



## Salmon mortality 2012

Interim investigation report into a Chinook salmon  
mortality event in Pelorus Sound

MPI Information Paper

Prepared for New Zealand King Salmon

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## Executive summary

1. The Ministry for Primary Industries (MPI) has undertaken a comprehensive range of tests and examinations of the samples submitted by aquaculture company New Zealand King Salmon (NZKS) from the Waihinau Bay farm in Pelorus Sound.
2. MPI's work has ruled out a number of exotic and endemic disease threats. No cause for the excess mortality has been identified. While the presence of a number of specific diseases can be excluded, no wide-ranging assurances about freedom from disease can be made in light of the unexplained mortality.
3. Ongoing work includes histopathology and virus isolation at the OIE Reference Laboratory in Norway and whole genomic sequence analysis at MPI's Animal Health Laboratory (AHL).

## Background

4. The purpose of this report is to provide New Zealand King Salmon Company (NZKS) an interim summary of the laboratory and epidemiological investigation into the mortality event at Waihinau Bay in March 2012. A final report will be generated following completion of all necessary testing and peer review of the process.
5. Domestic and international audiences of the Ministry for Primary Industries (MPI), including industry stakeholders and regulatory agencies expect competent and demonstrably thorough investigation. Initial objectives of the investigation in March were:
  - a. Determine if the mortality event in Chinook salmon reported from Waihinau Bay, Pelorus Sound is associated with an infectious agent.
  - b. Rule out OIE listed diseases which can cause Chinook salmon mortality.
6. The first objective was modified as of the 8th May to:
  - a. Determine if the mortality event in Chinook salmon reported from Waihinau Bay, Pelorus Sound is associated with an infectious agent, especially infectious salmon anaemia virus (ISAV).
7. On the 1<sup>st</sup> of March 2012 NZKS notified MPI of an abnormal mortality event occurring in 1kg Chinook salmon in Waihinau Bay, outer Pelorus Sound. Initial observations by NZKS included fish with lesions, reduced feed intake and many remaining fish exhibiting lethargy. An investigation was initiated and samples were submitted by NZKS for diagnostic testing. Current and historical mortality data provided by NZKS was examined for patterns to gain insight into the cause(s) of the event.

## Laboratory testing

8. All samples were initially sent to MPI's Animal Health Laboratory (AHL) for testing. AHL uses a combination of broad ranging tests (e.g. histopathology, virus isolation and bacterial culture) and pathogen specific tests (e.g. molecular based tests such as PCR).

9. As part of AHL standard procedures, a comprehensive diagnostic differential list specific for pathogens of Chinook salmon was compiled (see Table 1).
10. Samples were tested from three submissions of fish and provided no evidence for a bacterial or viral agent. Histopathology identified heart abnormalities in some fish, although changes were general and they did not point to a specific cause.

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**Table 1: Summary of diagnostic testing conducted at AHL**

Pathogen/Test	Result	Test type	Comment/Reference
<i>Aeromonas salmonicida</i>	NEGATIVE	PCR*	AHL IANZ** accredited method
<i>Yersinia ruckeri</i>	NEGATIVE	PCR	AHL IANZ accredited method
Viral haemorrhagic septicaemia virus	NEGATIVE	PCR	OIE – World Organisation for Animal Health
Infectious hematopoietic necrosis virus	NEGATIVE	PCR	OIE - World Organisation for Animal Health
Totivirus	NEGATIVE	PCR	Lovoll et al 2010
Reovirus	NEGATIVE	PCR	Palacios, et al., 2010
Renibacterium salmoninarum	NEGATIVE	PCR	AHL IANZ accredited method
General bacterial pathogens including <i>Vibrio</i> species	Nothing of clinical significance	Bacterial culture	AHL validated method
Cytopathic viruses	No cytopathic effects observed indicative of the presence of virus	Virus isolation	AHL IANZ accredited Virus isolation at OIE reference laboratory for ISA in Norway pending.
Histopathology	Inconsistent presence of abnormal heart pathology potentially indicative of the presence of a viral agent	Histopathology	Interpretation conducted by two independent pathologists – Gribbles and Brightwater Consulting Ltd Interpretation by OIE reference laboratory for ISA in Norway pending.
Electron Microscopy	No virus particles observed	Electron Microscopy	Selected heart samples with abnormal pathology
Mycoplasma	NEGATIVE	PCR	Not considered a likely cause; included for completeness
Iridovirus - Megalocytivirus - Ranavirus	NEGATIVE	PCR	Not considered a likely cause; included for completeness
Infectious pancreatic necrosis	NEGATIVE	PCR	Not considered a likely cause; included for completeness
Next generation sequencing		Whole genomic sequencing	No evidence of known pathogens detected, however detailed analysis is continuing.

\* PCR is a technique that detects DNA/RNA unique to a particular pathogen  
 \*\* IANZ – International Accreditation New Zealand is the national authority for the accreditation of testing and calibration laboratories



### ✓ Infectious salmon anaemia testing

- ✓ 11. During the course of MPI's investigation, it became public knowledge through a local newspaper that a Chinook salmon mortality event had occurred in Pelorus Sound. This generated public interest and resulted in speculation as to the cause of the mortality at an international level, including an enquiry from MPI's central government counterpart in Australia. Among this speculation were suggestions that Infectious Salmon Anaemia virus (ISAV) was responsible. ISAV is a significant economic disease of farmed salmon in the northern hemisphere and in Chile.
- ✓ 12. ISAV has not been reported in the scientific literature to cause disease in Chinook salmon and the clinical pathology of the affected fish did not suggest ISAV as a potential disease. However, ISAV belongs to Orthomyxoviridae - a family of viruses that are notable for rapid mutation and cross-species infection, as demonstrated by the influenza viruses. Increasing international interest and the potential reputational and trade impacts on New Zealand as a producer of salmon led to MPI arranging further testing for ISAV.
- ✓ 13. At the time, MPI's AHL did not have molecular (PCR) capability to detect ISAV, as it had not been considered a significant threat to New Zealand's Chinook-based salmon industry. When testing is not available at AHL, it is standard practise to contract the test to an international reference laboratory for that pathogen. The OIE-World Organisation for Animal Health has accredited two laboratories for ISAV testing, one in Canada and the other in Norway.
- ✓ 14. The OIE ISA reference laboratory at the National Veterinary Institute in Norway (NVI) was contacted in confidence to seek their availability to test for ISAV. Due to severe illness of the leading Norwegian ISA expert (not known by AHL at the time), there was a significant delay in their response to our request for assistance. MPI therefore decided to contact the OIE ISA reference laboratory at Atlantic Veterinary College in Canada. A response was immediate so MPI agreed that Atlantic Veterinary College would test samples for ISAV from New Zealand.
15. New samples (15 fish in total) were collected for further testing on the 20<sup>th</sup> March 2012, 20 days after the initial samples were submitted for testing. Samples from 7 fish were sent to the Atlantic Veterinary College for ISAV testing and samples from the other 8 fish were retained at AHL for further testing.
16. Atlantic Veterinary College used a combination of molecular tests as recommended by the OIE Manual of Diagnostic Tests for Aquatic Animals 2011 chapter for ISA, other published molecular tests for ISA as well as an unpublished test developed in-house at the Canadian laboratory. Atlantic Veterinary College reported to AHL that positive PCR results were generated from different tissues and from more than one fish, and using different tests.
- ✓ 17. AHL had retained histopathology samples and samples for electron microscopy from the same fish that were sent to Canada. Gross pathology and histopathology observations of the 7 fish sent to Canada indicated no evidence of ISAV. Further, heart tissue from all 7 fish was examined by AHL using an electron microscope and no evidence of ISA viral particles was identified.



- ✓ 18. The discrepancies between results generated at Atlantic Veterinary College and those determined at AHL meant that MPI needed to acquire a greater understanding of the tests undertaken at the Canadian laboratory to clarify why PCR positive results were produced from fish showing no other evidence of ISAV infection.
- ✓ 19. MPI sent an experienced staff member to visit Atlantic Veterinary College to observe and also request the underlying data that supported the positive results.
- ✓ 20. The lead scientist responsible for carrying out the tests at the Canadian laboratory has so far not provided any supporting information for the PCR positive results.
- ✓ 21. Following the positive results from Atlantic Veterinary College, discrepant analyses were undertaken. AHL established ISAV molecular tests as detailed in the OIE Manual of Diagnostic Tests for Aquatic Animals 2011 chapter for ISA and re-contacted NVI in Norway, who agreed to test samples for ISAV from New Zealand. Additional samples (representing 38 fish) and previously collected samples (representing 22 fish) were sent to NVI for ISAV testing.
- ✓ 22. The Norwegian laboratory was not able to detect ISAV in any of the samples by standard methods used at their laboratory for the detection of ISAV.
- ✓ 23. AHL has been unable to reproduce the results from Atlantic Veterinary College.
- ✓ 24. Both AHL and NVI have obtained NEGATIVE results for ISAV from samples using OIE recommended tests.

#### Next generation sequencing

25. As the heart pathology was suggestive of a possible viral agent, AHL undertook whole genomic sequencing to try and understand if there was a link between the pathology of the fish and an infectious agent.
26. Next generation sequencing was carried out on the hearts of two fish still showing pathology in May 2012, approximately two months after the peak mortality had occurred. A huge dataset was generated from the two fish. These results will require more detailed analyses and interpretation and will provide useful information for future discussions.

#### Laboratory testing pending

27. Test results still outstanding at the NVI in Norway include virus isolation for cytopathic viruses and histopathology interpretation. These tests results will be compared with the interpretations already conducted in New Zealand. MPI expects to receive these results within the next six weeks.

<sup>1</sup> Next generation sequencing is a technique that identifies a large proportion of all DNA (both fish and pathogen) and offers potential insight into what disease related processes and pathogens might be present.



## Epidemiological investigation

28. Mortality in March was higher than typically expected at all cages across the Waihinau Bay salmon farm site.
29. Within the affected site two cages stood out as having higher mortality in March and April 2012 than all other cages (see Appendix 1, Figure 1).
30. No obvious causes for the increased mortality were reported by the farm operator regarding environmental factors (e.g., higher than usual water temperature, decreased dissolved oxygen, algal blooms), or husbandry activities (e.g. grading, changes to feed).
31. No recent movements of fish were reported to have occurred at the site that may have provided a potential route of introduction of a pathogen.
32. When cumulative monthly mortalities were compared across sites, Waihinau Bay appeared significantly higher than other NZKS sites in February and March 2012,
- X 33. Cumulative monthly mortalities were also compared across sites in 2011.
- X 34.
35. NZKS may want to consider instigating a more comprehensive survey over time, including different sites and age classes to understand the significance of the heart pathology detected during this investigation.

### Summary

36. Higher mortalities in NZKS farmed Chinook salmon occurred during March and April 2012 at Waihinau Bay, Pelorus Sound.
37. PCR positive results for ISAV were received from the OIE reference laboratory at Atlantic Veterinary College in Canada but subsequent MPI requests for underlying data to support the results have not met with a response. MPI is continuing its efforts to obtain more information from the Atlantic Veterinary College.
38. There is a moderate to strong likelihood based on the investigation to date and ongoing communication of MPI with trading partners that there will be no adverse market access issues and the OIE appreciation of the health status of New Zealand's salmon will not change.



39. To date no probable cause of the mortality has been established and data from the Waihinau Bay farm indicates mortality rates have returned to baseline levels typically expected for farmed salmon in the Marlborough Sounds (i.e. the mortality event has ceased).
40. Comprehensive epidemiological analysis and diagnostic testing at the Animal Health Laboratory and the National Veterinary Institute in Norway has not revealed an infectious cause of disease.
41. However, histopathology revealed abnormal heart pathology that could be indicative of numerous causes, including infection with an intracellular pathogen.
42. In order to maintain New Zealand's reputation for animal health management, it is necessary to continue the investigation until reasonable causes for the mortality event have been examined (reasonable causes in reference to OIE listed diseases).
43. MPI recommends NZKS closely monitor the health of salmon smolt recently transferred to the Waihinau Bay site. Any unusual mortality events must be notified to MPI, as per the Standard for Health Surveillance in Approved Establishments for Export of Salmon to Australia (paragraph 4.4).
44. The current focus of the investigation is to clarify the ISAV status of the samples from Waihinau Bay, given the positive PCR results from the Atlantic Veterinary College and negative results from AHL and NVI. To date, PCR positive results for ISAV in samples from Waihinau Bay have not been replicated.
45. MPI recommends NZKS continue working alongside MPI and Aquaculture New Zealand to improve its biosecurity planning, management and reporting in order to satisfy international trade requirements.



— s9(2)(b)(ii)

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