence infectivity. For example, the infectivity of copepodids of *C. epidemicus* increased with increasing copepodid density and varied with the age of the copepodid, peaking after 3 or 4 days post hatching at 26 or 19°C, respectively, then declining over time (Hallett and Roubal 1995). Further, some fish appeared refractory to infection, while other individuals of the same host species were extremely susceptible to infection, resulting in an overdispersed distribution typical of that seen in many parasite/host relationships (Hallett and Roubal 1995).

Both empirical measurements and models have been used to estimate the additional infection pressure potentially exerted by marine farms containing salmon infected with sea-lice (mainly *L. salmonis* and *C. elongatus*). As the lifespan of the planktonic nauplii and infective copepodid stages of sea lice can be as long as 14 days at 10°C (Johnson and Albright 1991), significant transport and dispersion with surface currents is possible (Amundrud and Murray 2009, Brooks 2009). Some models suggest that sea lice infection pressure near infected marine farms can be 2 to 4 orders of magnitude higher than ambient background levels, and can exceed background levels at least 30 km from infected farms (Krkosek et al. 2005), and possibly up to 45 km (Johnsen et al. 2016). Dispersal distances of larvae of other marine species in relation to the range of typical coastal ocean current conditions suggested that sea lice larvae may disperse an average of 27 km (11–45 km range) over 5–15 days, depending on current velocity

(Costello 2006). Based on data from Johnson and Albright (1991), copepodids suffer mortality at an average rate of 1.0 - 2.9% per hour in seawater, depending on temperature and salinity (Stein et al. 2005, Bricknell et al. 2006), so while some infective stages can survive for long periods under optimal conditions, infection pressure still decreases rather rapidly with increasing distance from an infected marine farm (Amundrud and Murray 2009).

If chinook salmon in seacages did become infected with sea lice via host switching, there is a direct pathway for sea lice infective stages originating from infected salmon to enter the marine environment and infect wild fishes close to salmon farms, but also possibly up to 45 km away, depending on currents at the farm and a myriad of other factors (Krkosek et al. 2005, Amundrud and Murray 2009, Brooks 2009, Johnsen et al. 2016). Acknowledging the empirical evidence demonstrating that sea lice infections have not occurred in cultured chinook salmon in New Zealand at this time, but noting that some sea lice species are able to infect a broad range of susceptible hosts, and that host switching due to increased host density (Krkosek 2010) may be facilitated by activities such as artificial lighting and/or if the intensity of sea cage salmon farming in the Marlborough Sounds is increased in the future, the risk of exposure and establishment is non-negligible, and the likelihood of exposure of wild fish populations to sea lice is considered to be **Moderate**.

5.4.7 Consequence assessment

Many species of sea lice are already present in New Zealand's marine environment. All size classes of juvenile and adult fish can become infected with caligid copepods, and infections of some species with low host specificity, such as *C. epidemicus*, can have negative impacts on the health of individual fish and their populations in the wild, but only under extraordinary circumstances (Hewitt 1971). However, *C. epidemicus* does not occur in the South Island, and at this time water temperatures are likely to be too cold for it to become established there, though water temperatures may increase in the future consistent with global trends, and this needs to be taken into account. The potential for host switching to occur if a threshold intensity of fish farming is reached (Krkosek 2010) also needs to be considered. There is evidence that chinook salmon have established self sustaining populations in the Clarence and Wairou Rivers (N. Boustead, personal communication), which may indicate that establishment of sealice infections on cultured chinook salmon could result in interactions with migrations of wild salmon through the Marlborough Sounds region, although the extent of these potential interactions would be difficult to quantify. Because sea lice that infect seacaged salmonids only tend to occur at subclinical levels in wild non-salmonids (Jones et al. 2006a, 2006b), localized increases in prevalence and/or intensity of sea lice infections in wild marine fish near affected salmon farms are unlikely to have significant impacts on wild fish populations. No copepod parasites are listed by the OIE or in New Zealand's national reportable disease list, hence their presence is unlikely to have adverse impacts on trade. Considering all of these factors, establishment of sea lice in sea caged salmon would have mild biological consequences for wild fishes, and/or may cause some environmental effects, which would not be serious or irreversible. It is therefore estimated that the environmental consequences of introduction of sea lice via salmon in sea cages in the Marlborough Sounds would be **Low**.

5.4.8 Risk estimation

The unrestricted risk associated with sea lice infections is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Tables 6, 7). The unrestricted risk estimate for sea lice does not exceed the ALOP, suggesting that additional risk management for these disease agents is not required at this time.

Risk estimate for sea lice

Commodity type	**Seacaged salmon**
Combined likelihood of release and exposure	Low
Consequences of establishment and spread	Low
Risk estimation	Very Low Risk

5.5 Whirling Disease

5.5.1 Aetiologic agent: *Myxobolus cerebralis*, a myxosporean parasite of salmonid fishes.

5.5.2 OIE List: No **Reportable disease in New Zealand:** Yes

5.5.3 New Zealand's status: *Myxobolus cerebralis* has been reported from several species of salmonids in the South Island (Boustead 1993, 1996), but does not appear to have been recorded from the North Island (Anderson 1996, Diggles et al. 2002).

5.5.4 Epidemiology

Myxosporeans are economically important histozoic and coelozoic endoparasites which have adversely affected the culture of freshwater and marine fishes worldwide (Alvarez-Pellitero and Sitja-Bobadilla 1993, Moran et al. 1999a). The higher taxonomy of the group has been controversial in the past but the link between myxosporeans and cnidarians within basal metazoa has now been confirmed (Smothers et al. 1994, Holland et al. 2010), and it appears that myxosporeans are highly specialised parasitic cnidarians. The myxosporean parasite *Myxobolus cerebralis* infects cartilage of the skeletal system, including the cranium, affecting the auditory and nervous systems resulting in neurological changes and tail chasing behaviour in clinically diseased fish (particularly rainbow trout *Oncorhynchus mykiss*), resulting in what is termed whirling disease (Bartholomew and Reno 2002).

First reported in Germany in rainbow trout (*O. mykiss*) and brook trout (*Salvelinus fontinalis*) in the late 19th century, *M. cerebralis* has since been documented in temperate freshwater ecosystems around most of the world (Bartholomew and Reno 2002). The parasite is likely to have originated from European brown trout (*Salmo trutta*), which are resistant to whirling disease and are known sub-clinical carriers of *M. cerebralis*. In contrast, both wild and cultured salmonids in North America have suffered significant disease outbreaks since the parasite was first documented in the United States in Pennsylvania in 1956 (Bartholomew and Reno 2002). *Myxobolus cerebralis* is thought to have been transported to North America in the 1950s in either live brown trout imported into hatcheries as broodstock, or in frozen trout products imported from Europe and introduced into local waterways as bait or fishfeed (Nickum 1999, Bartholomew and Reno 2002). Since the original introduction, *M. cerebralis* has since spread through much of the United States through stocking of infected fingerlings into uninfected waterways (Bartholomew and Reno 2002), and angler activities (Budy et al. 2003, Gates et al. 2008, 2009). How and when *M. cerebralis* was introduced into New Zealand is not clear, however it was first detected in New Zealand at a trout hatchery near Dunedin in 1971 (Hewitt and Little 1972), and was thought to have been present many years before that, perhaps as early as 1952 (Hewitt and Little 1972, Boustead 1993, Bartholomew and Reno 2002). Since then *M. cerebralis* has been found in wild and cultured salmonids at several locations in the South Island in both clinically diseased rainbow trout as well as clinically healthy salmonids that were sampled for research or export certification (Hewitt 1972, Knowles 1992, Boustead 1993, 1996, Anderson 1993, 1995, 1996, 1997). It appears that *M. cerebralis* has not been recorded in the North Island (Anderson 1996, Diggles et al. 2002).

The lifecycle of *M. cerebralis* is indirect and requires tubificid oligochaetes as an intermediate host (Markiw and Wolf 1983, Wolf and Markiw 1984). Myxosporean triactinomyxon infective stages can be disseminated via translocation of oligochaete worms (Lowers and Bartholomew 2003, Hallett et al. 2006), and in regions where *M. cerebralis* has been introduced, sites with highest angler activity tend to have the highest prevalences of the parasite (Budy et al. 2003). Indeed, transfer of spores or other infective stages of the parasite via soil or other material lodged in fishing boots, waders or other angling equipment is a likely source of unexpected spread of the parasite through angler activity (Gates et al. 2008, 2009). *Myxobolus cerebralis* spores can tolerate freezing at -20 °C for a week (Arsan and Bartholomew 2008), to 3 months (El-Matbouli and Hoffmann 1991), while the triactinomyxon infective stages can be spread via translocation of oligochaetes (Hallett et al. 2006), hence imported salmon products, tubificids imported as ornamental fish food, and translocation of spores on angling equipment such as waders could all have been potential mechanisms by which the disease agent was introduced into New Zealand (Bartholomew and Reno 2002). *Myxobolus cerebralis* infects salmonids only and has not been known to infect any other groups of fishes (Anderson 1993).

5.5.5 Release assessment

Myxobolus cerebralis has been introduced and has become established in New Zealand. The parasite has been found in a range of salmonid species, including rainbow trout (*O. mykiss*), brown trout (*S. trutta*), brook trout (*S. fontinalis*), chinook salmon (*O. tshawytscha*) and Sockeye salmon (*O. nerka*) (see Boustead 1993). The tubificid oligochaetes that are suitable intermediate hosts for the parasite are ubiquitous in the aquatic freshwater environment, and because of this the disease agent occurs naturally in freshwater aquatic environments in several places in the South Island (Boustead 1993, Anderson 1996). As salmonids age their susceptibility to infection by *M. cerebralis* reduces markedly due to several factors, particularly the degree of ossification of cranial cartilage (Markiw 1992, Anderson 1993). Use of bore water and concrete raceways can significantly reduce the chances of exposure of young salmon to the disease agent (Knowles 1992, Anderson 1997). However, juvenile chinook salmon reared in freshwater in concrete raceways can still be exposed to infective stages of the parasite via their water supply and have become infected at very low prevalences and intensities in New Zealand (Boustead 1993, 1996), although clinical whirling disease has never been recorded in this species in New Zealand (Boustead 1993, 1996, Anderson 1996, 1997). Taking into account that *M. cerebralis* is rarely detected in cultured chinook salmon fingerlings, the likelihood estimation for salmon infected by *M. cerebralis* occurring in seacages in the Marlborough Sounds is considered to be **Low**.

5.5.6 Exposure assessment

Salmonids in freshwaters throughout the South Island of New Zealand are already at risk of exposure to *M. cerebralis* through natural pathways. However marine fishes are not susceptible to this disease agent, nor are the freshwater tubificid intermediate hosts required to complete the lifecycle likely to be present in the marine environment under salmon farms. However, it is possible that chinook salmon infected with *M. cerebralis* could escape from seacages, survive and re-enter freshwater, potentially allowing the opportunity for the lifecycle to be completed. There is evidence that chinook salmon have established self sustaining populations in the Clarence and Wairou Rivers, and some of these fish may

have originated as escapees from seacages (N. Boustead, personal communication). Because of this, even though the prevalence and intensity of *M. cerebralis* infections in chinook salmon smolt is very low (Boustead 1993, 1996), the risk of exposure and establishment of *M. cerebralis* in the environment of the Marlborough Sounds via introduction of live salmon cultured in seacages is non-negligible, and the likelihood of exposure and establishment of wild fish populations to *M. cerebralis* in adjacent freshwater rivers is considered to be **Extremely Low**.

5.5.7 Consequence assessment

Myxobolus cerebralis is already present in several locations in the South Island of New Zealand. This disease agent only infects salmonids and has caused clinical disease in New Zealand only on rare occasions in rainbow trout reared in earth or gravel ponds (Anderson 1997). It is possible that some of the chinook salmon in the Clarence and Wairou Rivers may have originated from salmon farms (N. Boustead, personal communication), which indicates that a potential pathway exists which could allow completion of the lifecycle of *M. cerebralis* if infected fish were released into seacages and subsequently escaped. However the likelihood of this pathway being successfully completed would appear remote, and needs to be measured against the significant risks of transfer of spores or other infective stages of the parasite via angling activity (Gates et al. 2008, 2009). This parasite is not listed by the OIE, though it remains a reportable disease in New Zealand. Considering all of these factors, transfer of *M. cerebralis* into the marine environment with sea caged salmon would have mild biological consequences for wild salmonids, and/or may cause minor environmental effects, which would not be serious or irreversible. It is therefore estimated that the environmental consequences of introduction of *M. cerebralis* via salmon in sea cages in the Marlborough Sounds would be **Very Low**.

5.5.8 Risk estimation

The unrestricted risk associated with whirling disease is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Tables 6, 7). The unrestricted risk estimate for whirling disease does not exceed the ALOP, suggesting that additional risk management for this disease agent is not required at this time.

Risk estimate for whirling disease

Commodity type	Seacaged salmon
Combined likelihood of release and exposure	Extremely Low
Consequences of establishment and spread	Very Low
Risk estimation	Negligible Risk

6.0 Recommendations for disease risk mitigation

6.1 Mitigating risks posed by infection with *Piscirickettsia*-like bacteria

This qualitative risk analysis has determined that the only disease of concern in cultured salmon that requires additional risk mitigation at this time is infection with *Piscirickettsia*-like bacteria (NZ-RLO) when it occurs in clinically diseased salmon. The emergence of this disease in farmed salmon in New Zealand in recent years confirms observations from overseas that new diseases tend to originate from wild fish populations, but emergence is generally only observed first in farmed fish populations (Jones et al. 2015). Because of this, maintenance of a high health status in farmed fish reduces both their susceptibility to putative pathogens carried by wild fish populations, and also reduces the risk of possible subsequent back spill of the same disease agents into wild fish populations (Jones et al. 2015).

Given that this analysis suggests the environmental risks only exceed the ALOP if populations of clinically diseased salmon are held in seacages, mitigation of the risk of disease outbreaks due to *Piscirickettsia*-like bacteria is important. As for other bacterial diseases, use of best practice husbandry methods to control known risk factors can reduce the liklihood of *Piscirickettsia* disease outbreaks. Perhaps the most important risk factor is water quality, which needs to be optimized via site selection and farm management to maximize the immune competence of the fish, which is especially important as NZ-RLO infected fish may not become clinically diseased if they are not stressed or infected with other pathogens (DAFF 2013). In the case of salmonids, this means maintaining dissolved oxygen levels above 6 mg/L (preferably above 6.5 mg/L), reducing temperature and salinity fluctuations and avoiding temperature extremes and exposure to pollutants (Ellard 2015). It would be reasonably expected that all these water quality objectives would more likely be achieveable at farm sites with higher water flows.

Other best practice risk reduction methods which should be employed to mitigate risks posed by *Piscirickettsia*-like bacteria include using broodstock that have never been exposed to seawater, rearing fish at optimal stocking densities, allowing farms in a given region to fallow at regular intervals, controlling ectoparasites that may act as vectors, and avoiding horizontal transmission between year classes by holding single year classes of fish at any given site (Fryer and Hedrick 2003). Of these best practices, regular rotational fallowing and single year class farming within each farm management area are not explicitly addressed by the current biosecurity management plan for NZ King Salmon (NZ King Salmon 2016). For more on these points, see section 6.2 below. Broodstock management which prevents use of broodstock fish originating from seawater is also not explicitly addressed in the current biosecurity management plan, although this policy is reportedly in place (Colin Johnston, personal communication 25 May 2016).

Obviously, this analysis has necessarily been based on those diseases that occur in New Zealand salmon at this point in time. Some of the disease agents of concern which presently do not occur in the South Island of New Zealand (e.g. sea lice *Caligus epidemicus*) may extend their range into the Marlborough Sounds in the future if current global warming trends continue as projected. The risk of host switching of other sea lice (e.g. *C. elongatus*) or emergence of other endemic diseases (e.g. *Kudoa* spp.) vectored by native marine fishes if a threshold intensity of chinook salmon farming is reached (Krkosek 2010)

also needs to be considered when planning for this industry. Furthermore, there have been many instances of disease emergence in finfish aquaculture around the world that have occurred due to lapses in biosecurity. Although New Zealand's biosecurity arrangements are amongst the best in the world, there have been several biosecurity leaks in recent years that have allowed exotic pests and diseases to establish in New Zealand waters (Smith et al. 2003, Stuart 2004, Kilroy et al. 2009, Lane et al. 2016). These examples demonstrate that a risk remains and exotic diseases could be introduced, and/or new endemic diseases could emerge in salmon aquaculture in New Zealand at some time in the future. Because of this, it is important to ensure that biosecurity planning is integral to management of the salmon farming industry in order to firstly avoid disease problems, and in a worse case scenario, to be able to effectively manage any new problems that may emerge (Munro et al. 2003, Murray and Peeler 2005, Gustafson et al. 2005, 2007, Kibenge et al. 2009, Mardones et al. 2009). As detailed above, the biosecurity protocols listed in the current biosecurity management plan for NZ King Salmon (NZ King Salmon 2016) effectively mitigate most, but not all, of the risks related to management of diseases such as the NZ-RLO. Effective disease surveillance is also an important activity that can help reduce the risks of establishment of new diseases, and the risks to the industry can be further mitigated through implementation of worlds best practice biosecurity management arrangements for finfish seacage farming. Some of the best practice management arrangements used in salmon farming to minimise disease risks are discussed in more detail in the sections below.

6.2 Number and location of seacages

Number of seacages: Density of hosts is one of the most significant epidemiological factors that drives disease emergence in aquaculture (Murray and Peeler 2005, Krkosek 2010). It is important, therefore, to ensure that the salmon farming industry in New Zealand is allowed access to sufficient farming area to maintain optimal stocking densities and minimise the likelihood of overstocking of individual seacages. It is also very important for the industry to have access to additional suitable sites to permit best practice biosecurity procedures such as regular rotational site fallowing. Furthermore, allowing access to sufficient farm sites provides several advantages that can minimise the risk of disease emergence and maximise the ability to control disease outbreaks, including the ability to operate independent farm management areas, and if sufficient farm sites are available, the flexibility to undertake year class farming.

Location of seacages: Site selection is critical to maintenance of high health status in seacage cultured fish. The restricted distribution of disease associated with the NZ-RLO (which historically has mainly been problematic at the low flow site at Waihinau Bay), suggests that environmental conditions at that site are more permissive for infection to occur and disease to emerge than at other sites, especially during summer when salmon may be stressed by high water temperatures. The proposed deletion of low flow, suboptimal farming sites such as Waihinau Bay and replacement of them with high flow sites would minimise risks of emergence of both infectious and non-infectious diseases and thus better allow the salmon farming industry to minimize risks to the environment posed by diseases of salmon.

6.2.1 Independent farm management areas

An independent farm management area can be defined as one or more farm sites that occur within a particular geographical area (a defined area) that share one or more characteristics of epidemiological significance, such as movements of water (e.g. tidal excursion), wild fish, aquaculture stock, equipment and staff. The size of a farm management area depends on many factors relating to hydrodynamic variables of water movements, dispersal dynamics of infective agents, movements of wild fish, movements of stock, staff, equipment and so on (McClure et al. 2005, Chambers and Ernst 2005, Gustafson et al. 2007, Dempster et al. 2009). Adoption of independent farm management areas allows development of integrated biosecurity and pest management strategies that can be optimised for maximum effectiveness (Chambers and Ernst 2005, Brooks 2009, Wilson et al. 2009, Snow 2011). The dispersal dynamics of sea lice, and both ISA-like (a labile virus) and IPN-like (a robust virus) viruses will be used here as examples for the purposes of exploring the utility of independent farm management areas for mitigating disease risks.

Empirical evidence and modelling from the northern hemisphere has shown that the risk of infection with viruses such as IPNV and ISAV increases significantly when non-infected salmon farms are within a 5 km distance of an infected salmon farm (Jarp and Karlsen 1997, Scheel et al. 2007, Wallace et al. 2008). For metazoans such as sea lice, the risk of infection is increased above background levels at least 8 to 18 km from lice infected salmon farms (Brooks 2009, Penston et al. 2011), and the maximum distance where increased infection pressure has been observed or modelled is around 30-45 km (Krkosek et al. 2005, Amundrud and Murray 2009, Penston et al. 2011, Johnsen et al. 2016). The markedly reduced infection pressure with distance associated with the viruses compared to the sea lice is likely to be due to the viruses requiring a certain minimum infectious dose before they are transmitted, compared to the sealice in which one infective stage is sufficient to cause infection. Furthermore, sealice infective stages are robust and survive for long periods in the plankton, and indeed they are non-infective for several days prior to moulting into the infective copepodid stage (Murray and Gillibrand 2006, Amundrud and Murray 2009). Taking these figures on dispersal dynamics as representative for disease agents that may emerge in New Zealand salmon culture in the future, and taking into account hydrodynamic conditions at the various existing salmon farming sites in the Marlborough Sounds (Gillespie et al. 2011), (which are similar in many aspects to hydrodynamic conditions found in lochs and fiords where sealice and viral diseases have been problematic elsewhere, see Murray and Gillibrand 2006, Gillibrand and Amundrud 2007), the following can be concluded:

The existing farm sites in Queen Charlotte Sound and Tory Channel (Clay Point, Te Pangu, Ngamahau, Ruakaka Bay, Otanerau Bay) are considered to be within an area sufficiently connected by water and wild fish movements to constitute an individual farm management area (Tory Channel Management Area). This assessment would appear to be consistent with the outcomes of the hydrodynamic analyses for Queen Charlotte Sound/Tory Channel done by MAF Biosecurity (2011a).

The existing farm sites at Waitata, Waihinau Bay, Richmond and Forsyth Bay are considered to be within an area sufficiently connected by water and wild fish movements to constitute an individual farm management area (Outer Pelorus Sound Management Area). This assessment would appear to be

consistent with the outcomes of the analysis for Waitata Reach done by MAF Biosecurity (2011a). The proposed relocation of salmon farm sites including deletion of low flow sites at Ruakaka Bay, Otanerau, Waihinau Bay and Forsyth Bay and implementing new sites at high flow locations at potential sites 42, 47, 82 and 156 at Tio Point in Tory Channel and potential sites 34, 106, 122, 124 and 125 in Outer Pelorus Sound would therefore allow the salmon farming industry in the Marlborough Sounds to develop 2 farm management areas that are epidemiologically independent of each other based on hydrodynamic principals.

6.2.2 Year class farming and site fallowing

Year class farming and site fallowing are methods of farm management used to control some of the risk factors associated with disease emergence and persistence in seacage aquaculture (Stewart 1998). The presence of multiple year classes of fish on any given site can allow disease agents to persist for long periods on site because holdover fish provide an avenue for transfer of pathogens between year classes (Gustafson et al. 2007). Management arrangements that allow only one year class of fish to be held in any given individual farm management area significantly reduce the risk of persistence and spread of disease agents (Gustafson et al. 2007, Brooks 2009). Because of this, year class farming is generally acknowledged to be worlds best practice for salmon farming. Furthermore, fallowing of seacage sites is often useful to reduce or eliminate residual infection pressure for disease agents such as viruses and parasites by removing their hosts (Gustafson et al. 2007, Penston et al. 2011). There is also evidence that fallowing can assist with mitigation of bacterial diseases (Stewart 1998) and indeed, fallowing can reduce the risk of emergence of new diseases which otherwise could adapt (switch) to new hosts if they are allowed to co-exist with them for long uninterrupted periods (Snow 2011). Synchronised fallowing within farm management areas has become compulsory in some salmon farming areas for these very reasons (Chang et al. 2007).

The proposed planning changes provide for deletion of 6 low flow farm sites and their replacement with high flow farm sites. If all proposed high flow sites were approved, this would provide up to 7 farm sites per farm management area. Assuming the total number of active farms remains the same as present in each area, this arrangement could allow the option to schedule regular (one year out of every 6 years) rotational site fallowing within each management area, which would slightly reduce the risk of disease emergence compared to a situation with no fallowing at all. The fact that chinook salmon reach market size within 18 months to 2 years after introduction into seacages, suggests that the proposed changes are an incremental improvement, but are not sufficient to allow worlds best practice which would entail regular fallowing combined with complete year class farming with only one year class in each independent farm management area at any given time. Worlds best practice could probably only be practically implemented if there were 3 epidemiologically independent farm management areas, each with sufficient farm sites to allow fallowing of all farms within each management area every third year. In view of current global warming trends which are likely to increase disease risks to the industry over time, the ideal situation of 3 independent farm management areas with compulsory fallowing of an area every 3rd year should be considered in future planning arrangements for the industry.

6.3 Buffer zones surrounding farming areas

Together with establishment of independent farm management areas, best practice management of the salmon aquaculture industry in the Marlborough Sounds should also include establishment of salmon farming-free buffer zones surrounding each of the independent farm management areas, to ensure their epidemiological independence. Buffer zones are extremely useful because they are an important form of prevention in that they reduce the connectivity between different farming areas that otherwise may occur through movements of water, shipping and other potential vectors such as wild fishes (Dempster et al. 2009). Buffer zones also allow integrated pest management strategies to be successfully implemented in each independent farm management area during disease outbreaks (McClure et al. 2005, Chambers and Ernst 2005, Gustafson et al. 2007). The widths of buffer zones should be defined based on epidemiological criteria, particularly those relating to movements of water (e.g. tidal excursion) and the dispersal dynamics (particularly the longevity of infective stages) of likely disease agents (Chambers and Ernst 2005, Gustafson et al. 2007). Again, the dispersal dynamics of sea lice, and ISA-like and IPN-like viruses will be used as examples of the types of metazoan and microbial disease agents that could emerge in New Zealand salmonid culture at some stage in the future.

As mentioned above, empirical evidence and modelling from the northern hemisphere has shown that the risk of infection with viruses such as IPNV and ISAV increases significantly when non-infected salmon farms are within 5 km of an infected salmon farm (Jarp and Karlsen 1997, Scheel et al. 2007, Wallace et al. 2008). Because of this, (and assuming similar hydrodynamic conditions between the Marlborough Sounds and salmon farming areas in other parts of the world, see Murray and Gillibrand 2006, Gillibrand and Amundrud 2007, Gillespie et al. 2011), a minimum 5 km buffer zone from the edge of one farm management area to the edge of the next farm management area would appear necessary to ensure their independence in the event of a viral disease outbreak. However, for metazoan parasites such as sea lice, the buffer zone may need to be much larger to be effective. For sealice, the risk of infection is increased above background levels at least 18 km from lice infected salmon farms (Brooks 2009), and the maximum distance where increased infection pressure has been observed or modelled is around 30-45 km (Krkosek et al. 2005, Costello 2006, Amundrud and Murray 2009, Penston et al. 2011, Johnsen et al. 2016). This suggests that the width of an ideal on-water buffer zone ("as the fish swims", not "as the crow flies") to ensure true independence of salmon farming management areas for all known disease agents of salmon would be between 18 and 45 km from the nearest farm in one farm management area to the nearest farm in an adjacent farm management area.

The proposed locations of the new high flow farm sites and deletion of the low flow farm sites would potentially increase connectivity between individual farms within each farm management area, but would also increase the width of the existing buffer zone between the two farm management areas to in excess of the ideal minimum of 45 km.

6.4 Harvesting

Harvesting is another process that presents an increased risk of disease transmission. Indeed, the spread of ISA in Scotland, and Norway (Murray et al. 2002, Munro et al. 2003, Thorud and Hastein 2003) and

also Canada (Gustafson et al. 2005) was associated with spread of effluent from well boats used for harvesting, and/or from processing plants. A brief summary of the best practice arrangements used for both of these activities is included below.

6.4.1 Harvesting method

There have been instances of spread of viral disease agents such as ISA during the harvesting process when live fish are transported from farming areas to centralised processing facilities in well boats (Murray et al. 2002). Munro et al. (2003) evaluated the relative risks of spread of ISA associated with various different harvesting methods commonly used in Scotland. The highest risks of transmission to neighbouring farms was likely to occur when live fish in seacages were towed to centralised processing plants (Munro et al. 2003). Any harvesting methods that could allow escape of live fish were also considered to be higher risk. In contrast, the harvesting methods currently used by NZ King Salmon (rested slaughter at the cage site using anaesthetic followed by bleeding into an ice slurry in a harvest tub and transport of harvest tubs by barge to the wharf for road transport to landbased processing plants) were considered to represent a moderate risk of spread of disease to neighbouring farms, but the lowest risk of spread of disease en-route to the processing plant (Munro et al. 2003).

The current harvesting methods used by NZ King Salmon are therefore compatible with the process of establishment of independent farm management areas, provided that there is no movement of harvest barges from one farm management area to another. This would be accommodated by ensuring in the biosecurity management plan that all fish harvested from the Tory Channel Management Area are landed only in Picton, and that separate barges and equipment are used to service the Outer Pelorus Sound Management Area and that salmon from there are only landed at Havelock. Such an arrangement would be consistent with worlds best practice management for this activity from both minimisation of disease risk and maximisation of product quality and animal welfare (Gregory 2008, Tuckey et al. 2009, 2010).

6.4.2 Processing premises

Spread of viral diseases such as ISA has been documented where several companies within a salmon farming area utilise a central processing premise (Munro et al. 2003). The increased risk of disease transmission under these circumstances is associated with sharing of contaminated harvesting and processing equipment such as well boats, harvest barges, grading equipment and harvesting tubs, as well as activities such as transport of live fish to the vicinity of the processing plant (Munro et al. 2003). The highest risks were associated with holding live fish in cages adjacent to processing plants, and discharge of untreated effluent from processing plants (Munro et al. 2003).

NZ King Salmon is a vertically integrated company that utilises its own landbased processing plants with no discharge of effluent back into the sea. NZ King Salmon also does not share harvesting barges, tubs, and other transport equipment with other salmon farming companies. Because of this, NZ King

Salmon is in a good position to minimise risk of cross contamination of equipment during harvest, transport and processing.

7.0 Conclusions

This risk analysis found that infection with *Piscirickettsia*-like bacteria (NZ-RLO) in clinically diseased chinook salmon held in seacages represents the only disease of concern in cultured salmon that requires additional risk mitigation measures at this time. The emergence of this disease in salmon farmed at the low flow site in Waihinau Bay in Pelorus Sound demonstrates the increased risk of disease emergence at farm sites where environmental conditions are suboptimal. Thus, the proposed planning changes to relocate several salmon farms situated in low water flow sites in the Marlborough Sounds to high water flow sites is likely to reduce risks to the environment that presently exist due to infection with *Piscirickettsia*-like bacteria (NZ-RLO) in clinically diseased salmon.

However, this assessment by necessity was based on the diseases of salmon presently known to occur in New Zealand at this point in time. Given that new diseases can emerge and biosecurity leaks can occur, we cannot assume that the disease status of chinook salmon in New Zealand will not change at some stage in the future. Because of this, it is notable that the proposed deletion of low flow, suboptimal farming sites and replacing them with high flow sites would better allow the salmon farming industry to minimize risks to the environment and industry development posed by diseases of salmon. The proposed changes, if approved, would allow the industry to mitigate several disease risk factors that contribute to emergence of infectious diseases, provide an option to implement regular farm fallowing (if all proposed high flow sites were approved), while permitting establishment of 2 epidemiologically independent farm management areas separated by ideal buffer zones. These arrangements would allow the salmon farming industry to enhance its existing biosecurity controls and implement integrated pest management strategies if required.

The farms in the Tory Channel (Clay Point, Te Pangu, and Ngamahau and potential sites 42, 47, 82 and 156 at Tio Point) could be managed as one farm management area (Tory Channel Management Area). The farms proposed for Pelorus Sound (Waitata, Richmond and potential sites 34, 106, 122, 124 and 125) could be managed as a second farm management area (Outer Pelorus Sound Management Area). Given the density of individual farms in each management area remains relatively low by world standards, the increased number of farms within each management area is not a major concern (particularly if any extras sites are used to instigate regular farm fallowing), provided water quality remains optimal (by virtue of improved site selection), on-farm stocking densities remain optimal, and the biosecurity practices outlined in the biosecurity management plan are maintained.

In conclusion, I encourage the deletion of the 6 sub optimal sites and relocation of salmon farms to the proposed high flow locations, not only to reduce risks of outbreaks of NZ-RLO and emergence of other infectious and non-infectious diseases, but also to allow the establishment of ideal on-water buffer zones that will allow utilisation of 2 truly independent farm management areas, and possibly the option for site fallowing within each farm management area.

8.0 References

Altinok I (2004). The infectious route of *Yersinia ruckeri* is affected by salinity. *Bulletin of the European Association of Fish Pathologists* 24: 253-259.

Alvarez-Pellitero P, Sitja-Bobadilla A (1993). Pathology of Myxosporea in marine fish culture. *Diseases of Aquatic Organisms* 17: 229-238.

Amos KH, Gustafson L, Warg J, Whaley J, Purcell M, Rolland J, Winton J, Snekvik K, Meyers T, Stewart B, Kerwin J, Blair M, Bader J, Evered J (2014). U.S. Response to a Report of Infectious Salmon Anemia Virus in Western North America. *Fisheries* 39: 501-506.

Amundrud TL, Murray AG (2009). Modelling sea lice dispersion under varying environmental forcing in a Scottish sea loch. *Journal of Fish Diseases* 32: 27–44.

Anderson CD (1993). Epidemiological aspects of *Myxobolus cerebralis*, the agent of salmonid whirling disease. *Surveillance* 20(2): 19-24.

Anderson CD (1995). Surveillance for fish diseases. *Surveillance* 22(4): 13

Anderson CD (1996). Distribution of salmonid diseases in New Zealand. *Surveillance* 23(4): 23-24.

Anderson CD (1997). Fish facts from New Zealand. *Microbiology Australia* May 1997. 20-21.

Anderson CD (1998). Survey of New Zealand Salmonids for OIE List B viruses. *Surveillance* 25(4): 9-10.

Anderson CD, Knowles G, de Lisle G (1994). A survey for *Yersinia ruckeri* and *Aeromonas salmonicida* in farmed and wild fish. *Surveillance* 2(3): 39-40.

Arkush KD, Mendoza L, Adkison MA, Hedrick RP (2003). Observations on the life stages of *Sphaerothecum destruens* n. g., n. sp., a Mesomycetozoean fish pathogen formerly referred to as the rosette agent. *Journal of Eukaryotic Microbiology* 50: 430–438.

Arsan EL, Bartholomew JL (2008). Potential for dissemination of the nonnative salmonid parasite *Myxobolus cerebralis* in Alaska. *Journal of Aquatic Animal Health* 20: 136-149.

Asche F, Hansen H, Tveteras R, Tveteras S (2010). The Salmon Disease Crisis in Chile. *Marine Resource Economics* 24: 405–411.

Austin B, Austin DA (2007). Bacterial fish pathogens: disease in farmed and wild fish. 4th edition. Springer. 552 pgs.

Bain MB, Cornwell ER, Hope KM, Eckerlin GE, Casey RN, Groocock GH, Getchell RG, Bowser PR, Winton JR, Batts WN, Cangelosi A, Casey JW (2010). Distribution of an invasive aquatic pathogen (Viral Hemorrhagic Septicemia Virus) in the Great Lakes and its relationship to shipping. *PLoS ONE* 5(4): e10156. doi:10.1371/journal.pone.0010156

Bartholomew JL, Reno PW (2002). The history and dissemination of Whirling Disease. *American Fisheries Society Symposium* 29: 3-24.

Benthic Standards Working Group (2014). Best Management Practice guidelines for salmon farms in the Marlborough Sounds: Benthic environmental quality standards and monitoring protocol. Nov. 2014. http://www.kingsalmon.co.nz/kingsalmon/wp-content/uploads/2014/02/Best-practice-guidelines-for-salmon-farm-management-seabed-health-Nov-....pdf

Bermingham ML, Mulcahy MF (2007). *Neoparamoeba* sp. and other protozoans on the gills of Atlantic salmon *Salmo salar* smolts in seawater. *Diseases of Aquatic Organisms* 76: 231–240.

Birkbeck TH, Rennie S, Hunter D, Laidler LA, Wadsworth S (2004). Infectivity of a Scottish isolate of *Piscirickettsia salmonis* for Atlantic salmon *Salmo salar* and immune response of salmon to this agent. *Diseases of Aquatic Organisms* 60: 97–103.

Bisset KA (1948). Seasonal changes in the normal bacterial flora of freshwater fish. *Journal of Hygiene* 46: 94-97.

Boustead NC (1982). Fish diseases recorded in New Zealand, with a discussion on potential sources and certification procedures. MAF Fisheries Research Division Occasional Publication No. 34. 19 pgs.

Boustead NC (1989). A guide to diseases of salmon in New Zealand. New Zealand Freshwater Fisheries Report No. 112. MAFFish, Christchurch. 87 pgs.

Boustead NC (1993). Detection and New Zealand distribution of Myxobolus cerebralis, the cause of whirling disease of salmonids. New Zealand Journal of Marine and Freshwater Research 27: 431-436.

Boustead, NC (1996). New Zealand's experience with Whirling Disease. In: Bergersen, EP & Knopf (eds) Whirling Disease Workshop proceedings. Colorado Cooperative Fish and Wildlife Research Unit. Pgs 78-87.

Bovo G, Hill B, Husby A, Hastein T, Michel C, Olesen NJ, Storset A and Midtlyng P (2005). Work package 3 report: Pathogen survival outside the host, and susceptibility to disinfection. VESO, P.O. Box 8109 Dep., N-0032 Oslo, Norway. Available at: www.crl-fish.eu/upload/sites/eurl-fish/links/fisheggtrade%20wp_3.pdf

Bricknell IR, Dalesman S, O'shea B, Pert CC, Mordue J (2006). The effect of environmental salinity on sea lice (*Lepeophtheirus salmonis*) settlement success. *Diseases of Aquatic Organisms* 71: 201–212.

Bridle AR, Crosbie PB, Cadoret K, Nowak BF (2010). Rapid detection and quantification of *Neoparamoeba perurans* in the marine environment. *Aquaculture* 309: 56–61.

Brooks KM (2009). Considerations in developing an integrated pest management programme for control of sea lice on farmed salmon in Pacific Canada. *Journal of Fish Diseases* 32: 59–73.

Bruno DW, Noguera PA, Black J, Murray W, Macqueen DJ, Matejusova I (2014). Identification of a wild reservoir of salmonid alphavirus in common dab *Limanda limanda*, with emphasis on virus culture and sequencing. *Aquaculture Environment Interactions* 5: 89–98.

Budy P, Thiede GP, de la Hoz EA, Vatland S (2003). Logan River Whirling Disease Study: Factors Affecting Trout Population Dynamics, Abundance, and Distribution in the Logan River, Utah. 2004 Annual Report to Utah Division of Wildlife Resources No. F-47–R. Salt Lake City, UT: Utah Division of Wildlife Resources, 69 pgs.

Burge CA et al. (2016) Complementary approaches to diagnosing marine diseases: a union of the modern and the classic. *Philosophical Transactions of the Royal Society B* 371: 20150207.

Buschmann AH, Riquelme VA, Hernandez-Gonzalez MC, Varela D, Jimenez JE, Henriquez LA, Vergara PA, Guinez R, Filun L (2006). A review of the impacts of salmonid farming on marine coastal ecosystems in the southeast Pacific. *ICES Journal of Marine Science* 63: 1338-1345.

Byrnes T (1987). Caligids (Copepoda: Caligidae) found on the bream (*Acanthopagrus* spp.) of Australia. *Journal of Natural History* 21: 363- 404.

Carson J, Wilson T (2009). Yersinosis in fish. Australian and New Zealand Standard Diagnostic Procedure. http://www.scahls.org.au/__data/assets/pdf_file/0010/1516519/Yersiniosis.pdf

Chambers C, Ernst I (2005). Dispersal of the skin fluke *Benedenia seriolae* (Monogenea: Capsalidae) by tidal currents and implications for seacage farming of *Seriola* spp. *Aquaculture* 250: 60-69.

Chang BD, Page FH, Losier RJ, Lawton P, Singh R, Greenberg DA (2007). Evaluation of bay management area scenarios for the southwestern New Brunswick salmon aquaculture industry: Aquaculture Collaborative Research and Development Program final project report. *Canadian Technical Report of Fisheries and Aquatic Sciences* 2722: 69 pgs.

Chang FH, Anderson C, Boustead NC (1990). First record of *Heterosigma* (Raphidophyceae) bloom with associated mortality of cage reared salmon in Big Glory Bay, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 24: 461-469.

Chang FH, Chiswell SM, Uddstrom MJ (2001). Occurrence and distribution of *Karenia brevisulcata* (Dinophyceae) during the 1998 summer toxic outbreaks on the central east coast of New Zealand. *Phycologia* 40: 215-221.

Clifford MA, Eder KJ, Werner I, Hedrick RP (2005). Synergistic effects of esfenvalerate and infectious hematopoietic necrosis virus on juvenile chinook salmon mortality. *Environmental Toxicology and Chemistry* 24: 1766-1772.

Colquhoun DJ, Duodu S (2011). *Francisella* infections in farmed and wild aquatic organisms. *Veterinary Research* 42: 47

Corbeil S, Hyatt AD, Crane MSJ (2005). Characterisation of an emerging rickettsia-like organism in Tasmanian farmed Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 64: 37-44.

Corbeil S, Crane MSJ (2009). SCAHLS Australian and New Zealand Standard Diagnostic Procedure: *Piscirickettsia salmonis*. . http://www.scahls.org.au/Procedures/Documents/AQANZSDP/Piscirickettsia_salmonis.pdf

Costello MJ (2006). Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology* 22: 475-483.

Costello MJ (2009). How sea lice from salmon farms may cause wild salmonid declines in Europe and North America and be a threat to fishes elsewhere. *Proceedings of the Royal Society B* 276: 3385–3394.

Crane MJ and 8 other authors. (2000). First isolation of an aquatic birnavirus from farmed and wild fish species in Australia. *Diseases of Aquatic Organisms* 43: 1-14.

Crockford M (2007). Partial characterization of pilchard herpesvirus and the associated disease in pilchards. Thesis presented for the degree of Doctor of Philosophy at Murdoch University. 108 pgs.

Crosbie PB, Bridle AR, Leef MJ, Nowak BF (2010). Effects of different batches of *Neoparamoeba perurans* and fish stocking densities on the severity of amoebic gill disease in experimental infection of Atlantic salmon, Salmo salar L. *Aquaculture Research* 41: e505–e516.

DAFF (2013). Disease strategy: Piscirickettsiosis (Version 1). In: Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT.

Dale OB, Orpetveit I, Lyngstad TM, Kahns S, Skall HF, Olesen NJ, Dannevig BH (2009). Outbreak of viral haemorrhagic septicaemia (VHS) in seawater farmed rainbow trout in Norway caused by VHS virus Genotype III. *Diseases of Aquatic Organisms* 85: 93-103.

Davies RL (1990). O-serotyping of *Yersinia ruckeri* with special emphasis on European isolates. *Veterinary Microbiology* 22: 299-307.

Davies KR, McColl KA, Wang LF, Yu M, Williams LM, Crane MJ (2010). Molecular characterisation of Australasian isolates of aquatic birnaviruses. *Diseases of Aquatic Organisms* 93: 1-15.

Dempster T, Uglem I, Sanchez-Jerez P, Fernandez-Jover D, Bayle-Sempere J, Nilsen R, Bjørn PA (2009). Coastal salmon farms attract large and persistent aggregations of wild fish: an ecosystem effect. *Marine Ecology Progress Series* 384: 1-14.

Diamant A (1997). Fish to fish transmission of a marine myxosporean. *Diseases of Aquatic Organisms* 30: 99-105.

Diggles BK (2011). Environmental Assessment Report – Disease Risks. DigsFish Services Report DF11-02. Prepared for NZ King Salmon 5 August 2011.

Diggles BK, Landos M (2010). Australian Aquatic Veterinary Emergency Plan – AQUAVETPLAN Disease Strategy – Viral Haemorrhagic Septicaemia. Version 2.0. Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT. 71 pgs.

Diggles BK, Carson J, Hine PM, Tait M (2000). *Vibrio* species associated with mortalities in hatchery reared turbot (*Colistium nudipinnis*) and brill (*C. guntheri*) in New Zealand. *Aquaculture* 183: 1-12.

Diggles BK, Hine PM, Handley S, Boustead NC (2002). A handbook of diseases of importance to aquaculture in New Zealand. NIWA Science and Technology Series No. 49. 200 pgs.

Douglas-Helders M, Nowak BF, Zilberg D, Carson J (2000). Survival of *Paramoeba pemaquidensis* on dead salmon: implications for management or cage hygiene. *Bulletin of the European Association of Fish Pathologists* 20: 167–169.

Douglas-Helders GM, Dawson DR, Carson J, Nowak BF (2002). Wild fish are not a significant reservoir for *Neoparamoeba pemaquidensis* (Page, 1987). *Journal of Fish Diseases* 25: 569–574.

DPIPWE (2015). Sampling for POMV, TSRV and Tasmanian RLO PCR. Tasmanian Department of Primary Industries, Parks, Water & Environment Animal Health Laboratory.

Dykova I, Figueras A, Peric Z (2000). *Neoparamoeba* Page, 1987: light and electron microscopic observations on six strains of different origin. *Diseases of Aquatic Organisms* 43: 217–223.

Egidus E (1987). Vibriosis: pathogenicity and pathology. A review. *Aquaculture* 67: 15-28.

El-Matbouli M, Hoffmann RW (1991). Effects of freezing, aging, and passage through the alimentary canal of predatory animals on the viability of *Myxobolus cerebralis* spores. *Journal of Aquatic Animal Health* 3: 260–262.

Ellard K (2015). Discussion paper relating to potential infectious disease risks affecting farmed salmonids within Macquarie Harbour. Advisory document to the Tasmanian Chief Veterinary Officer. Tasmanian Department of Primary Industries, Parks, Water & Environment. 17 pgs.

Elston RA, Meyers TR (2009). Effect of viral haemorrhagic septicaemia virus on Pacific herring in Prince William Sound, Alaska, from 1989 to 2005. *Diseases of Aquatic Organisms* 83: 223-246.

Ferguson HW, Delannoy CM, Hay S, Nicolson J, Sutherland D, Crumlish M (2010). Jellyfish as vectors of bacterial disease for farmed salmon (*Salmo salar*). *Journal of Veterinary Diagnostic Investigation* 22: 376–382.

Fletcher WJ, Jones JB, Pearce AF, Hosja W (1997). Environmental and biological aspects of the mass mortality of pilchards (Autumn 1995) in Western Australia. Fisheries Department of WA Fisheries Research Report No. 106. 112 pgs.

Fryer JL, Lannan CN, Garces LH, Larenas JJ, Smith PA (1990). Isolation of a rickettsiales-like organism from diseased coho salmon *Oncorhynchus kisutch* in Chile. *Fish Pathology* 25: 107–114.

Fryer JL, Lannan CN, Giovannoni SJ, Wood ND (1992). *Piscirickettsia salmonis* gen. nov., sp. nov., the causative agent of an epizootic disease in salmonid fishes. *International Journal of Systematic Bacteriology* 42: 120–126.

Fryer JL, Hedrick RP (2003). *Piscirickettsia salmonis*: a Gram-negative intracellular bacterial pathogen of fish, *Journal of Fish Diseases* 26: 251–262.

Gates KK, Guy CS, Zale AV, Horton TB (2008). Adherence of *Myxobolus cerebralis* myxospores to waders: implications for disease dissemination. *North American Journal of Fisheries Management* 28: 1453–1458.

Gates KK, Guy CS, Zale AV, Horton TB (2009). Angler awareness of aquatic nuisance species and potential transport mechanisms. *Fisheries Management and Ecology* 16: 448-456.

Genna RL, Mordue W, Pike AW, Mordue AJ (2005). Light intensity, salinity, and host velocity influence presettlement intensity and distribution on hosts by copepodids of sea lice, *Lepeophtheirus salmonis*. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2675–2682.

Gillespie P, Knight B, MacKenzie L, Cornelisen C (2011). Biophysical water column effects. Draft Cawthron Institute Report prepared for New Zealand King Salmon Ltd. 38 pgs.

Gillibrand PA, Amundrud TL (2007). A three-dimensional model study of the tidal circulation and buoyancy effects in a Scottish fjord: Loch Torridon. *Journal of Geophysical Research – Oceans* 112 (C0), 5030, doi:10.1029/2006JC003806.

Glover KA, Nilsen F, Skaala O, Taggart JB, Teale AJ (2001). Differences in susceptibility to sea lice infection between a sea run and a freshwater resident population of brown trout. *Journal of Fish Biology* 59: 1512-1519.

Gottesfeld AS, Proctor B, Rolston LD, Carr-Harris C (2009). Sea lice, *Lepeophtheirus salmonis*, transfer between wild sympatric adult and juvenile salmon on the north coast of British Columbia, Canada. *Journal of Fish Diseases* 32: 45–57.

Gozlan RE, Whipps CM, Andreou D, Arkush CD (2009). Identification of a rosette-like agent as *Sphaerothecum destruens*, a multi-host fish pathogen. *International Journal for Parasitology* 39: 1055–1058.

Graham DA, Fringuelli E, Wilson C, Rowley HM, Brown A, Rodger H, McLoughlin MF, McManus C, Casey E, McCarthy LJ, Ruane NM (2010). Prospective longitudinal studies of salmonid alphavirus infections on two Atlantic salmon farms in Ireland; evidence for viral persistence. *Journal of Fish Diseases* 33: 123–135.

Gregory NG (2008). Animal welfare at markets and during transport and slaughter. *Meat Science* 80: 2-11.

Grenfell BT, Dobson AP (1995) Ecology of infectious diseases in natural populations. Cambridge University Press, Cambridge. 521 pgs.

Grimnes A, Jakobsen PJ (1996). The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. *Journal of Fish Biology* 48: 1179–1194.

Gustafson LL, Ellis SK, Bartlett CA (2005). Using expert opinion to identify risk factors important to infectious salmon-anemia (ISA) outbreaks on salmon farms in Maine, USA and New Brunswick, Canada. *Preventive Veterinary Medicine* 70: 17–28.

Gustafson LL, Ellis SK, Beattie MJ, Chang BD, Dickey DA, Robinson TL, Marenghi FP, Moffett PJ, Page FH (2007). Hydrographics and the timing of infectious salmon anemia outbreaks among Atlantic salmon (*Salmo salar* L.) farms in the Quoddy region of Maine, USA and New Brunswick, Canada. *Preventive Veterinary Medicine* 78: 35–56.

Hallett SL, Roubal FR (1995). Experiments on the infection dynamics of *Caligus epidemicus* (Copepoda: Caligidae) on the small marine fish, *Ambassis marianus* (Günther). *Journal of Fish Diseases* 18: 59-66.

Hallett SL, Atkinson SD, Erseus C, El-Matbouli M (2006). Myxozoan parasites disseminated via oligochaete worms as live food for aquarium fishes: descriptions of aurantiactinomyxon and raabeia actinospore types. *Diseases of Aquatic Organisms* 69: 213-225.

Håstein T, Gudding R, Evensen O (2005). Bacterial vaccines for fish - an update of the current situation worldwide. *Developments in Biologicals* 121: 55-74.

Hayward CJ, Bott NJ, Nowak BF (2009). Seasonal epizootics of sealice, *Caligus* spp., on southern bluefin tuna , *Thynnus maccoyi* (Castelnau), in a long term farming trial. *Journal of Fish Diseases* 32: 101-106.

Heuch PA, Parsons A, Boxaspen K (1995). Diel vertical migration — a possible host finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 681–689.

Hevroy EM, Boxaspen K, Oppedal F, Taranger GL, Holm JC (2003). The effect of artificial light treatment and depth on the infestation of the sea louse *Lepeophtheirus salmonis* on Atlantic salmon (*Salmo salar* L.) culture. *Aquaculture* 220: 1-14.

Hewitt GC (1963). Some New Zealand parasitic copepoda of the family Caligidae. Transactions of the Royal Society *of New Zealand* 4: 61-115.

Hewitt GC (1971). Two species of *Caligus* (Copepoda, Caligidae) from Australian waters, with a description of some developmental stages. *Pacific Science* 25: 145-164.

Hewitt GC (1972). Survey of New Zealand trout hatcheries for Whirling disease caused by *Myxosoma cerebralis*. *New Zealand Journal of Marine and Freshwater Research* 6: 463-468.

Hewitt GC, Little RW (1972). Whirling disease in New Zealand trout caused by *Myxosoma cerebralis* (Hofer, 1903) (Protozoa: Myxosporidia). *New Zealand Journal of Marine and Freshwater Research* 6: 1-10.

Hine PM (1995). Herpesviruses associated with mortalities among pilchards (*Sardinops neopilchardus*) around New Zealand. *Surveillance* 22(3): 45-48.

Hine PM, Boustead NC (1974). A guide to disease in eel farms. MAF Fisheries Research Division Occasional Publication No. 6. 28 pgs.

Hine PM, Jones JB, Diggles BK (2000). A checklist of parasites of New Zealand fishes, including previously unpublished records. NIWA Technical Report 75. 94 pgs.

Ho JS, Kim IH, Cruz-Lacierda ER, Nagasawa K (2004). Sea lice (Copepoda: Caligidae) parasitic on marine cultured and wild fishes of the Philippines. *Journal of the Fisheries Society of Taiwan* 31: 235-249.

Holland JW, Okamura B, Hartikainen H, Secombes CJ (2010). A novel minicollagen gene links cnidarians and myxozoans. *Proceedings of the Royal Society, Biological Sciences B* 278: 546-553.

House ML, Bartholomew JL, Winton JR, Fryer L (1999). Relative virulence of three isolates of *Piscirickettsia salmonis* for coho salmon, *Oncorhynchus kisutch*. *Diseases of Aquatic Organisms* 35: 107–113.

Isshiki T, Kusuda R (1987). Susceptibility of different marine fish fingerlings to yellowtail ascites virus (YAV). *Fish Pathology* 22: 191-194.

Isshiki T, Nagano T, Suzuki S (2001). Infectivity of aquabirnavirus strains to various marine fish species. *Diseases of Aquatic Organisms* 46: 109-114

Jackson D, Deady S, Hassett D, Leahy Y (2000). *Caligus elongatus* as parasites of farmed salmonids in Ireland. *Contributions to Zoology* 69: http://dpc.uba.uva.nl/ctz/vol69/nr01/art07

Jarp J, Karlsen E (1997). Infectious salmon anaemia (ISA) risk factors in sea-cultured Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 28: 79– 86.

Johnsen BO, Jensen AJ (1994). The spread of furunculosis in salmonids in Norwegian rivers. *Journal of Fish Biology* 45: 47-55.

Johnsen IA, Asplin LC, Sandvik AD, Serra-Llinares RM (2016). Salmon lice dispersion in a northern Norwegian fjord system and the impact of vertical movements. *Aquaculture Environment Interactions* 8: 99-116.

Johnson SC, Albright LJ (1991). Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda, Caligidae) under laboratory conditions. *Journal of the Marine Biological Association of the United Kingdom* 71: 425–436.

Johnson SC, Albright LJ (1992a). Comparative susceptibility and histopathology of the response of naive Atlantic, chinook and coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Diseases of Aquatic Organisms* 14: 179–193.

Johnson SC, Albright LJ (1992b). Effects of cortisol implants on the susceptibility and the histopathology of the responses of naive coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Diseases of Aquatic Organisms* 14: 195-205.

Jones JB (1988). New Zealand parasitic Copepoda; genus *Caligus* Muller, 1785 (Siphonostomatoida: Caligidae). *New Zealand Journal of Zoology* 15: 397-413.

Jones SR, Prosperi-Porta G, Kim E, Callow P, Hargreaves NB (2006a). The occurrence of *Lepeophtheirus salmonis* and *Caligus clemensi* (Copepoda: Caligidae) on three-spine stickleback *Gasterosteus aculeatus* in coastal British Columbia. *Journal of Parasitology* 92: 473–480.

Jones SR, Kim E, Dawe S (2006b). Experimental infections with *Lepeophtheirus salmonis* (Krøyer) on threespine sticklebacks, *Gasterosteus aculeatus* L. and juvenile Pacific salmon, *Oncorhynchus* spp. *Journal of Fish Diseases* 29: 489–495.

Jones SRM, Bruno DW, Madsen L, Peeler EJ (2015). Disease management mitigates risk of pathogen transmission from maricultured salmonids. *Aquaculture Environment Interactions* 6: 119–134

Kabata Z (1979). Parasitic copepoda of British Fishes. Ray Society, London. 468 pgs.

Kabata Z (1984). Diseases caused by metazoans: Crustaceans. In: Kinne O (ed). Diseases of Marine Animals, Vol. IV, Part 1, Introduction, Pisces. Biologische Anstalt Helgoland, Hamburg. Pgs. 321-399.

Kent ML (2000). Marine netpen farming leads to infections with some unusual parasites. *International Journal for Parasitology* 30: 321-326.

Kent ML, Poppe TT (1998). Diseases of seawater netpen-reared salmonid fishes. Pacific Biological Station, Fisheries and Oceans Canada. 137 pgs.

Kent ML, Sawyer TK, Hedrick RP (1988). *Paramoeba pemaquidensis* (Sarcomastigophora: Paramoebidae) infestation of the gills of coho salmon *Oncorhynchus kisutch* reared in sea water. *Diseases of Aquatic Organisms* 5: 163-169.

Kent ML, Dawe SC, Speare DJ (1995). Transmission of *Loma salmonae* (Microsporea) to chinook salmon in sea water. *Canadian Veterinary Journal* 36: 98-101.

Kent ML, Traxler GS, Kieser D, Richard J, Dawe SC, Shaw RW, Prosperi-Porta G, Ketcheson J, Evelyn TPT (1998). Survey of Salmonid Pathogens in Ocean-Caught Fishes in British Columbia, Canada. *Journal of Aquatic Animal Health* 10: 211-219.

Kibenge FS, Godoy MG, Wang Y, Kibenge MJ, Gherardelli V, Mansilla S, Lisperger A, Jarpa M, Larroquete G, Avendano F, Lara M, Gallardo A (2009). Infectious salmon anaemia virus (ISAV) isolated from the ISA disease outbreaks in Chile diverged from ISAV isolates from Norway around 1996 and was disseminated around 2005, based on surface glycoprotein gene sequences. *Virology Journal* 6: 88.

Kibenge MJT, Iwamoto T, Wang Y, Morton A, Routledge R, Kibenge FSB (2016). Discovery of variant infectious salmon anaemia virus (ISAV) of European genotype in British Columbia, Canada. *Virology Journal* 13:3, DOI 10.1186/s12985-015-0459-1

Kilroy C, Larned ST, Biggs BJF (2009). The non-indigenous diatom *Didymosphenia geminata* alters benthic communities in New Zealand rivers. *Freshwater Biology* 54: 1990-2002.

Knowles GJ (1992). Diagnosis of whirling disease in Canterbury fish farms. *Surveillance* 19(4): 27-28.

Krkosek M, Lewis MA, Volpe JP (2005). Transmission dynamics of parasitic sea lice from farm to wild salmon. *Proceedings of the Royal Society B* 272: 689–696.

Krkosek M (2010). Host density thresholds and disease control for fisheries and aquaculture. *Aquaculture Environment Interactions* 1: 21-32.

Lane HS, Webb SC, Duncan J (2016). *Bonamia ostreae* in the New Zealand oyster *Ostrea chilensis*: a new host and geographic record for this haplosporidian parasite. *Diseases of Aquatic Organisms* 118: 55-63.

Larenas JJ, Bartholomew J, Troncoso O, Fernandez S, Ledezma H, Sandoval N, Vera P, Contreras J, Smith P (2003). Experimental vertical transmission of *Piscirickettsia salmonis* and in vitro study of attachment and mode of entrance into the fish ovum. *Diseases of Aquatic Organisms* 56: 25–30.

Lin CL, Ho JS, Chen SN (1996). Developmental stages of *Caligus epidemicus* Hewitt, a copepod parasite of tilapia cultured in brackish water. *Journal of Natural History* 30: 661-684.

Lom J, Dykova I (1992). Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science 26. Elsevier. 315 pgs.

Lowers JM, Bartholomew JL (2003). Detection of myxozoan parasites in oligochaetes imported as food for ornamental fish. *The Journal of Parasitology* 89: 84-91.

Lumsden JS, Morrison B, Yason C, Russell S, Young K, Yazdanpanah A, Huber P, Al-Hussinee L, Stone D, Way K (2007). Mortality event in freshwater drum (*Aplodinotus grunniens*) from Lake Ontario, Canada associated with viral hemorrhagic septicemia virus, type IV. *Diseases of Aquatic Organisms* 76: 99–111.

MAF Biosecurity (2011a). Aquaculture Readiness Data Phase II. MAF Technical Paper No: 2011/68. July 2011. http://www.biosecurity.govt.nz/files/publications/technical-papers/aquatic-animal-pest2.pdf

MAF Biosecurity (2011b). Disease affecting Southland kanakana/lamprey. . www.biosecurity.govt.nz/files/pests/kanakana-factsheet.pdf

Mardones FO, Perez AM, Carpenter TE (2009). Epidemiologic investigation of the re-emergence of infectious salmon anemia virus in Chile. *Diseases of Aquatic Organisms* 84: 105-114.

Margolis L, Boyce NP (1990). Helminth parasites from North Pacific anadromous chinook salmon *Oncorhynchus tshawytscha*, established in New Zealand. *Journal of Parasitology* 76: 133-135.

Markiw ME (1992). Experimentally induced whirling disease. I. Dose response of fry and adults of rainbow trout exposed to the *Triactinomyxon* stage of *Myxobolus cerebralis*. *Journal of Aquatic Animal Health* 4: 40-43.

Markiw ME, Wolf K (1983). *Myxosoma cerebralis* (myxozoa: Myxosporea) etiologic agent of salmonids whirling disease requires a tubificid worm (Annelida: Oligochaeta) in its lifecycle. *Journal of Protozoology* 30: 561-564.

Mauel MJ, Miller DL (2002). Piscirickettsiosis and piscirickettsiosis-like infections in fish: a review. *Veterinary Microbiology* 87: 279-289.

MacKenzie K, Longshaw M, Begg GS, McVicar AH (1998). Sea lice (Copepoda: Caligidae) on wild sea trout (*Salmo trutta*) in Scotland. *ICES Journal of Marine Science* 55: 151-162.

MacKinnon BM (1998). Host factors important in sea lice infections. *ICES Journal of Marine Science* 55: 188-192.

Marine Harvest (2008). Sustainability Report 2008. . http://www.marineharvest.com/Documents/Marine%20Harvest%20-%20Sustainability%20Report%20-%202008.pdf

Matthews RA (2005). *Ichthyophthirius multifiliis* Fouquet and Ichthyophthiriosis in freshwater teleosts. *Advances in Parasitology* 59: 159-241.

Mauel MJ, Miller DL (2002). Piscirickettsiosis and piskirickettsiosis-like infections in fish: a review. *Veterinary Microbiology* 87: 279-289.

McAllister PE (1993). Salmonid fish viruses. In: Stoskopf MK (ed). *Fish Medicine*. W B Saunders Co., Philadelphia. pgs. 380-408.

McAllister PE, Owens WJ (1995). Assessment of the virulence of fish and molluscan isolates of infectious pancreatic necrosis virus for salmonid fish by challenge of brook trout, *Salvelinus fontinalis* (Mitchill). *Journal of Fish Diseases* 18: 97-103.

McAllister PE, Bebak J, Wagner BA (2000). Susceptibility of arctic char to experimental challenge with infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV). *Journal of Aquatic Animal Health* 12: 35-43.

McColl KA, Davies KR, Young JG, Crane MS (2009). Aquatic birnavirus infections of finfish. Australian and New Zealand Standard Diagnostic Procedure. .
http://www.scahls.org.au/__data/assets/pdf_file/0009/1516491/Aquatic_birnaviruses.pdf

McClure CA, Hammell KL, Dohoo IR (2005). Risk factors for outbreaks of infectious salmon anemia in farmed Atlantic salmon, *Salmo salar*. *Preventative Veterinary Medicine* 72: 263-280.

McDowall RM (1978). New Zealand Freshwater Fishes. A guide and natural history. Heinemann. 230 pgs.

McDowall RM (1994). Gamekeepers for the nation. The story of New Zealand's acclimatization societies 1861-1990. Canterbury University Press. 508 pgs.

McIntyre L, Rawdon T, Johnston C (2010). Animal health surveillance. Salmonid surveillance during 2009. *Surveillance* 37(2): 16-19.

Michel C, Faivre B, de Kinkelin P (1986). A clinical case of enteric redmouth in minnows (*Pimephales promelas*) imported in Europe as baitfish. *Bulletin of the European Association of Fish Pathologists* 6: 97-99.

Mitchell SO, Rodger HD (2011). A review of infectious gill disease in marine salmonid fish. *Journal of Fish Diseases* 34: 411-432.

Molinet C, Cáceres M, Gonzalez MT, Carvajal J, Asencio G, Díaz M, Díaz P, Castro MT, Codjambassis H (2011). Population dynamic of early stages of *Caligus rogercresseyi* in an embayment used for intensive salmon farms in Chilean inland seas. *Aquaculture* 312: 62-71.

Moran JDW, Kent ML (1999). *Kudoa thyrsites* (Myxozoa: Myxosporea) infections in pen reared Atlantic salmon in the northeast Pacific Ocean with a survey of potential non- salmonid reservoir hosts. *Journal of Aquatic Animal Health* 11: 101-109.

Moran JDW, Whitaker DJ, Kent ML (1999a). A review of the myxosporean genus *Kudoa* Meglitsch, 1947, and its impact on the international aquaculture industry and commercial fisheries. *Aquaculture* 172: 163-196.

Moran JDW, Whitaker DJ, Kent ML (1999b). Natural and laboratory transmission of the marine myxozoan parasite *Kudoa thyrsites* to Atlantic salmon. *Journal of Aquatic Animal Health* 11: 110-115.

Morrison RN, Crosbie PB, Cook MT, Adams MB, Nowak BF (2005). Culture derived *Neoparamoeba pemaquidensis* fails to elicit amoebic gill disease (AGD) in Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 66: 135–144.

Mortensen SH (1993). Passage of infectious pancreatic necrosis virus (IPNV) through invertebrates in an aquatic food chain. *Diseases of Aquatic Organisms* 16: 41-45.

Mortensen SH, Bachere E, Gall G, Mialhe E (1992). Persistence of infectious pancreatic necrosis virus (IPNV) in scallops *Pecten maximus*. *Diseases of Aquatic Organisms* 12: 221-227.

MPI (2013). Salmon mortality investigation. REW-1017 Pelorus Sound. MPI Technical Paper 2013/19. July 2013. 16 pgs.

MPI (2015). Unusual mortality rates in Marlborough farmed salmon. October 2015. http://mpi.govt.nz/document-vault/10037

MPI (2016).Legal controls to manage the spread of an unwanted organism in Marlborough farmed salmon. http://mpi.govt.nz/document-vault/12004

Muroga K (2001). Viral and bacterial diseases of marine fish and shellfish in Japanese hatcheries. *Aquaculture* 202: 23-44.

Munday BL, Foster CK, Roubal FR, Lester RJG (1990). Paramoebic gill infection and associated pathology of Atlantic salmon, *Salmo salar*, and rainbow trout, *Salmo gairdneri*, in Tasmania. In: FO. Perkins, TC Cheng (eds) Pathology in Marine Science. Academic Press. pgs 215-222.

Munday BL, Lange K, Foster CK, Lester RJG, Handlinger J (1993). Amoebic gill disease of sea caged salmonids in Tasmanian waters. *Tasmanian Fisheries Research* 28: 14-19.

Munday BL, Zilberg D, Findlay V (2001). Gill disease of marine fish caused by infection with *Neoparamoeba pemaquidensis*. *Journal of Fish Diseases* 24, 497–507.

Munro PD, Murray AG, Fraser DI, Peeler J (2003). An evaluation of the risk of infectious salmon anaemia transmission associated with different salmon harvesting methods in Scotland. *Ocean and Coastal Management* 46: 157–174.

Munro ES, Millar CP, HastingsTS (2010). An analysis of levels of infectious pancreatic necrosis virus in Atlantic salmon, *Salmo salar* L., broodstock in Scotland between 1990–2002. *Journal of Fish Diseases* 33: 171-177.

Murray AG, Peeler EJ (2005). A framework for understanding the potential for emerging diseases in aquaculture. *Preventative Veterinary Medicine* 67: 223–235.

Murray AG, Gillibrand PA (2006). Modelling salmon lice dispersal in Loch Torridon, Scotland. *Marine Pollution Bulletin* 53: 128-135.

Murray AG, Smith RJ, Stagg RM (2002). Shipping and the spread of infectious salmon anemia in Scottish aquaculture. *Emerging Infectious Diseases* 8: 1–5.

Murray AG, Amundrud TL, Gillibrand PA (2005). Models of hydrodynamic pathogen dispersal affecting Scottish salmon production: modelling shows how Scotland eradicated ISA, but not IPN. *Bulletin of the Aquaculture Association of Canada* 105: 79–86.

Nakajima K, Inouye K, Sorimachi M (1998). Viral diseases in cultured marine fish in Japan. *Fish Pathology* 33: 181-188.

Nickum D (1999). *Whirling disease in the United States: a summary of progress and research and management*, Trout Unlimited. 35 pgs. http://whirlingdisease.montana.edu/pdfs/TU_Report_99.pdf

Noga EJ (2010). Fish disease: diagnosis and treatment. Second edition. Wiley Blackwell. 519 pgs.

Nomura T, Yoshimizu M, Kimura T (1993). An epidemiological study of furunculosis in salmon propagation in Japanese rivers. *Fisheries Research* 17: 137-146.

Novoa B, Figueras A, Puentes CF, Ledo A, Toranzo AE (1993). Characterization of a birnavirus isolated from diseased turbot cultured in Spain. *Diseases of Aquatic Organisms* 15: 163-169.

Nylund A, Plarre H, Karlsen M, Fridell F, Ottem KF, Bratland A, Sæther PA (2007). Transmission of infectious salmon anaemia virus (ISAV) in farmed populations of Atlantic salmon (*Salmo salar*). *Archives of Virology* 152: 151–179.

NZ King Salmon (2016). The New Zealand King Salmon Company Ltd. Biosecurity Management Plan. 24 March 2016.

Oines O, Heuch PA (2007). *Caligus elongatus* Nordmann genotypes on wild and farmed fish. *Journal of Fish Diseases* 30: 81–91.

Ostland VE, Hicks BD, Daly JG (1987). Furunculosis in baitfish and its transmission to salmonids. *Diseases of Aquatic Organisms* 2: 163-166.

Penston MJ, McBeath AJ, Millar CP (2011). Densities of planktonic *Lepeophtheirus salmonis* before and after an Atlantic salmon farm relocation. *Aquaculture Environment Interactions* 1: 225-232.

Pike AW, Wadsworth SL (1999). Sea lice on salmonids: their biology and control. *Advances in Parasitology* 44: 233-337.

Pulkkinen K, Suomalainen LR, Read AF (2010). Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. *Proceedings of the Royal Society B* 277: 593-600.

Ramsay JM, Speare DJ, Dawe SC, Kent ML (2002). Xenoma formation during microsporidial gill disease of salmonids caused by *Loma salmonae* is affected by host species (*Oncorhynchus tshawytscha, O. kisutch, O. mykiss*) but not by salinity. *Diseases of Aquatic Organisms* 48: 125-131.

Reno PW (1999). Infectious pancreatic necrosis and associated aquatic birnaviruses. In: Woo PTK, Bruno DW (eds.) *Fish Diseases and Disorders, Volume 3: Viral, Bacterial and Fungal Infections*, pgs 1-55.

Roberts RJ (2001). Fish Pathology. 3rd edition. W.B. Saunders. 472 pgs.

Rohde K (1984). Diseases caused by metazoans: Helminths. In: Kinne O (ed). Diseases of Marine Animals, Vol. IV, Part 1, Introduction, Pisces. Biologische Anstalt Helgoland, Hamburg. Pgs. 193-320.

Rolland JB, Winton JR (2003). Relative resistance of pacific salmon to infectious salmon anaemia virus. *Journal of Fish Diseases* 26: 511-520.

Roubal FR (1994). Histopathology caused by *Caligus epidemicus* Hewitt (Copepoda: Caligidae) on captive *Acanthopagrus australis* (Gunther) (Pisces: Sparidae). *Journal of Fish Diseases* 17: 631-640.

Roubal FR, Lester RJG, Foster CK (1989). Studies on cultured and gill attached *Paramoeba* sp. (Gymnamoebae: Paramoebidae) and the cytopathology of paramoebic gill disease in Atlantic salmon , *Salmo salar* L., from Tasmania. *Journal of Fish Diseases* 12: 481-492.

Rucker RR (1966). Redmouth disease of rainbow trout (*Salmo gairdneri*). *Bull. Off. Int. des Epiz.* 65: 825-830.

San Diego-McGlone ML, Azanza RV, Villanoy CL, Jacinto GS (2008). Eutrophic waters, algal bloom and fish kill in fish farming areas in Bolinao, Pangasinan, Philippines. *Marine Pollution Bulletin* 57: 295-301.

SCAAH (2015). Sub-committee on Aquatic Animal Health SCAAH Newsletter #5, June 2015. http://apfa.com.au/wp-content/uploads/2015/01/SCAAH-Newsletter-2015-7-July-2015.pdf

Scheel I, Aldrin M, Frigessi A, Jansen PA (2007). A stochastic model for infectious salmon anemia (ISA) in Atlantic salmon farming. *Journal of the Royal Society Interface* 4: 699–706.

Sina MA and 27 other authors (2005). The New Higher Level Classification of Eukaryotes with Emphasis on the Taxonomy of Protists. *Journal of Eukaryotic Microbiology* 52: 399-432.

Skall HF, Olesen NJ and Mellergaard S (2005a). Viral haemorrhagic septicaemia virus in marine fish and its implications for fish farming – a review. *Journal of Fish Diseases* 28: 509–529.

Skall HF, Olesen NJ and Mellergaard S (2005b). Prevalence of viral haemorrhagic septicaemia virus in Danish marine fishes and its occurrence in new host species. *Diseases of Aquatic Organisms* 66: 145–151.

Slocombe RF (1980). Induction of ichthyosporidiosis in brown trout (Salmo trutta) by feeding of raw mullet. *Australian Veterinary Journal* 56(12): 585-587.

Smith PJ, Webber WR, McVeah SM, Inglis GJ, Gust N (2003). DNA and morphological identification of an invasive swimming crab *Charybdis japonica* (A. Milne-Edwards 1891) in New Zealand waters, *New Zealand Journal of Marine and Freshwater Research* 37: 753–762.

Smothers JF, von Dohlen CD, Smith LH, Spall RD (1994). Molecular evidence that the myxozoan protists are metazoans. *Science* 265: 1719–1721.

Snow M (2011). The contribution of molecular epidemiology to the understanding and control of viral diseases of salmonid aquaculture. *Veterinary Research* 42: 56.

Snow M, Black J, Matejusova I, McIntosh R, Baretto E, Wallace IS, Bruno DW (2010). Detection of salmonid alphavirus RNA in wild marine fish: implications for the origins of salmon pancreas disease in aquaculture. *Diseases of Aquatic Organisms* 91: 177−188.

Sommer AI, Strand MA, Rasmussen E, Mennen S (2004). Susceptibility of spotted wolffish *Anarhichas minor* to experimental infection with nodavirus and infectious pancreatic necrosis virus. *Diseases of Aquatic Organisms* 59: 101-108.

Sparboe O, Koren C, Hastein T, Poppe T, Stenwig H (1986). The first isolation of *Yersinia ruckeri* from farmed Norwegian salmon. *Bulletin of the European Association of Fish Pathologists* 6: 41-42.

Stein A, Bjorn PA, Heuch PA, Elston DA (2005). Population dynamics of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon and sea trout. *Marine Ecology Progress Series* 290: 263–275.

Steinum T, Kvellestad A, Rønneberg LB, Nilsen H, Asheim A, Fjell K, Nygard SM, Olsen AB, Dale OB (2008). First cases of amoebic gill disease (AGD) in Norwegian seawater farmed Atlantic salmon, *Salmo salar* L., and phylogeny of the causative amoeba using 18S cDNA sequences. *Journal of Fish Diseases* 31: 205–213.

Stewart JE (1998). Sharing the Waters: An Evaluation of Site Fallowing, Year Class Separation and Distances Between Sites for Fish Health Purposes on Atlantic Salmon Farms. *Canadian Technical Report of Fisheries and Aquatic Sciences* 2218: 56 pgs.

Stuart MD (2004). Review of research on *Undaria pinnatifida* in New Zealand and its potential impacts on the eastern coast of the South Island. Department of Conservation Internal Series 166. 40 pgs.

Swearer SE, Robertson DR (1999). Life history, pathology and description of *Kudoa ovivora* n. sp. (Myxozoa, Myxosporea): an ovarian parasite of Carribbean labroid fishes. *J. Parasitol.* 85: 337-353.

Thorsen BK, Enger O, Norland S, Hoff K A (1992). Long-Term Starvation Survival of *Yersinia ruckeri* at Different Salinities Studied by Microscopical and Flow Cytometric Methods. *Applied and Environmental Microbiology* 58: 1624-1628.

Thorud KE, Hastein T (2003). Experiences with regulatory responses to Infectious Salmon Anaemia in Norway. In: Miller O et al. (eds). International response to infectious salmon anemia: prevention, control, and eradication: proceedings of a symposium; 3–4 September 2002; New Orleans, LA. Tech. Bull. 1902. Washington, DC: U.S. Dept. Agric.Animal and Plant Health Inspection Service. 194 p.

Thrush MA, Murray AG, Brun E, Wallace S, Peeler EJ (2011). The application of risk and disease modelling to emerging freshwater diseases in wild aquatic animals. *Freshwater Biology* 56: 658–675.

Tisdall D, Phipps J (1987). Isolation and characterization of a marine birnavirus from returning quinnat salmon (*Oncorhynchus tshawyscha*) in the South Island of New Zealand. *New Zealand Veterinary Journal* 35: 217-218.

Tobback E, Decostere A, Hermans K, Haesebrouck F, Chiers K (2007). *Yersinia ruckeri* infections in salmonid fish. *Journal of Fish Diseases* 30: 257-268.

Todd CD (2007). The copepod parasite (*Lepeophtheirus salmonis* (Krøyer), *Caligus elongatus* Nordmann) interactions between wild and farmed Atlantic salmon (*Salmo salar* L.) and wild sea trout (*Salmo trutta* L.): a mini review. *Journal of Plankton Research* 29: i61-i71.

Toranzo AE, Magarinos B, Romalde JL (2005). A review of the main bacterial fish diseases in mariculture systems. *Aquaculture* 246: 37-61.

Tubbs L, Wybourne BA, Lumsden JS (2010). Nodular gill disease causing proliferative branchitis and mortality in chinook salmon (*Oncorhynchus tshawytscha*). *New Zealand Veterinary Journal* 58: 59-61.

Tuckey NP, Forster ME, Gieseg SP (2009). Lipid oxidation is inhibited by isoeugenol exposure in chinook salmon (*Oncorhynchus tshawytscha*) fillets during storage at 15°C. *Journal of Food Science* 74: C333–C338.

Tuckey NP, Forster ME, Gieseg SP (2010). Effects of rested harvesting on muscle metabolite concentrations and K-values in chinook salmon (*Oncorhynchus tshawytscha*) fillets during storage at 15°C. *Journal of Food Science* 75: C459–C464.

Uglem I, Dempster T, Bjørn PA, Sanchez-Jerez P, Økland F (2009). High connectivity of salmon farms revealed by aggregation, residence and repeated movements of wild fish among farms. *Marine Ecology Progress Series* 384: 251-260.

Unwin MJ, Rowe DK, Poortenaar CW, Boustead NC (2005). Suppression of maturation in 2- year-old chinook salmon (*Oncorhynchus tshawytscha*) reared under continuous photoperiod. *Aquaculture* 246 (1-4): 239-250.

Urquhart K, Murray AG, Gregory A, O'Dea M, Munro LA, Smail DA, Shanks AM, Raynard MS (2008). Estimation of infectious dose and viral shedding rates for infectious pancreatic necrosis virus in Atlantic salmon, *Salmo salar* L., post-smolts. *Journal of Fish Diseases* 31: 879-887.

Venmathi Maran BA, Seng LT, Ohtsuka S, Nagasawa K (2009). Records of *Caligus* (Crustacea: Copepoda: Caligidae) from marine fish cultured in floating cages in Malaysia with a redescription of the male of *Caligus longipedis* Bassett-Smith, 1898. *Zoological Studies* 48: 797-807.

Vossbrinck CR, Andreadis TG, Debrunner-Vossbrinck BA (1998). Verification of intermediate hosts in the life cycles of microsporidia by small subunit rDNA sequencing. *Journal of Eukaryotic Microbiology* 45: 290-292.

Wallace IS, Gregory A, Murray AG, Munro ES, Raynard RS (2008). Distribution of infectious pancreatic necrosis virus in wild marine fish from Scottish waters with respect to clinically infected aquaculture sites producing Atlantic salmon, *Salmo salar* L.. *Journal of Fish Diseases* 31:177-86.

Wechsler SJ, Woods LC, Kraeuter JN, Hetrick FM, McAllister PE (1987). Transmission of infectious pancreatic necrosis virus in striped bass, *Morone saxatilis*. *Journal of Fish Diseases* 10: 29-34.

Wells A, and 11 other co-authors (2007). Physiological consequences of "premature freshwater return" for wild sea-run brown trout (Salmo trutta) post smolts infested with sealice (*Lepeophtheirus salmonis*). *Canadian Journal of Fisheries and Aquatic Sciences* 64: 1360-1369.

Whipps, CM, Adlard, RD, Bryant, MS, Lester, RJG, Findlay, V and Kent, ML (2003) First report of three *Kudoa* species from Eastern Australia: *Kudoa thyrsites* from Mahi mahi (*Coryphaena hippurus*), *Kudoa amamiensis* and *Kudoa minithyrsites* n. sp. from sweeper (*Pempheris ypsilychnus*). *Journal of Eukaryotic Microbiology* 50: 215-219.

Whittington RJ, Cullis B (1988). The susceptibility of salmonid fish to an atypical strain of *Aeromonas salmonicida* that infects goldfish, *Carassius auratus* (L.) in Australia. *Journal of Fish Diseases* 11: 461-470.

Whittington RJ, Gudkovs N, Carrigan MJ, Ashburner LD, Thurstan SJ (1987). Clinical, microbiological and epidemiological findings in recent outbreaks of goldfish ulcer disease due to atypical *Aeromonas salmonicida* in south-eastern Australia. *Journal of Fish Diseases* 10: 353-362.

Whittington RJ, Crockford M, Jordan D, Jones B (2008). Herpesvirus that caused epizootic mortality in 1995 and 1998 in pilchard, *Sardinops sagax neopilchardus* (Steindachner), in Australia is now endemic. *Journal of Fish Diseases* 31: 97-105.

Wilson A, Magill S, Black KD (2009). Review of environmental impact assessment and monitoring in salmon aquaculture. In FAO. Environmental impact assessment and monitoring in aquaculture. FAO Fisheries and Aquaculture Technical Paper. No. 527. Rome, FAO. pgs. 455–535.

Wolf K, Markiw ME (1984). Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of the invertebrate and vertebrate hosts. *Science* 225: 1449-1452.

Wolf K, Snieszko SF, Dunbar CE, Pyle E (1960). Virus nature of infectious pancreatic necrosis in trout. *Proceedings of the Society for Experimental Biology and Medicine* 104: 105–108.

Yasuda H, Ooyama T, Iwata K, Tun T, Yokoyama H, Ogawa K (2002). Fish to fish transmission of *Myxidium* spp. (Myxozoa) in cultured tiger puffer suffering from emaciation disease. *Fish Pathology* 37: 29-33.

Young ND, Crosbie PBB, Adams MB, Nowak BF, Morrison, RN (2007). *Neoparamoeba perurans* n. sp. an agent of amoebic gill disease in Atlantic salmon (*Salmo salar* L.) *International Journal for Parasitology* 37: 1469-1481.

Young ND, Dykova I, Snekvik K, Nowak BF, Morrison, RN (2008). *Neoparamoeba perurans* is a cosmopolitan aetiological agent of amoebic gill disease. *Diseases of Aquatic Organisms* 78: 217-223.

Zainathan SC (2012). Detection of aquareovirus in farmed Tasmanian Atlantic salmon (*Salmo salar*). Thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy, University of Tasmania, April 2012. 300 pgs

Zubchenko AV, Karaseva TA (2002). *Ichthyophonus hof*eri as one of the possible causes of increased marine mortality in post-smolts of Atlantic salmon. NPAFC Technical report No. 4. 90-92.

Appendix 1 – Risk Assessment Methodology

Release assessment

The likelihood that a hazard would be translocated into the environment is determined through the release assessment stage of the process. The only hosts considered in the risk analysis component of this document are chinook salmon (*Oncorhynchus tshawytscha*) cultured in seacages. The release pathway for spread of disease from the host into the New Zealand marine environment is direct via faeces, urine, blood, mucus and other fluids shed by cultured salmon into the water. The risk assessment for a particular hazard was concluded if the release assessment determined that the likelihood of release of that hazard was negligible.

Table 4. Nomenclature for the qualitative likelihood estimations used in this RA.

Likelihood	Definition
High	The event would be very likely to occur
Moderate	The event would occur with an even probability
Low	The event would be unlikely to occur
Very Low	The event would be very unlikely to occur
Extremely low	The event would be extremely unlikely to occur
Negligible	The event would almost certainly not occur

Exposure assessment

The exposure assessment examines the likelihood of wild aquatic animals in an uninfected jurisdiction being exposed to the hazards via infected seacaged salmon and determines the likelihood of the establishment of the hazard. The likelihood of exposure will depend on several factors relating to the capacity of the disease agent to survive in the environment in an infective form, the availability of susceptible hosts, the ease of infection of susceptible hosts, and the likelihood of subsequent transmission of infection to others within a population. In determining the likelihood of exposure of susceptible hosts to disease agents carried by salmon, the following key factors were considered relevant:

1. *Route of Infection (Oral/Contact):* Viable infective stages must be ingested by a susceptible host or otherwise come into contact with susceptible fish or invertebrate species. Infection may occur via the digestive tract, or through direct contact with contaminated water via the skin and gills or integument.

2. *Infective Dose:* There must be sufficient quantities of viable infective stages to induce an infection following ingestion or contact via the skin and gills or integument.

Once a hazard is released into the environment, the likelihood of whether the disease agent would survive, infect susceptible hosts, and become established within a population was expressed qualitatively using the likelihood estimations in Table 4, based on information available in the scientific (and other) literature, unpublished data, as well as the professional judgment of the analyst. The likelihoods for the release and exposure assessments were combined using the matrix of 'rules' for combining descriptive likelihoods, as shown in Table 5.

Table 5. Matrix of rules for combining descriptive likelihoods for the release and exposure assessments.

Likelihood of exposure

Likelihood of release	**High**	**Moderate**	**Low**	**Very Low**	**Extremely low**	**Negligible**
High	High	Moderate	Low	Very Low	Extremely low	Negligible
Moderate		Low	Low	Very Low	Extremely low	Negligible
Low			Very Low	Very Low	Extremely low	Negligible
Very Low				Extremely low	Extremely low	Negligible
Extremely low					Negligible	Negligible
Negligible						Negligible

The risk assessment for a particular hazard was concluded if the exposure assessment determined that the probability of establishment was negligible.

Consequence assessment

The consequence assessment estimates the likely magnitude of the consequences of establishment and/or spread of a disease agent into the environment and the possible effects of the disease agent on aquatic animals, the environment, industry and the economy. The qualitative terms used to describe the consequences of establishment of an unwanted disease agent in this RA are defined in Table 6. These descriptions are based on information available in other RAs, the scientific literature, unpublished data, as well as the professional judgment of the analyst. For each disease of concern, the consequence assessment determined the likelihood of occurrence and the associated impact for each of two main outbreak scenarios. Either:

1. The disease agent becomes established and spreads throughout populations of susceptible species in Marlborough Sounds and beyond. This scenario assumes that if a disease agent were to establish in a local population it would eventually spread to its natural geographical limits, or;

2. An index case occurs and infection may even spread to co-habiting animals, but the agent does not persist in the environment.

Only the first scenario was considered to represent establishment of the disease agent, because the second scenario would go undetected.

Table 6. Definition of terms used to describe consequences of establishment of disease agents.

Consequence	Definition
Extreme	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.
Very Low	Establishment of disease would have mild 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.

The risk assessment for a particular hazard was concluded if the consequence assessment determined that the consequences of introduction were negligible.

Risk estimation

Risk estimation is the final step involved with each assessment and would be used to determine whether the extent of the unrestricted risk presented by each disease agent to the environment and aquatic animals of New Zealand was sufficient to require risk management. 'Unrestricted risk' means the estimated risk if the current industry practices remain unchanged. Risk was assessed using the risk estimation matrix in Table 7 which uses a combination of the qualitative answers given for the combined likelihoods of release and exposure and the significance of the consequences of establishment of a disease agent to provide an estimate of the risk involved, ranging from 'negligible' through to 'extreme'. The appropriate level of protection (ALOP) for the environment adopted in this RA is expressed in qualitative terms. The ALOP is expressed as providing a high level of sanitary or phytosanitary protection whereby risk is reduced to a **very low** level, but not to zero. This definition of ALOP, and its illustration by way of a risk estimation matrix is shown below in Table 7.

Table 7. Risk estimation matrix showing the ALOP utilized for this RA (white squares = very low risk). Any diseases which fall to the right of the ALOP during the RA will require additional risk management (red font).

Likelihood of establishment and spread	Negligible	Very Low	Low	Moderate	High	Extreme
High	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Ext. Low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk

Consequences of establishment and spread

If either the likelihood of establishment and spread, or the significance of the consequences of establishment and spread were considered to be negligible, it was considered the unrestricted risk posed by the disease agent was negligible (rising to very low for extreme consequences of establishment), and there would be no need to implement any additional risk management steps (Table 7). If the consequences of establishment and spread were considered to be very low, even a high probability of

establishment and spread was tolerable without the need for risk management. If the likelihood of establishment and spread were considered to be very low, even high consequences of establishment and spread were tolerated without the need for risk management, but extreme consequences of establishment and spread were considered to exceed the ALOP, and risk management would be required (Table 7). Alternatively, if the likelihood of establishment and spread was high, even if the consequences of establishment and spread were considered to be low, this scenario would exceeded the ALOP and require risk management (Table 7).

Risk mitigation

If the unrestricted risk estimation for any disease agent is determined to be unacceptable (that is above very low), the threats posed by the disease agent will be ranked (high, medium, low) based on the likelihood that it would pose a disease risk when introduced into the Marlborough Sounds with cultured salmon. The ranking process will take into account not only the types of disease agents harboured by cultured salmon, but also the quantity of the salmon being cultured. For any diseases with risk estimation rankings that exceed the ALOP, risk mitigation measures may be necessary to reduce the risk estimate back to within the ALOP. The risk mitigation processes examined as part of this RA process will relate only to option evaluation.

Option evaluation

The RA will identify the options available for mitigating any risks that may exceed the ALOP.