BOOK OF ABSTRACT

INTERNATIONAL CONFERENCE

PUERTOVARASCHILE2026

Organized by

INCAR²
Interdisciplinary Center for Aquaculture Research Applied Research

November 9 ~ 11 2026

Saludo Editorial



Estimadas y estimados colegas:

Es un honor darles la bienvenida a la Primera Conferencia Internacional sobre Piscirickettsiosis (SRS 2026), un encuentro que marca un hito para la investigación científica y la industria salmonicultora, al reunir en Puerto Varas a investigadores, especialistas, representantes del sector productivo y autoridades en torno a uno de los principales desafíos sanitarios de la acuicultura.

La piscirickettsiosis, causada por Piscirickettsia salmonis, continúa representando una problemática de gran relevancia para la salmonicultura chilena y mundial. Su complejidad epidemiológica, sus consecuencias productivas y sus implicancias para la salud y el bienestar de los peces hacen indispensable avanzar hacia una comprensión integral de la enfermedad y el desarrollo de estrategias de prevención y control cada vez más efectivas y sostenibles.

Enfrentar este desafío requiere fortalecer la investigación científica, promover enfoques interdisciplinarios y, especialmente, estrechar la colaboración entre la academia, la industria y las instituciones públicas. Estamos convencidos de que el conocimiento adquiere su mayor valor cuando logra traducirse en soluciones concretas para los desafíos que enfrenta nuestra sociedad.

Con este propósito, desde el Centro Interdisciplinario para la Investigación Acuícola – Investigación Aplicada (INCAR²) hemos impulsado la realización de esta primera conferencia internacional, como un espacio de encuentro, intercambio de conocimientos y discusión científica que contribuya a fortalecer las capacidades de investigación y la cooperación nacional e internacional en torno al SRS.

El programa científico reúne tres conferencias magistrales, 39 presentaciones orales y 16 pósteres, organizados en áreas fundamentales para comprender y abordar la enfermedad: epidemiología y medio ambiente, genética y genómica, estrategias de prevención y control, y bienestar animal. Esta diversidad temática refleja la complejidad del desafío sanitario y la necesidad de integrar distintas disciplinas, metodologías y perspectivas para avanzar en su solución.

El presente Libro de Resúmenes reúne las contribuciones científicas que forman parte de esta primera edición. Más allá de constituir un registro de los trabajos presentados, esperamos que esta publicación contribuya a difundir nuevos conocimientos, identificar oportunidades de investigación y estimular futuras colaboraciones que permitan acelerar el desarrollo de soluciones innovadoras para la salmonicultura.

Para INCAR², esta conferencia representa también una expresión concreta de nuestra misión institucional: generar conocimiento científico de excelencia, impulsar la investigación aplicada y promover la transferencia de tecnologías y soluciones que contribuyan a una acuicultura más sostenible, resiliente y competitiva.

Quisiera expresar nuestro sincero agradecimiento a los investigadores y autores que han compartido sus trabajos, a los conferencistas invitados, a los integrantes de los comités científico y organizador, y a las instituciones y empresas que han apoyado la realización de este encuentro.

Esperamos que esta primera conferencia sea el inicio de un espacio permanente de colaboración científica internacional, capaz de generar nuevas preguntas, fortalecer redes de investigación y contribuir, desde la evidencia y la innovación, a enfrentar uno de los desafíos más importantes para el futuro de la salmonicultura.

Dr. Cristián Gallardo-Escárate
Director
Centro Interdisciplinario para la Investigación Acuícola – Investigación Aplicada (INCAR²)

Puerto Varas, Chile
9 de noviembre de 2026

Editorial Welcome



Dear Colleagues,

It is an honor to welcome you to the First International Conference on Piscirickettsiosis (SRS 2026), an event that marks a significant milestone for scientific research and the salmon farming industry. Bringing together researchers, specialists, industry representatives, and public authorities in Puerto Varas, this conference provides an opportunity to address one of the most important health challenges in the chilean aquaculture.

Piscirickettsiosis, caused by Piscirickettsia salmonis, remains a major challenge for salmon farming in Chile and worldwide. Its epidemiological complexity, impacts on production, and implications for fish health and welfare underscore the urgent need to advance our understanding of the disease and develop increasingly effective and sustainable prevention and control strategies.

Addressing this challenge requires strengthening scientific research, promoting interdisciplinary approaches, and, above all, fostering closer collaboration among academia, industry, and public institutions. We believe that scientific knowledge achieves its greatest value when it can be translated into practical solutions to the challenges facing our society.

With this vision, the Interdisciplinary Center for Aquaculture Research – Applied Research (INCAR²) has organized this first international conference as a forum for scientific exchange, knowledge sharing, and discussion, aimed at strengthening research capabilities and fostering national and international cooperation in the field of SRS.

The scientific program features 3 keynote lectures, 39 oral presentations, and 14 poster presentations, covering key areas essential to understanding and addressing the disease: epidemiology and the environment, genetics and genomics, prevention and control strategies, and animal welfare. This thematic diversity reflects the complexity of the health challenge and highlights the importance of integrating different disciplines, methodologies, and perspectives to advance effective solutions.

This Book brings together the scientific contributions presented at this inaugural conference. Beyond serving as a record of the research shared during the event, we hope this publication will help disseminate new knowledge, identify research opportunities, and encourage future collaborations that accelerate the development of innovative solutions for the salmon farming industry.

For INCAR², this conference also represents a tangible expression of our institutional mission: to generate scientific knowledge of excellence, advance applied research, and promote the transfer of technologies and solutions that contribute to a more sustainable, resilient, and competitive aquaculture sector.

I would like to express our sincere gratitude to the researchers and authors who have contributed their work, our invited keynote speakers, the members of the Scientific and Organizing Committees, and the institutions and companies whose support has made this event possible.

We hope this first conference will mark the beginning of a lasting platform for international scientific collaboration—one that inspires new research questions, strengthens research networks, and contributes, through scientific evidence and innovation, to addressing one of the most important challenges facing the future of salmon farming.

Dr. Cristián Gallardo-Escárate
Director
Interdisciplinary Center for Aquaculture Research –
Applied Research (INCAR²)

Puerto Varas, Chile
November 9, 2026


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Puerto Varas, ciudad sede de la I Conferencia Internacional de SRS 2026.

Puerto Varas, Host City of the 1st International Conference on Piscirickettsiosis (SRS 2026).

Ubicada a orillas del lago Llanquihue, en el sur de Chile, Puerto Varas es la puerta de entrada a la Patagonia chilena. Rodeada de montañas nevadas, volcanes, lagos, bosques y parques nacionales, la ciudad goza de una ubicación privilegiada y es un reconocido destino turístico.

Located on the shores of Lake Llanquihue in southern Chile, Puerto Varas is considered the gateway to Chilean Patagonia. Surrounded by snow-capped mountains, volcanoes, lakes, forests, and national parks, the city enjoys a privileged location and is a renowned tourist destination.







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Scientific Program

MONDAY	NOVEMBER 9
15:00–19:00	Registration
15:00–18:00	Poster setup and presentation projection tests
18:00–19:30	**Icebreaker**

TUESDAY	NOVEMBER 10
8:00–8:30	Registration
8:30–10:00	Opening Ceremony
10:10–10:40	**Keynote Talk: Dr. Fernando Mardones** **From evidence to action: Scientific Advances in *Piscirickettsia salmonis* Towards an Integrated Disease Management Strategy**
10:40–11:10	Coffee break
11:10–12:10	**Session I: Environmental Epidemiology** (Chairperson: Manuel Estay / Rolando Ibarra) **10 years of epidemiological surveillance in salmon farming in chile: a 2014-2024 retrospective study of infectious disease mortality risk** *Benjamín Diethelm-Varela, Pontificia Universidad Católica de Chile, Chile* **Bayesian back-calculation of the onset and temporal trajectory of reconstructed fatal Piscirickettsia salmonis infections from mortality records in two Chilean salmon farms.** *Rodrigo Montes Aste, INCAR², Chile* **SRS and the evolution of ai: a vision from adjacent aquaculture diseases** *Agustín Piña, Manolin, United States* **Risk factors associated with srs mortality at month 8 and at the end of the production cycle in atlantic salmon in the Los Lagos and Aysén regions of Chile** *Alex Moyano Escobar, Aquabench, Chile*
12:15–12:30	Official Conference Photograph
12:30–14:00	Lunch
14:00–14:30	**Keynote Talk: Dr. Frank Asche** **Social and economic impacts of disease in the salmon industry**

14:30–16:00	**Session II: Genetics and Genomics** (Chairperson: Yeny Leal / Jorge Mancilla) **Fine-mapping SRS resistance in atlantic salmon to improve genomic selection and identify gene-editing targets** *Rayner Gonzalez-Prendes, Hendrix Genetics, The Netherlands* **Exploring genome-wide presence–absence variation across atlantic salmon families with contrasting resistance to *Piscirickettsia salmonis*** *Diego Valenzuela, INCAR², Chile* **The fewer, the better: The role of transcriptional frontloading in atlantic salmon resistance to *Piscirickettsia salmonis*** *Alberto Sandoval, INCAR², Chile* **A major QTL for resistance against salmonid rickettsial septicaemia in coho salmon (*Oncorhynchus kisutch*) maps to a narrow region on chromosome 21, implicating two candidate genes** *Thomas Moen, AquaGen, Norway* **Unraveling the genomic basis and convergent evolution of resistance to *Piscirickettsia salmonis* in Atlantic (*Salmo salar*) and Coho (*Oncorhynchus kisutch*) Salmon** *Victor Martinez, Universidad de Chile, Chile* **Automated phenotyping strengthens genetic evaluation of growth and SRS resistance in salmon breeding programs** *Osiel Guzmán, Caleta Bay, Chile*
16:00–16:30	Coffee break
16:30–17:45	**Session III: SRS Control Tools** (Chairperson: Antonio Casuso / Carolina San Martín) **Systematic literature review of costs associated with different management strategies aiming to reduce pathogen infestations in salmon farming.** *Jorge Dresdner-Cid, INCAR², Chile* **Nutritional conditioning to enhance bioresilience against salmonid rickettsial septicaemia** *Carolina San Martin, NML-GROUP, Chile* **A model for cost-benefit analysis of antimicrobial use in salmon aquaculture** *Manuel Estay, INCAR², Chile* **Development of new live attenuated vaccine for global SRS control** *Jaime Tobar, Vaxxinova, Norway* **Development of a recombinant BCG-based vaccine expressing a chimeric protein against *Piscirickettsia salmonis*** *Esteban Valdevenito, Pontificia Universidad Católica de Chile, Chile*
18:00–19:30	Conference Reception

WEDNESDAY	NOVEMBER 11
9:00–9:10	Daily welcome
9:15–9:45	**Keynote Talk: Dr. Hamish Rodger** **Global lessons, local solutions: Piscirickettsiosis in Europe and its main challenges. How the PisciNor® project and international collaborative research contribute to knowledge to control the disease in Chile and the world.**
10:00–11:15	**Session IV: SRS Control Tools** (Chairperson: Diego Valenzuela / Carolina San Martín) **Beyond SRS: lessons from emerging bacterial diseases in atlantic salmon** *Bjorn Krossoy, VAXXINOVA, Norway* **Global developments in vaccination against SRS** *Marius Karlsen, PHARMAQ, Norway* **Development and validation of a functional feed to increase resilience against *Piscirickettsia salmonis* in atlantic salmon (*Salmo salar*)** *Gabriela Carril, BioMar AS, Norway* **INNBOOST®: Bringing precision medicine to aquaculture, a new recombinant protein-based feed additive to prevent SRS outbreaks.** *Jorn Bethke, AQUIT, Chile* **Strategies and best management practices in Chilean salmon aquaculture: evidence from the Pincoy initiative** *Pablo Ibieta, Iniciativa Pincoy, Chile*
11:15–11:45	Coffee break
11:45–13:00	**Session V: Environmental Epidemiology** (Chairperson: Rodrigo Montes / Daniel Jiménez) **Freshwater health history and host physiological-inmunological condition as potential determinants of SRS susceptibility after seawater transfer: preliminary evidence.** *Jorge Mancilla, Mowi, Chile* **Treatment management determinant of antibiotic use against salmon rickettsial syndrome** *Daniel Jiménez, Aquabench S.A., Chile* **Prisma-SRS: an evidence-based decision-support calculator for piscirickettsiosis risk and intervention prioritisation at the farm level in Chilean salmon aquaculture** *Fernando Mardones, University of Ediburgh, Scotland* **Antimicrobial susceptibility surveillance of *Piscirickettsia salmonis* in Chile: regional, genogroup, and temporal trends (2017–2025).** *Cristian Valenzuela, Instituto de Fomento Pesquero, Chile* **Bayesian back-calculation of the onset and temporal trajectory of reconstructed fatal *Piscirickettsia salmonis* infections from mortality records in two Chilean salmon farms** *Rodrigo Montes, INCAR², Chile*

13:00–14:00	Lunch
14:00–15:15	**Session VI: SRS Control Tools** (Chairperson: Yeny Leal / Marcos Mancilla) **AI applied to the discovery and in vitro validation of natural antibacterials against *Piscirickettsia salmonis*: from 500,000 individual compounds identified to an effective combination with a low minimum inhibitory concentration (MIC)** *Francisco Arancibia, ISR-BIO, Chile* **Contemporary *Piscirickettsia salmonis* field isolates demand updated challenge models: a standardized platform based on LF-89 and EM-90 genogroups** *Pablo Ibieta, TekBios Ltda., Chile* **Smoltvision case study; positive synergy with the use of ALPHA JECT LIVAC SRS vaccine.** *Jorge Infante, PHARMAQ Analytiq, Chile* **Yelcho experimental trial: evaluation of vaccines and vaccination strategies for improved immunity against SRS** *Daniel Woywood, Aquabench, Chile* **Seawater immersion booster vaccination reduces SRS-associated mortality under commercial atlantic salmon farming conditions** *Carlos Saffie, Veterquimica, Chile*
15:15–16:45	**Session VII: Genetics and Genomics** (Chairperson: Diego Valenzuela / Alejandro Heisinger) **Genomic selection for SRS resistance increases survival in the field** *Thomas Moen, AquaGen, Norway* **Transposable element activation and alternative splicing reprogramming during piscirickettsiosis: a comparative genomic study in rainbow trout (*Oncorhynchus mykiss*)** *Rodrigo Vidal, Universidad de Santiago de Chile, Chile* **Single-nuclei RNA sequencing reveals the immune cell landscape of atlantic salmon head kidney during *Piscirickettsia salmonis* infection.** *Yeny Leal, INCAR², Chile* **Major Locus and candidate genes controlling survival against SRS in coho salmon** *Jose Manuel Yañez, Universidad de Chile, Chile* **Comparative metabolomic and metagenomic profiling of atlantic salmon families with contrasting resistance to *Piscirickettsia salmonis* across freshwater and seawater stages** *Constanza Saez-Vera, INCAR², Chile* **GWAS meta-analysis of resistance against *Piscirickettsia salmonis* in coho salmon (*Oncorhynchus kisutch*)** *Constanza Urzúa, Universidad de Chile, Chile*

16:45–17:15	Coffee break
17:15–18:15	**Session VIII: Animal Welfare** (Chairperson: Fernando Mardones / Ruben Avendaño) **NUTRIPATH: integrating immunonutrition and multi-layered diagnostics to support health and welfare in challenged salmonids** *Byron Morales, Norwegian University of Life Sciences, Norway* **Spatial clustering of antimicrobial use and *Piscirickettsia salmonis* mortality in Chilean salmon aquaculture: a risk-based framework for targeted amr surveillance** *Roberto Montt, CERES BCA, Chile* **Temporal host–pathogen signatures in atlantic salmon skin mucus during experimental *Piscirickettsia salmonis* infection** *Marcos Mancilla, ADL Diagnostic Chile, Chile* **The YELCHO alliance: a new model of public-private governance and engagement with the animal health sector** *Fernando Flores, Executive Director, Alianza Yelcho, Chile*
18:15–19:30	Closing Ceremony and Social event

Note: *Presentation titles are preserved in their original language. Verify the November 10 photograph/lunch overlap, missing titles, and speaker affiliations before publication.*

Posters Presentation

Evaluation of peruvian maca (*Lepidium meyenii*) extract on immunomodulation, antioxidant defense and epithelial barrier integrity against *Piscirickettsia salmonis* in rtgut-gc cells	*Natacha Santibáñez*
Transposable element activation and alternative splicing reprogramming during piscirickettsiosis: a comparative genomic study in rainbow trout (*Oncorhynchus mykiss*)	*Rodrigo Vidal*
Identification and genomic characterization of the type 1 secretion system (T1SS) in *Piscirickettsia salmonis*	*Matías Vega*
Repeated exposure to sub-inhibitory concentrations of florfenicol induce the expression of antibiotic resistance-associated proteins in biofilm-embedded *Piscirickettsia salmonis*.	*Cristian Oliver*
Explainable GNN disease module detection in the salmo salar macrophage response to *Piscirickettsia salmonis*	*Cristian Figueroa*
Non-pharmacological solution to modulate the immunogenicresponse to srs in salmonid species	*Ángel Valenzuela*
In silico identification, transcriptomic profiling, and characterization of cell wall hydrolases from *Piscirickettsia salmonis* for the development of antibiofilm platforms	*Adrián Rodríguez*
Histopathological characterization of granulomatous lesions associated with *Piscirickettsia salmonis* in atlantic salmon (*Salmo salar*) from seawater farming sites	*Denis Cárdenas*
Vaccination at different life stages determines the immune response to *Piscirickettsia salmonis* in atlantic salmon: a transcriptome analysis	*Antonio Casuso*
Integrative transcriptomics uncovers the mechanisms of hint3-Mediated SRS resistance in coho salmon (*Oncorhynchus kisutch*)	*Jessica Dorner*
A morpholino-based approach to investigate trim33l, a candidate gene associated with salmonid rickettsial septicaemia resistance in atlantic salmon	*Marcela González*
Assessment of the antimicrobial potential of a characterized vitis vinifera vine extract against *Piscirickettsia salmonis*	*Claudia Mardones*
Mechanistic insights into cytoskeletal Involment in *P. salmonis* traslocation across epithelial RTGutGC cells	*Raibel Suarez*
The pangenome of *Piscirickettsia salmonis* reveal, different evolutionary trajectories given host-pathogen interaction and environmental production factors.	*Víctor Martínez*

Keynote Lecture



"From evidence to action: Scientific Advances in Piscirickettsia salmonis Towards an Integrated Disease Management Strategy"

Dr. Fernando Mardones, an academic at the University of Edinburgh and an international authority on aquaculture health.

"Global lessons, local solutions: Piscirickettsiosis in Europe and its main challenges. How the PisciNor® project and international collaborative research contribute to knowledge to control the disease in Chile and the world."

Dr. Hamish Rodger
Founding Partner of VET-Aqua International, dedicated to research, consulting, and training in aquatic animal health.





"Social and economic impacts of disease in the salmon industry"

Dr. Frank Asche
Distinguished Professor, Natural Resource Economics
School of Forest, Fisheries, and Geomatics Sciences
University of Florida

FINE-MAPPING SRS RESISTANCE IN ATLANTIC SALMON TO IMPROVE GENOMIC SELECTION AND IDENTIFY GENE-EDITING TARGETS

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Abstract

Salmon Rickettsial Syndrome (SRS), caused by *Piscirickettsia salmonis*, is the main infectious disease affecting Chilean Atlantic salmon farming and a major cause of mortality and economic loss. Current control combines vaccination, biosecurity, and therapeutic treatment. Breeding for higher resistance is a complementary and durable approach that can reduce losses and lower the need for treatment over time. Selecting for SRS resistance, and potentially editing the underlying genes, requires knowledge of the genomic regions that control it. Here we combined a genome-wide association study, higher-density imputation, and ongoing WGS fine-mapping. The aim is to turn broad peaks into specific variants and candidate genes that can be used in breeding programs and functional studies. Binary SRS mortality was analyzed in 8,052 Atlantic salmon (3,659 cases and 4,393 controls) genotyped with a custom medium-density SNP array using LDAK. Significance was assessed at two levels: a false discovery rate across all markers (genome-wide) and within each chromosome (chromosome-wide, $q < 0.05$). Lead SNPs were remapped to the Ssal_v3.1 assembly, and candidate genes and enriched pathways were searched within 500 kb of each. A higher-density imputed set (6,030 fish, ~59,000 SNPs) was then used to confirm these signals and refine their resolution. Whole-genome sequence from selected ancestors is currently being generated and will be projected into all phenotyped animals to fine-map the QTL intervals. In this first screening, five genome-wide significant regions were detected, on chromosomes SSA2, SSA4, SSA9, SSA10, and SSA19, with the strongest signals on SSA10 (81.73 Mb) and SSA2 (54.77 Mb) (Figure 1). Chromosome-wide testing detected additional regions on SSA2, SSA8, and SSA10, consistent with a polygenic architecture. The most robust positional candidates were evpla (SSA2), dnajc18 (SSA4), banp (SSA10) and epb41l3a (SSA19), genes related to epithelial and cytoskeletal integrity, protein homeostasis, and transcriptional control; pathway analysis pointed to innate immune signaling (Toll-like receptor, NF-κB, and interferon). Day-to-death resistance is moderately heritable ($h^2 \approx 0.39$ in the routine genetic evaluation), confirming that it responds to selection, whereas the five lead SNPs jointly explained only a small proportion of the phenotypic variance (about 1–2%), reflecting a few small-effect loci on a largely polygenic background.

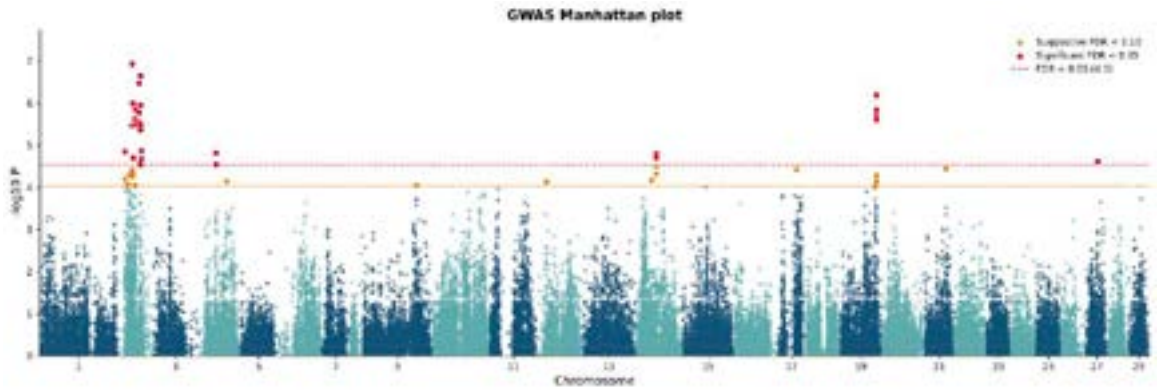


Figure 1. Genome-wide association analysis for SRS mortality**.** Manhattan plot showing -log10(*p*) values across chromosomes. The red dashed line indicates the genome-wide significance threshold (q-value < 0.05), while the orange dashed line indicates the chromosome-wide significance threshold (q-value < 0.10).

SRS resistance in Atlantic salmon is heritable and polygenic, based on a small set of robust candidate regions and immune-related genes. Because resistance responds to selection, genomic selection is a durable tool that complements vaccination, biosecurity, and health management and, by reducing the disease burden, can lower the need for treatment over time. Standard genomic selection already captures usable signal, and the ongoing WGS fine-mapping is expected to narrow these regions, improve prediction, and identify the likely causal variants within them, or reveal additional small-effect variants that lower-density panels cannot capture. These variants, and the genes surrounding them, are useful for functional validation and, subsequently, gene editing.

Keywords: *Piscirickettsia salmonis*; disease resistance; GWAS; heritability; genomic selection; sustainable disease control.

Acknowledgments: This study was carried out through a collaboration between CSIRO and Hendrix Genetics, which provided funding and data.

EXPLORING GENOME-WIDE PRESENCE–ABSENCE VARIATION (PAVS) BETWEEN ATLANTIC SALMON FAMILIES WITH CONTRASTING RESISTANCE TO *Piscirickettsia salmonis*

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Abstract

Salmonid rickettsial septicemia (SRS), caused by *Piscirickettsia salmonis*, remains one of the main health challenges affecting Atlantic salmon (*Salmo salar*) aquaculture, particularly in Chile. Although selective breeding programs have contributed to improving disease resistance, the genomic mechanisms underlying differences in susceptibility to *P. salmonis* remain incompletely understood. Most genomic studies have focused on single-nucleotide polymorphisms (SNPs), whereas the contribution of structural genomic variation, particularly gene presence–absence variation (PAV), has received considerably less attention. These variants may influence phenotypic differences by modifying gene content and affecting biological functions associated with host defense and pathogen response. Here, we investigated genome-wide PAV patterns in Atlantic salmon belonging to two distinct genetic backgrounds characterized by contrasting tolerance to SRS. Whole-genome sequencing data were analyzed using a coverage-based approach to identify genomic regions showing variable presence across individuals. Sequencing reads were mapped against the Atlantic salmon reference genome, and normalized read coverage was evaluated across genomic windows to identify candidate PAV regions. Comparative analyses were designed to characterize variation within and between genetic backgrounds and to explore potential associations between PAV profiles and disease tolerance. Candidate genomic regions were further examined to identify overlapping genes and biological functions potentially involved in host–pathogen interactions. This approach aims to determine whether differences in genomic content contribute to the contrasting responses to *P. salmonis* observed between Atlantic salmon genetic backgrounds. By exploring a relatively understudied component of genomic diversity, this study provides a novel approach for identifying candidate structural variants that may contribute to SRS resistance. These findings may complement SNP-based approaches and support the future development of genomic markers for selective breeding programs aimed at improving disease resistance in Atlantic salmon aquaculture.

SPATIAL CLUSTERING OF ANTIMICROBIAL USE AND *Piscirickettsia salmonis* MORTALITY IN CHILEAN SALMON AQUACULTURE: A RISK-BASED FRAMEWORK FOR TARGETED AMR SURVEILLANCE

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Abstract

The rapid expansion of Chilean salmon aquaculture has been accompanied by high levels of antimicrobial use (AMU), primarily for the control of *Piscirickettsia salmonis*, the etiological agent of Salmonid Rickettsial Septicaemia (SRS). Increasing reports of reduced susceptibility to florfenicol raise concerns regarding antimicrobial resistance (AMR) and the sustainability of current disease management strategies. Although national surveillance programs exist, they operate largely at regional scales and do not incorporate spatially explicit risk differentiation within production areas. This study proposes a geospatial framework for risk-based surveillance of antimicrobial use (AMU) in Chilean marine salmon farming. Using national-level production, mortality, and antimicrobial consumption data from all marine grow-out farms in Los Lagos and Aysén regions between 2015 and 2024, AMU, quantified for each farm and productive cycle using a standardized indicator equivalent to the Chilean Index of Antimicrobial Consumption (ICA), and SRS mortality per productive cycle was quantified. Spatial analyses were conducted to evaluate the geographic distribution and spatial dependence of AMU and SRS mortalities across marine grow-out farms. Farm-level geographic coordinates were used to construct spatial weight matrices representing neighbourhood structure among farms. To characterize spatial relationships among farming sites, maritime distances between farms were estimated between all pairs of farms. Global spatial autocorrelation of AMU and SRS mortality indicators was first assessed using Moran's I statistic. Following, Local Indicators of Spatial Association (LISA) were then applied to identify localized spatial clusters. In both analyses, neighbourhood relationships among farming sites were defined using a maritime distance threshold of 11 km. Farms separated by distances equal to or less than this threshold were considered neighbours. Based on the sign and statistical significance of the local LISA statistic, farming sites were classified into four cluster types: high–high (high values surrounded by high values), low–low (low values surrounded by low values), high–low (high-value sites surrounded by low-value neighbours), and low–high (low-value sites surrounded by high-value neighbours). For AMU and SRS mortality, Moran's I indicated significant positive spatial autocorrelation in both Los Lagos and Aysén regions, suggesting that similar values of cycle-level median SRS mortality tend to cluster geographically rather than being randomly distributed. Local analysis using LISA allowed the identification and quantification of hotspots (high-high clusters) and coldspots (low-low clusters) for both SRS mortality and AMU, identifying high and low-risk areas and supporting the implementation of targeted surveillance and risk-based management strategies. In Los Lagos region, the ACS with the highest concentration and spatial clustering of hotspots for SRS mortality were ACS 17B and ACS 9C. For AMU, the highest number and clustering of hotspots were observed in ACS 17B and 9B. The ACS with the highest concentration and clustering of coldspots for both variables was ACS 1, followed by ACS 17A. Although no coldspots for AMU were identified in ACS 9A, the spatial distribution of coldspots for both variables remained broadly consistent across the region. In Aysén region, for SRS mortality, the ACS with the highest concentration and clustering of hotspots were ACS 18C, 22D, and 32. Similarly, for AMU, the highest number and clustering of hotspots were observed in ACS 18C. For SRS mortality, the highest concentration of coldspots was observed in ACS 20, followed by ACS 30A, 32, and 19A. Similarly, for AMU, ACS 20 showed the highest concentration of coldspots, followed by ACS 30A.

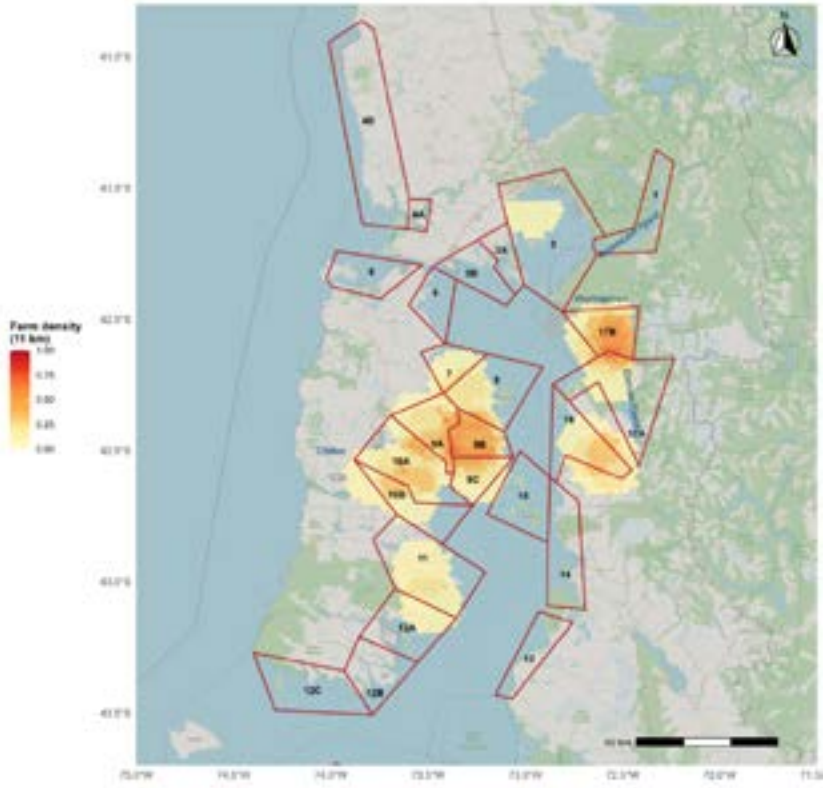


Figure 1. Spatial density of antimicrobial use (ICA) hotspots in the Los Lagos region. The heatmap represents the normalised density of farming sites classified as high–high LISA clusters (hotspots) for the antimicrobial consumption index (ICA).

This study demonstrates that AMU and SRS-associated mortality in Chilean salmon aquaculture are inherently spatial processes, characterized by well-defined clusters of high and low risk across production areas. By integrating production, health, and spatial data, the proposed framework identifies priority zones where disease burden and AMU are concentrated, providing a robust basis for risk-based surveillance and targeted intervention. These findings highlight the limitations of current generalist approaches to antimicrobial stewardship, which overlook spatial heterogeneity and may fail to address localized drivers of disease and treatment intensity. In contrast, a spatially explicit perspective enables more efficient allocation of surveillance and control efforts, with the potential to achieve disproportionate reductions in AMU by focusing on high-risk areas. Beyond its application to SRS, the methodology developed here offers a scalable and transferable tool for the management of infectious diseases in aquaculture systems. Incorporating spatial risk into decision-making represents a critical step toward the strategic management of diseases, reducing antimicrobial dependence, and advancing more sustainable aquaculture production systems aligned with One Health principles.

Keywords: Antimicrobial resistance; spatial clustering; hotspot analysis; LISA; risk-based surveillance; florfenicol; salmonid rickettsial septicaemia; SRS; One Health; Chile.

Acknowledgments: The authors gratefully acknowledge the salmon farming companies and industry representatives who contributed operational data and provided feedback during the development of this work. We also thank the staff of the Servicio Nacional de Pesca y Acuicultura (SERNAPESCA) for their support in accessing official production, health, and antimicrobial use records through public transparency mechanisms.

SYSTEMATIC LITERATURE REVIEW OF COSTS ASSOCIATED WITH DIFFERENT MANAGEMENT STRATEGIES AIMING TO REDUCE PATHOGEN INFESTATIONS IN SALMON FARMING

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Abstract

Antibiotic resistance in animal production is a global priority, so exploring alternative strategies to reduce antimicrobial use (AMU) is crucial. The aim of this paper is to perform a review of published scientific papers that inform on the costs associated with the implementation of alternative methods/ strategies to reduce pathogen infestations in salmon farming, and the costs associated with these methods/strategies as compared with the costs of the antimicrobial use (AMU) strategy. This information is important to calculate the cost-effectiveness of different methods/strategies. Our goal is to identify and compare the costs of the main strategies used to control pathogen infestations. We use several search engines to recover studies with relevant information, a 2020 PRISMA guideline protocol, and JBI Sumari external platform to manage the review. The number of articles in main search platforms of published studies on the economic costs of alternative methods/ strategies to reduce pathogen infestations in salmon farming is meagre. We found very few articles that discuss the economic costs of the methods used to reduce bacterial diseases in salmon farming. When the search requirement included having detailed information about the identification of the specific costs items that were included in the total monetary and non-monetary costs of disease control, article availability was almost inexistent. If the cost effectiveness determinants of different alternative methods/strategies to reduce AMU in salmon farming are scarcely known, it becomes difficult to discuss the economic convenience of reducing the use of antimicrobials. Moreover, the definition of the scope of the costs that should be included in the cost-effectiveness analysis is missing in the literature and discussed in the paper. More research on these subjects is urgently required**.**

Keywords: Salmon farming; antimicrobial use; costs.

A MODEL FOR COST-BENEFIT ANALYSIS OF ANTIMICROBIAL USE IN SALMON AQUACULTURE

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Abstract

Antimicrobial use (AMU) in salmon aquaculture is regulated through veterinary prescription limits backed by imperfect monitoring and monetary fines. Yet, the economic incentives farmers face to exceed those limits have received little formal treatment. The aim of this research is to assess the deterrent effect that the existing regulatory instruments have on the compliance behavior of producers. This paper adapts the regulatory-compliance framework of Sutinen and Kuperan (1999) to a salmon farm choosing AMU to maximize expected profit over a saltwater production cycle. Mortality is modeled as a linear function of antimicrobial intensity, so the expected gain from exceeding the prescribed dose reduces to a compact expression combining the net marginal value of additional treatment, the size of the violation, and the expected fine. The mortality–AMU parameter is estimated by instrumental variables on firm-level production, mortality and antimicrobial data for Chile, 2022–2024. The model is then parameterized with industry price and cost data, the regulated dosage, the statutory fine and a one-percent detection probability and evaluated for violations of up to ten percent above prescription. At central parameter values the expected gain from non-compliance is positive over most of the range considered and remains positive even at the maximum statutory fine; it turns negative only for the smallest farms or when the estimated mortality response approaches zero. The results suggest that, at current detection probabilities, the fine schedule alone is unlikely to deter AMU above prescription among large producers, and that monitoring intensity is the more effective policy margin.

Keywords: antimicrobial use; salmon aquaculture; regulatory compliance; cost-benefit analysis.

10 YEARS OF EPIDEMIOLOGICAL SURVEILLANCE IN SALMON FARMING IN CHILE: A 2014-2024 RETROSPECTIVE STUDY OF INFECTIOUS DISEASE MORTALITY RISK

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Abstract

The expansion of the salmon farming industry in Chile has been accompanied by several sustainability challenges, chiefly among them the rising threat of infectious diseases. Currently, the disease with the highest share of mortality risk is salmonid rickettsial septicemia (SRS), caused by the Gram-negative bacterium *Piscirickettsia salmonis*. SRS and other infectious diseases impose significant economic losses and place pressure on part of producers to employ large amounts of antimicrobial agents, which may contribute to antimicrobial resistance and environmental degradation. Management of infectious diseases through an integrated disease management strategy which prioritizes primordial and primary prevention is essential. However, implementing such a strategy demands a detailed understanding of the epidemiology of these diseases including, importantly, of their risk determinants. In a previous study, we identified infestations with the ectoparasite *Caligus rogercresseyi* as a predictor of SRS mortality risk (Diethelm-Varela et al., *J. Fish Dis.* 2025. DOI: 10.1111/jfd.70097), highlighting the importance of primary prevention toward achieving better outcomes in salmon farming. In Chile, epidemiological surveillance and infectious disease control plans are based on current guidelines of the National Fisheries Service (Sernapesca). These guidelines have resulted in the creation of an extensive database, the Sistema Integrado de Fiscalización de la Acuicultura (SIFA), which aggregates all historical mortality data for infectious diseases. This database represents a unique opportunity to leverage detailed epidemiological data to achieve a better understanding of the main infectious diseases affecting salmon farming. Consequently, the main aim of this study was to provide a comprehensive epidemiological characterization of the main infectious diseases affecting the Chilean salmon farming system. To fulfill this aim, we conducted a retrospective cohort epidemiological study that details 10 years of Chilean salmon farming, from 2014 to 2024, focusing on the six most relevant infectious diseases in terms of their contribution to overall mortality risk in salmonids: SRS, tenacibaculosis, bacterial kidney disease (BKD), heart and skeletal muscle inflammation (HSMI), infectious pancreatic necrosis (IPN), and amebiasis. For this study, we performed a retrospective cohort study design. The date range was from June 2014 to November 2024. Our observation unit was the production week at salmonid farms during a production cycle. Our data covered a total of 2659 production cycles from 811 salmon farms distributed across 88 farming "neighborhoods", located across the Los Ríos, Los Lagos, Aysén, and Magallanes regions. The three farmed species included in this study were Atlantic salmon, rainbow trout, and Coho salmon. Our main data source was the salmonid mortality data from the SIFA database, which includes mortality reports from all non-infectious and infectious causes, the latter of which we modeled as our outcome variables. Namely, our dependent variables of interest were the weekly mortality risk for: SRS, tenacibaculosis, BKD, HSMI, IPN, and amebiasis. We also employed data on *C. rogercresseyi* infestations from SIFA, and water surface temperature, salinity, and downward radiation intensity from the European Climate Data Store, as predictors. For each disease, we produced descriptive statistics and constructed time series, through which we described the general patterns, trend, and seasonality of the weekly mortality risk over the study period. Using spatial analysis, we characterized the emerging phenomenon of SRS-tenacibaculosis coinfections. Through a negative binomial regression modeling

approach, employing mixed effects to account for the hierarchical structure of the system, we identified, for each disease, the effect that several putative predictors have on infectious disease mortality risk.

Our descriptive statistics indicate a high prevalence of high-load *Caligus* infestations, with 57.54% of cycles experiencing at least one infestation event with a parasite load greater than 1 egg-laying female parasite per fish, indicating that this parasite remains as a relevant source of morbidity in farmed salmonids. The time series analysis of weekly mortality risk reveals that SRS has remained the main source of mortality risk throughout the entire study period, but tenacibaculosis has displayed a sustained increase in risk since 2018, becoming the second highest source of infectious disease mortality risk in the 2018-2024 interval. Spatial analysis indicates that SRS-tenacibaculosis coinfections have become a common occurrence since 2018, and time series analysis suggests that for typical cycles, a coinfection is preceded by rising *Caligus* infestation loads, suggesting this ectoparasite acts as a risk determinant for coinfectious outbreaks. Our negative binomial models showed that several factors act as risk determinants for the six infectious diseases we modeled, with temperature, salinity, time elapsed along the production cycle, physical trauma events, and *C. rogercresseyi* infestations being significant risk predictors. Analysis of our goodness-of-fit metrics also indicates that, for all diseases, most of the variance of the outcome variable is explained by the nested structure of the farming system, suggesting a key role of the structural design of the system toward understanding mortality risk with, for example, hydrodynamic connectivity within farms plausibly playing a role. This study provides a thorough 10-year epidemiological assessment of the six most important diseases in Chilean salmon farming: SRS, tenacibaculosis, BKD, HSMI, IPN, and amebiasis. We obtained insights on the overall trend and seasonal patterns of these diseases. Our study highlights SRS and tenacibaculosis as the two main diseases of the Chilean salmon farming system, with the first being the main source of infectious mortality, and the latter representing an emergent threat to salmon farming. The phenomenon of SRS-tenacibaculosis coinfections are described for the first time here in a field study, and we show that they are preceded by *Caligus* infestations. Furthermore, for each disease, we studied the effect that several putative predictors have on the weekly mortality risk of each disease, identifying novel risk determinants which could assist in primary prevention. Crucially, we determined that most of the infectious disease mortality risk is explained by the nested structure of the salmon farming system, arguing for the need to re-assess the system's design to achieve better outcomes, and thus highlighting the importance of primordial prevention. Overall, our results provide novel insights for infectious disease prevention in salmon farming which producers and authorities can employ to design better control strategies.

Keywords: Salmon farming; infectious disease epidemiology; retrospective cohort study; SRS; tenacibaculosis.

Acknowldegments: The authors acknowledge the Asociación Nacional de Investigación y Desarrollo de Chile (ANID) for the financial support of this research. In particular, BDV is a beneficiary of the ANID National Doctoral Scholarship # 21221163.

FROM HYDRODYNAMIC CONNECTIVITY TO ACTIVE SURVEILLANCE: A HYBRID STOCHASTIC-DETERMINISTIC MODEL FOR PISCIRICKETTSIOSIS RISK IN CHILEAN SALMON FARMING

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Abstract

Piscirickettsiosis remains a major health constraint for marine salmon farming in Chile. Its causative bacterium, *Piscirickettsia salmonis*, can be transmitted through seawater, making disease risk dependent not only on farm-level conditions but also on the hydrodynamic connectivity among farms. Conventional surveillance based on observed disease and mortality may therefore detect transmission only after it is underway. We present a hybrid modelling framework that combines mechanistic oceanographic simulations with a stochastic epidemiological model and show how it can support an active, risk-based surveillance and early-warning system for the Chilean salmon farming industry. Here, disease risk refers to the weekly probability that a farm is in an infected state, given upstream exposure and local oceanographic conditions. The framework synthesizes a case study covering January 2015 to December 2016 in the Los Lagos Region. Weekly production and piscirickettsiosis mortality records from 217 farm sites and 318 production cycles were combined with hourly predictions of currents, seawater temperature and salinity from a calibrated three-dimensional hydrodynamic model. Particle-tracking simulations represented the survival and passive transport of *P. salmonis* and generated weekly 217 x 217 farm-to-farm connectivity matrices. Potential connectivity was combined with reported mortality over a six-week influence window to calculate two exposure metrics: the number of upstream infected farms and cumulative exposure to upstream mortality weighted by hydrodynamic connectivity. A stochastic, state-based model then estimated weekly transitions among disease-free, infected and recovered states as functions of these metrics, temperature, salinity and stocking week. Building on the fitted model, we designed an active surveillance workflow linking modelled potential connectivity, near-real-time sanitary data and targeted confirmation of *p. salmonis* in fish tissues and/or seawater at downstream farms.

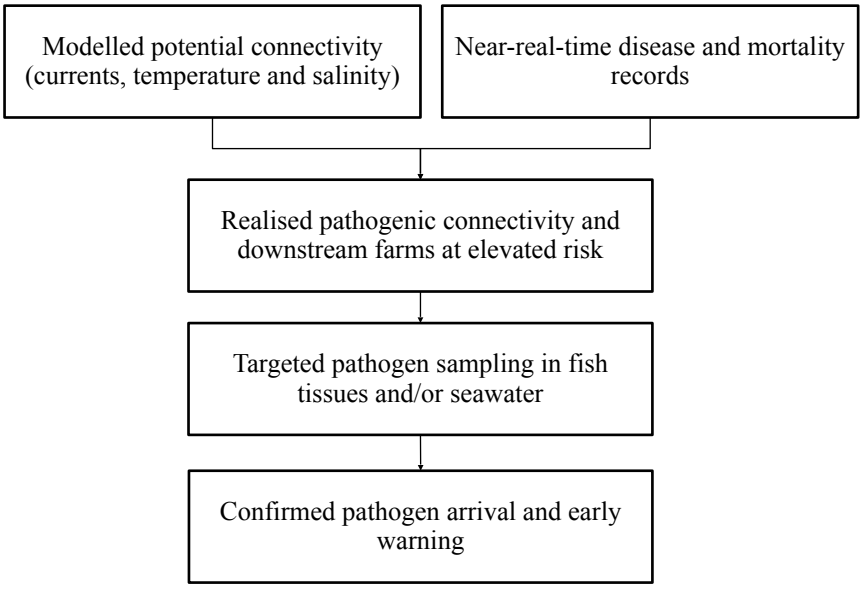


Figure 1. Proposed workflow for active surveillance of waterborne piscirickettsiosis transmission.

Farm sites were potentially connected to a mean of 15.3 farms per week; this decreased to 2.45 upstream farms when only sites reporting piscirickettsiosis mortality were considered. The strongest determinants of infection probability were, in descending order, the number of upstream infected farms, cumulative connectivity-weighted mortality, salinity and temperature, while stocking week had little influence. Observed prevalence became significantly higher after production week 10 in farms exposed to waterborne infection. It was approximately 60% by week 15 and exceeded 90% after week 25; in the model application, observed and predicted prevalence stabilized near 90% around week 40. In contrast, isolated farms with no estimated exposure to upstream infection reached only about 10% prevalence by weeks 54-56. Higher salinity and temperature were also associated with higher prevalence, although their effects were weaker than hydrodynamic exposure. These results show that distance alone is an incomplete proxy for transmission risk and that time-varying pathogenic connectivity can identify downstream farms requiring intensified surveillance. The hybrid framework provides a practical basis for moving from reactive case reporting to risk-based surveillance. When an upstream outbreak is reported, modelled currents can identify downstream farms at elevated risk, guide targeted water or tissue sampling, and support earlier warnings. The same information can inform coordinated vaccination, treatment, harvest, fallowing and quarantine decisions, and may help reduce reinfection and antibiotic use. Integration with continuous environmental and sanitary data streams would allow probabilities to be updated as conditions change, providing a core component of an adaptive digital twin for fish-health management. Prospective implementation should, however, validate predictions against pathogen measurements and refine uncertain processes, including bacterial shedding, infectious dose, survival in seawater, farm practices, and co-infections.

Keywords: piscirickettsiosis; hydrodynamic connectivity; stochastic epidemiology; risk-based surveillance; digital twin.

Acknowledgments: The original case study was developed within the SERNAPESCA Program for the Sanitary Management of Aquaculture (projects FIE-V014 and FIE-201707240090), funded through public-private arrangements involving the Chilean Ministry of Economy, Development and Tourism and SalmonChile.

EVALUATION OF PERUVIAN MACA (*Lepidium meyenii*) EXTRACT ON IMMUNOMODULATION, ANTIOXIDANT DEFENSE AND EPITHELIAL BARRIER INTEGRITY AGAINST *Piscirickettsia salmonis* IN RTGUT-GC CELLS

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Abstract

In modern salmonid aquaculture, maintaining functional intestinal epithelial barrier integrity and immune homeostasis is critical to withstand environmental stressors, nutritional variations, and pathogen invasion. *Piscirickettsia salmonis*, the causative agent of Piscirickettsiosis, remains one of the most severe bacterial threats to the salmon industry in Chile, inducing localized mucosal degradation, systemic pathology, and high mortality. The fish gut serves not only as a primary site for nutrient absorption but also as a physical and immunological barrier against systemic colonization. Phytogenic feed additives derived from medicinal plants, such as Peruvian Maca (*Lepidium meyenii*), are rich in bioactive compounds including glucosinolates, polyphenols, macaenes, and unique alkaloids with recognized immunomodulatory, antioxidant, and tissue-protective properties. However, the effects of Peruvian Maca extract on fish intestinal cells have not been reported to date, and its precise temporal kinetics and functional mechanisms remain unknown. Therefore, the objective of this study was to evaluate the immunomodulatory, antioxidant, and barrier-protective effects of *Lepidium meyenii* extract in rainbow trout intestinal (*RTgut-GC*) cells challenged with *P. salmonis*. To evaluate time- and dose-dependent effects, rainbow trout intestinal epithelial cells (*RTGut-GC*) were exposed to increasing concentrations of Peruvian Maca ethanolic extract (0, 0.5, 1, 5, 10, and 15 µg/mL) for 6, 24 and 48 h. The relative mRNA expression of key pro- and anti-inflammatory cytokines (*il-1β*, *tnf-α*, *tgf-β*), master antioxidant factors and enzymes (*nrf2*, *ho-1*, *cat*) and structural/functional gut barrier markers (*zo-1*, *e-cad*, *muc2*) was quantified by RT-qPCR normalized against *ef-1α* and *rps20* as reference genes. In addition, a protective pathogen challenge model was evaluated: *RTgut-GC* cells were pretreated with Maca extract (5 µg/mL) for 24 h prior to infection with *P. salmonis* (strains LF-89^T or EM-90; MOI: 10), and the relative mRNA expression of the same genes mentioned above was quantified by RT-qPCR. For functional barrier translocation assays, *RTgut-GC* cells were cultured on Transwell inserts for 28 days to establish fully differentiated, polarized monolayers with tight cell-cell junctions. Monolayers were pretreated with Maca (5 µg/mL for 24 h) and challenged with *P. salmonis* (strains LF-89^T or EM-90; MOI: 10). At 7 days post-infection (dpi), 100 µL aliquots were sampled from the basolateral chamber, plated on TS-Hem Austral agar, and incubated for 7–10 days to determine bacterial translocation rates (CFU/mL).

Baseline exposure revealed that Maca extract reduced the expression of inflammatory markers *il-1β* and *tnf-α* across all time points, peaking at 24 h at 5, 10, and 15 µg/mL ($p < 0.05$). Concurrently, *tgf-β* displayed an increase at 24 h followed by a decrease at 48 h at 5, 10, and 15 µg/mL ($p < 0.05$). Antioxidant markers showed a downregulation of *ho-1* across all times at 5, 10, and 15 µg/mL, while *nrf2* decreased at 10 and 15 µg/mL at 24 and 48 h ($p < 0.05$). Conversely, *cat* expression increased at 6 h, decreased at 24 h, and increased again at 48 h across most tested doses ($p < 0.05$). Regarding barrier junction markers, *zo-1* was induced only at 6 h ($p < 0.05$), *e-cad* decreased at higher concentrations, and *muc2* was upregulated after 48 h at 15 µg/mL ($p < 0.05$). In the infection model, *P. salmonis* (LF-89^T and EM-90) strongly induced *il-1β* and *tnf-α* expression across all time points; however, 24 h Maca pretreatment (5 µg/mL) significantly attenuated this pro-inflammatory induction ($p < 0.05$). Conversely, *tgf-β* expression dropped during infection but was restored nearly to control levels with Maca pretreatment ($p < 0.05$). For antioxidant markers, *ho-1* and *nrf2* showed no significant differences, whereas infection decreased *cat* expression at the earliest time point, and Maca pretreatment restored it to control levels ($p < 0.05$). Finally, in the Transwell translocation model, Maca pretreatment at 5 µg/mL for 24 hours, significantly reduced basolateral bacterial counts (CFU/mL, $p < 0.05$) compared to infection-only controls for both LF-89^T and EM-90 strains (Figure 1).

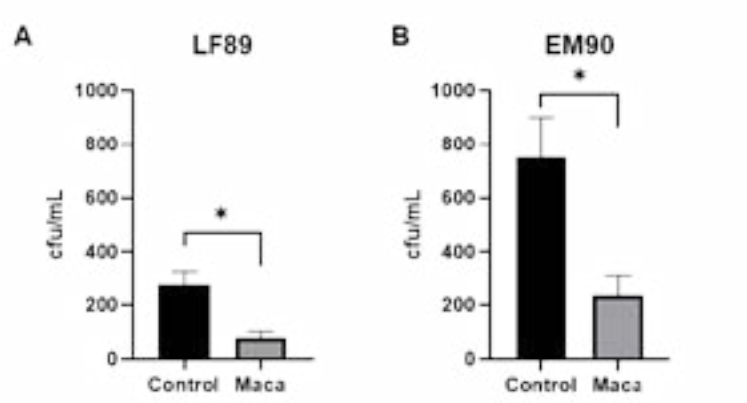


Figure 1. Effect of Maca extract on *P. salmonis* translocation. RTGut-GC cells cultured in Transwell systems were pretreated with 5 µg/mL Maca extract for 24 h and subsequently infected with *P. salmonis* LF-89^{T} (A) and EM-90 (B) (MOI: 10) for 7 days. Aliquots were collected from the basolateral compartment to quantify CFU/mL. Bars represent mean ± SD (n = 4). Asterisks (*) indicate statistically significant differences compared to the control calculated by Student's t-test ($p < 0.05$).

Peruvian Maca (*Lepidium meyenii*) extract exerts a protective effect on fish intestinal epithelial cells by blunting pathogen-induced pro-inflammatory cascades (*il-1β*, *tnf-α*), restoring immunoregulatory (*tgf-β*) and antioxidant (*cat*) responses, and enhancing mucosal barrier markers (*muc2*). Crucially, these molecular modifications translate into a significant functional reduction of *P. salmonis* translocation across *RTgut-GC* monolayers. These findings demonstrate for the first time the potential of Peruvian Maca as an effective phytogenic feed additive to reinforce mucosal immunity and control of Piscirickettsiosis in salmon farming.

Keywords: *RTgut-GC*; *Piscirickettsia salmonis*; *Lepidium meyenii*; Gut barrier integrity; Bacterial translocation; Mucosal immunity.

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VACCINATION AT DIFFERENT LIFE STAGES DETERMINES THE IMMUNE RESPONSE TO *Piscirickettsia salmonis* IN ATLANTIC SALMON: A TRANSCRIPTOME ANALYSIS

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Abstract

Vaccination programs in the salmon aquaculture industry are typically conducted during the freshwater stage when salmon weigh approximately 20 g. In Chile, data suggest that commercially available bacterial and viral vaccines provide suboptimal protection in seawater, particularly against *Piscirickettsia salmonis*. Optimizing the vaccination period relative to the production cycle could enhance immunological protection in seawater environments. This study aimed to evaluate parr and post-smolt salmon vaccination, and its effects on transcriptome modulation during *P. salmonis* infection. Atlantic salmon were vaccinated with a commercial vaccine during the parr and post-smolt stages, at weights of 20 g and 100 g, respectively. After 800 accumulated thermal units, vaccinated salmon were challenged by intraperitoneal injection with 0.1 ml of *P. salmonis* LF89 ($1x10^9$ cells/ml), and non-infected control groups for both stages were included. At 14 days post-infection (dpi), head kidney and gill tissue samples were collected for mRNA sequencing via Nanopore technology. RNAseq analysis and differentially expressed genes (DEGs) were performed, using the Atlantic salmon genome as a reference. DEGs were defined using a threshold of absolute fold change > 1.5 and FDR p-value < 0.05. DEGs were further analyzed through GO and KEGG pathway enrichment. Additionally, a mortality curve was constructed. Among infected fish, vaccinated parr showed a 3-day delay in mortality onset compared to vaccinated post-smolt, though both reached 86% cumulative mortality. Transcriptomic analysis identified 270 exclusive DEGs in vaccinated parr and 73 in vaccinated post-smolt, with 29 DEGs shared between both groups, revealing distinct transcriptional responses according to vaccination stage. Parr-vaccinated fish exhibited an upregulation of immune-related genes, particularly those linked to innate immunity, such as *tlr4* and *il-1β*, with KEGG enrichment in Toll-like receptor signalling and cytokine-cytokine receptor interaction pathways, suggesting early immune priming. Additionally, stress-response genes in the gills, such as *hsp70*, were more highly expressed in parr salmon, consistent with an increased cellular stress response. In contrast, the post-smolt group showed a marked upregulation of pro-inflammatory cytokines, including *TNF-α*, *IL-6*, and *IL-12*, with KEGG pathways related to Th1/Th2 cell differentiation highly enriched, consistent with an adaptive immune response. This study demonstrates that vaccination elicits distinct transcriptomic responses in Atlantic salmon, with life-stage specific immune mechanisms activated, potentially informing future vaccine strategies to enhance disease resistance in aquaculture.

Keywords: Vaccines; transcriptome; Atlantic salmon; *Piscirickettsia salmonis.*

Acknowledgments: FONDECYT-ANID 3240484, 1210852, 11220307; CIA-ANID grant #250009.

SINGLE-NUCLEI RNA SEQUENCING REVEALS THE IMMUNE CELL LANDSCAPE OF ATLANTIC SALMON HEAD KIDNEY DURING *Piscirickettsia salmonis* INFECTION

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Abstract

Salmonid Rickettsial Septicemia (SRS), caused by the intracellular bacterium *Piscirickettsia salmonis,* is highly impactful, accounting for 54.2% of infectious disease-related salmonid mortalities. In Chile, two major *P. salmoni*s genogroups, represented by LF-89 and EM-90 strains, have been described, with genomic differences in pathogenicity and transcriptional profiles reported. Despite the widespread use of commercial vaccines, protection against SRS remains limited, highlighting the need to better understand host immune cellular responses to distinct *P. salmonis* genogroups and the mechanisms underlying pathogen-host interactions. The aim of this study was to characterize systemic immune responses associated with distinct *P. salmonis* genogroups in Atlantic salmon, integrating snRNA-seq. Here, fish were assigned to four experimental groups: (1) control injected with PBS; (2) infection with 0.1 mL of an LD50 inoculum of EM-90; (3) infection with 0.1 mL of an LD50 inoculum of LF-89; (4) co-infection with both genogroups. Frozen head kidney samples were collected at 12 days post-infection (dpi). Single-nuclei RNA sequencing (snRNA-seq) was performed to identify immune cell populations and characterize cell-type-specific transcriptional responses. snRNA-seq revealed more transcriptionally distinct cell clusters in the LF-89 group than in control fish, suggesting infection-associated remodeling of the head kidney cellular landscape. Preliminary cell annotation based on *de novo* marker genes revealed major immune and non-immune populations, including macrophages, granulocytes, B cells, endothelial cells, erythroid progenitors, and steroidogenic interrenal cells. The LF-89 group exhibited greater cellular heterogeneity than the control group, whereas the mixed infection group contained distinct inflammatory macrophage and granulocyte populations, as well as specialized endothelial and erythroid cell populations. These findings provide the first cell-resolution characterization of the Atlantic salmon head kidney during infection with different *P. salmonis* infection scenarios and establish a cellular framework to understand host immune responses and identify potential biomarkers associated with SRS.

Keywords: *Piscirickettsia salmonis*; Atlantic salmon; single-nuclei RNA-seq; head kidney; immune cell atlas.

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MECHANISTIC INSIGHTS INTO CYTOSKELETAL INVOLMENT IN *Piscirickettsia salmonis* TRASLOCATION ACROSS EPITHELIAL RTGutGC CELLS

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Abstract

Piscirickettsiosis is a systemic infectious disease caused by *Piscirickettsia salmonis*, a Gram-negative, biofilm-forming bacterium that infects the main salmonid species farmed in Chile. Pathogenesis begins in the skin, gills, and gut, reaching target cells such as macrophages. Several studies have described the detailed infection mechanisms in macrophages, which are the primary cells for intracellular replication. We previously demonstrated that *P. salmonis* modulates immune and intercellular junction gene expression, leading to disruption of intestinal morpho-functionality in the rainbow trout intestinal epithelial cell line RTgutGC. The aim of this study was to determine the role of cytoskeletal dynamics in the infection process of *P. salmonis* and its ability to translocate across RTgutGC epithelial cells. To this end, RTgutGC cells were preincubated with inhibitors targeting tight junctions and cytoskeletal function: myosin light chain kinase inhibitor (ML-7, 5 µM), Cytochalasin D (25 nM), and Chlorpromazine (10 µM, a clathrin-mediated endocytosis inhibitor), and subsequently infected with *P. salmonis* strains LF-89^T and an isolated EM-90. Intracellular bacterial load was assessed using absolute qPCR quantification, TEER measurements, and CFU counts from bacterial translocation across the epithelial barrier. Results indicate that infection of RTgutGC cells by *P. salmonis* LF-89^T and EM-90 occurs within the first three hours, with differences observed between genogroups. Bacterial infection was influenced by cytoskeletal dynamics; however, the most striking finding was that disruption of cytoskeletal function enabled bacterial translocation, with distinct effects depending on the genogroup. For LF-89^T, the strongest effect was induced by Cytochalasin D, which increased bacterial translocation approximately one hundred-fold compared to the control, followed by ML-7 and Chlorpromazine. In the case EM-90, the greatest effect was observed with ML-7, which increased translocation nearly three hundred-fold compared to the control, followed by Cytochalasin D and Chlorpromazine. These findings suggest that cytoskeletal dynamics play an active role in the infective process of *P. salmonis* in RTgutGC intestinal epithelial cells with genogroup-specific differences.

Keywords: *Piscirickettsia salmonis*; RTGutGC; infection; cytoskeleton dynamic.

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TRANSPOSABLE ELEMENT ACTIVATION AND ALTERNATIVE SPLICING REPROGRAMMING DURING PISCIRICKETTSIOSIS: A COMPARATIVE GENOMIC STUDY IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Abstract

Piscirickettsiosis, caused by *Piscirickettsia salmonis*, is the most costly infectious disease of Chilean salmonid aquaculture, affecting both Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). We previously reported, in independent genome-wide analyses of Atlantic salmon head-kidney transcriptomes, (i) widespread transcriptional activation of transposable elements (TEs) following *P. salmonis* infection, with 4550 differentially expressed TE loci (84.4% upregulated, dominated by TIR/TcMar and LINE families), and (ii) extensive infection-induced alternative splicing (AS) reprogramming, with 158,668 splicing events and hundreds of differentially spliced genes (DSG) enriched in spliceosome and RNA-binding functions. Whether this TE activation–AS coupling reported in Atlantic salmon represents a salmonid-conserved regulatory response, or a species-specific phenomenon, remains unknown. Rainbow trout, which shares the salmonid-specific whole-genome duplication and a comparably TE-rich genome, but diverged from Atlantic salmon over 50 million years ago, offers a suitable model to test the conservation of this mechanism. Head-kidney RNA-seq libraries were generated from control and *P. salmonis* (LF-89)-challenged rainbow trout (3 and 9 days post-infection, n = 45 total), and processed with the same pipeline previously applied to Atlantic salmon. A species-specific rainbow trout TE consensus library was built with EarlGrey/RepeatMasker against the reference genome USDA_OmykA_1.1. Locus-specific TE expression (ExplorATE, DESeq2) and differential alternative splicing events (DASE; rMATS, $|\Delta PSI| \geq 10\%$, FDR <0.01) were quantified in parallel. BEDTools was used to intersect intronic coordinates of DSG with annotated TE loci (±2 kb of splice junctions), and Pearson correlation/linear models tested the association between locus-specific TE expression (TPM) and PSI values of proximal AS events. FIMO/JASPAR scanning identified cryptic splice-site and SR-protein binding motifs within TE sequences overlapping DASE junctions. Results were directly benchmarked against the previously published Atlantic salmon TE and AS datasets to assess cross-species conservation. Rainbow trout showed a comparably marked TE transcriptional activation upon infection, with 3912 differentially expressed TE loci (79.6% upregulated), dominated by TIR/TcMar and LINE/L2 families, closely mirroring the class-level composition reported in Atlantic salmon. In parallel, 142,850 AS events were detected, generating 287 DSG enriched in RNA processing and spliceosome functions, consistent in magnitude with the Atlantic salmon dataset. Intronic TE insertions were significantly enriched within trout DSG relative to non-DSG genes (35.7% vs. 18.9% of introns harboring an annotated TE; ~1.9-fold enrichment, $p < 0.001$), essentially replicating the enrichment previously found in Atlantic salmon (38.2% vs. 19.4%). A total of 53 TE–AS event pairs displayed significant positive correlation between local TE expression and PSI values ($|r| \geq 0.7$, $p < 0.05$) in trout, versus 61 in salmon, with 19 orthologous gene pairs showing coupled TE–AS regulation in both species. TE sequences overlapping DASE junctions in trout were similarly enriched for cryptic splice-donor/acceptor motifs and SR-protein (SRSF7, hnRNPM) binding sites. Functional enrichment of genes under coupled TE–AS regulation converged on innate immune signaling (Toll-like receptor, apoptosis) and RNA metabolic processes in both species, and the 19 shared orthologous pairs were significantly overrepresented in spliceosome-associated transcripts, indicating conservation of the proposed feed-forward circuit in which TE activation modulates the splicing of splicing-regulatory genes.

This comparative analysis provides the first evidence in rainbow trout that *P. salmonis* infection triggers coupled TE activation and alternative splicing reprogramming, closely paralleling the pattern previously described in Atlantic salmon. The conservation of this TE–AS regulatory layer across two divergent salmonid hosts strengthens the hypothesis that mobilome activation is a genuine, non-species-specific component of the salmonid response to Piscirickettsiosis, and highlights shared TE-linked splicing events as candidate cross-species biomarkers for resistance/susceptibility phenotyping and future genetic-selection programs.

Keywords: Transposable elements; Alternative splicing; *Piscirickettsia salmonis*; *Oncorhynchus mykiss*; Comparative salmonid genomics.

IDENTIFICATION AND GENOMIC CHARACTERIZATION OF THE TYPE 1 SECRETION SYSTEM (T1SS) IN *Piscirickettsia salmonis*

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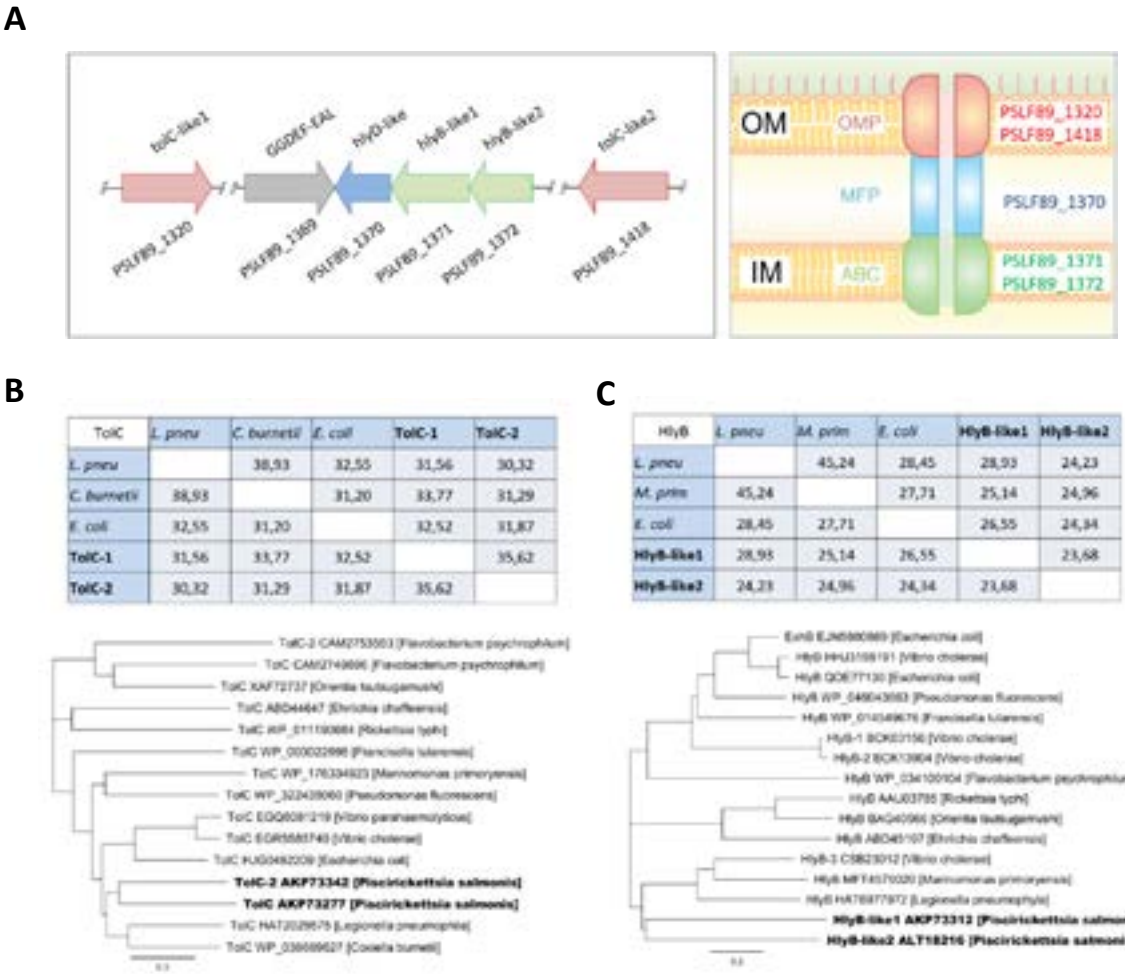
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Abstract

Piscirickettsia salmonis is the etiologic agent of piscirickettsiosis, the most prevalent bacterial disease affecting farmed fish, which generates great economic losses in the Chilean salmon aquaculture. Despite the impact in fish health and the high mortality, several molecular mechanisms involved in the secretion of virulence factors are still partially identified. The type 1 secretion system (T1SS) is a key molecular machinery involved in exporting proteins and toxins in Gram-negative bacteria. Thus, the aim of this study was to identify and characterize *in silico* the structural components of the T1SS in *P. salmonis* genome. The protein sequences of all predicted ORFs for NZ_CP011849.2 (LF-89 ^{T}) and NZ_CP038208.1 (EM-90 genogroup) were obtained from GenBank and then analyzed with TXSScan (v1.1.0) for identification of secretion system components. Identified proteins were analyzed with CDD (v3.2.0) for domain annotation. Phylogenetic analyses were performed using MAFFT (v7.0). Both *P. salmonis* genomes (LF-89^{T} and EM-90) harbored two copies of the *tolC* gene, encoding predicted proteins that differed by 67 residues and sharing 35.62% sequence identity. Similarly, two copies of a gene associated with *hlyB* were identified, differing by 139 residues and sharing 23.68% sequence identity. Phylogenetic analyses revealed that all structural components clustered with their respective orthologs in *Legionella pneumophila*, *Coxiella burnetii*, and *Escherichia coli*. Finally, one ORF encoding a protein harboring RTX domains were identified in both LF-89^{T} and EM-90 genomes. This protein exhibited low sequence identity (13.98%) to the HlyA toxin of *E. coli*, and it was found in LF-89^{T} biofilm proteome. This suggests the need for further characterization to determine whether it function as an effector of the T1SS. These results reveal the presence of genes associated with T1SS in *P. salmonis*, including duplication and sequence divergence of key components, TolC and HlyB. Although an RTX domain-containing protein was identified, its low sequence identity to known T1SS effectors, suggest that further characterization is required to determine whether it functions as a T1SS effector.

			LF-89^{T} (NZ_CP011849.2)			EM-90 (NZ_CP038208.1)		
Component	Identified domain [Acc. no.] (e-value)	Requirement	Locus	CDS	Length (aa)	Locus	CDS	Length (aa)
TolC	tolC [PRK09465] (8.59e-97)	Required	PSLF89_1320 (tolC-like1)	AKP73177.1	419	E3220_RS01875	WGZ72970.1	418
	tolC [PRK09465] (6.38e-89)		PsLF89_1418 (tolC-like2)	AKP73342.2	486	E3220_RS06900	WGZ71333.1	480
HlyB	type_I_sec_LssB [TIGR03375] (2.70e-106)	Required	PSLF89_1371 (HlyB-like1)	AKP73312.1	706	E3220_RS02045	WGZ70555.1	706
	type_I_sec_HlyB [TIGR01846] (7.80e-91)		PSLF89_1372 (HlyB-like2)	AJT18216.1	567	E3220_RS02050	WGZ70556.1	567
HlyD	type_I_HlyD [TIGR01843] (1.35e-108)	Required	PSLF89_1370	AKP73311.1	449	E3220_RS02040	WGZ70554.1	449
GGDEF-EAL dom containing protein	Cyclic di-GMP metabolism protein [COG5001] (0.00)	Regulatory	PSLF89_1369	AKP73130.2	715	E3220_RS02035	WGZ70553.1	715
HlyA	RTX toxin-related [COG5293] (1.31e-24)	Effector (?)	PSLF89_1786	AKP73634.1	1290	E3220_05330	WGZ71113.1	1337

Table 1. T1SS components identified in *P. salmonis* genomes (LF-89^{T}:NZ_CP011849.2; EM-90: NZ_CP038208.1). The accession number of the most probable putative domain identified by CDD (v3.2.0) is indicated.



TolC	*L. pneu*	*C. burnetii*	*E. coli*	TolC-1	TolC-2
L. pneu		38,93	32,55	31,56	30,32
C. burnetii	38,93		31,20	33,77	31,29
E. coli	32,55	31,20		32,52	31,87
TolC-1	31,56	33,77	32,52		35,62
TolC-2	30,32	31,29	31,87	35,62	

HlyB	*L. pneu*	*M. prim*	*E. coli*	HlyB-like1	HlyB-like2
L. pneu		45,24	28,45	28,93	24,23
M. prim	45,24		27,71	25,14	24,96
E. coli	28,45	27,71		26,55	24,34
HlyB-like1	28,93	25,14	26,55		23,68
HlyB-like2	24,23	24,96	24,34	23,68	

Figure 1. Identification of T1SS components in *P. salmonis*. A) Schematic representation of T1SS operon components. Percentage identity of the putative TolC (B) and HlyB (C) components from LF-89^T with homologous proteins from *E. coli* and the 2 phylogenetically closest proteins, and phylogenetic analyses of these components in organisms with a confirmed T1SS presence.

Keywords: T1SS; Virulence factor; TolC duplication; Comparative genomics.

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AI APPLIED TO THE DISCOVERY AND IN VITRO VALIDATION OF NATURAL ANTIBACTERIALS AGAINST *Piscirickettsia salmonis*: FROM 500,000 INDIVIDUAL COMPOUNDS IDENTIFIED TO AN EFFECTIVE COMBINATION WITH A LOW MINIMUM INHIBITORY CONCENTRATION (MIC)

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Abstract

Piscirickettsiosis, caused by *Piscirickettsia salmonis*, is the main cause of infectious mortality and antimicrobial consumption in Chilean salmon farming. In this work, we developed an AI-guided virtual screening system to prioritize and accelerate the identification of natural compounds derived from plants with anti-*P. salmonis* activity as functional additive candidates validated *in vitro*. A protein-ligand co-embedding model was trained on 625,776 pairs from BindingDB and ChEMBL, generating 20 variants (four data partitions and five featurizers). The CORAL-AI PANGEA production model was Cold Drug/Morgan+ProtBert which measures generalization to new molecules. PANGEA defined the GyrA subunit of DNA gyrase (UniProt A0A9Q6LNP5) as the target and screened 500,000 compounds (COCONUT) with probability filters >60% and a price <150 USD/kg. From this analysis, 14 candidates were selected and tested against an isolate of *P. salmonis* genogroup LF89, determining the MIC (80% inhibition) and MBC. From the tested candidates, a total of 5 presented MIC values of 10.4; 31.25; 62.5; 62.5 and 500 µg/mL, of which 3 were bactericidal, with MBC values of 125 µg/mL and 2 bacteriostatic. In the case of the equimolar combination of 2 candidates proposed by PANGEA, the lowest MIC in the panel was reached at 8.03 µg/mL with a bacteriostatic effect. Additionally, the combination of products generated an IC50 of 2.19 and an IC80 of 4.16 µg/mL compared to an IC50 of 3.46 and IC80 of 6.18 µg/mL for the best individual candidate. These results indicate a statistically significant reduction of 36.8% and 32.6% in IC50 and IC80, respectively, and a relative potency of 1.58-fold. The shift in IC50 and IC80 in the combination of candidate compounds suggests a clear indication of a synergistic effect between the compounds.
In summary, the co-embedding model reduced 500,000 identified compounds to 14 candidates (0.0028%) in 1 day and the in vitro validation generated bactericidal and bacteriostatic profiles distinguishable by potency and solubility, criteria that can be used to formulate functional additives against piscirickettsiosis. Future studies aim to scientifically validate the interaction between the compounds, expand the study to other isolates and genogroups of *P. salmonis*, and determine the effect of these compounds on the infectious process in macrophages and epithelial cell lines of salmonids, which, together with intestinal absorption analyses, will allow for better inclusion in the diet and the precise design of trials with fish.

Acknowledgments: ANID INCAR2 CIA250009.

TEMPORAL HOST–PATHOGEN SIGNATURES IN ATLANTIC SALMON SKIN MUCUS DURING EXPERIMENTAL *Piscirickettsia salmonis* INFECTION

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Abstract

Piscirickettsiosis, caused by *Piscirickettsia salmonis*, remains a major constraint to Atlantic salmon production in Chile. Host transcriptional responses to this intracellular pathogen have been extensively characterized in internal tissues, but it remains unclear whether host-response transcripts recovered non-lethally from skin mucus capture the temporal progression of infection. This study characterized mucus-derived host transcriptional signatures together with *P. salmonis* detection during experimental infection, asking whether a non-lethal mucosal sample could provide complementary information on both pathogen presence and the host response during the preclinical-to-clinical transition. Atlantic salmon (*Salmo salar*; mean weight 160 g) were maintained in seawater (33 ppt) at 14 °C and challenged by cohabitation with the EM-90-like *P. salmonis* isolate. Two tanks were maintained under identical conditions (120 fish/tank; Trojan:cohabitant ratio 2:3). One tank was assigned to serial sampling, with three cohabitant fish collected at baseline and every seven days through day 49 (n = 24), whereas the second tank remained undisturbed to follow disease progression. Skin mucus, kidney and other internal tissues were collected. Bacterial signal was assessed by qPCR. *IFNγ, TNFα, IL-1β, IRF3, IRF7, IFNβ, STING* and *DDX41* were quantified by RT-qPCR in technical duplicate; replicate Ct values were averaged per fish. Kidney and mucus profiles were normalized to ELF using the ΔCt method, and fold changes relative to T0 were calculated using ΔΔCt. Temporal structure across the full marker panel was additionally examined using changes in target Ct values over time and principal component analysis (PCA). The challenge showed an early preclinical phase followed by systemic infection and evident disease. In the undisturbed tank, all Trojan fish had died by day 23 post-infection, confirming rapid establishment of the challenge, while cumulative mortality among cohabitants reached 62.5% (25/40) by day 49, confirming progression to a clinically relevant outcome. *P. salmonis* signal in skin mucus increased over time and correlated with signals in kidney (r = 0.70), liver (r = 0.71) and spleen (r = 0.69), indicating that the external mucosal compartment tracked systemic infection. Host transcripts were consistently measurable in mucus, and the eight-marker profile displayed a pronounced temporal structure by PCA (PC1+PC2: 89.7% of variance in mucus and 85.0% in kidney). During the preclinical window, an early IFNβ-associated shift was detected at day 7, followed by progressive changes involving IL-1β and interferon-regulatory/cytosolic-sensing markers as infection advanced. This temporal sequence is compatible with an initial sensing/interferon-associated mucosal response followed by broader immune activation as bacterial burden and tissue involvement increase. By day 42, *TNFα* and *IFNγ* showed the strongest mucus responses (ELF-normalized log2FC = 7.7 and 7.5, respectively), supporting a marked inflammatory response as infection progressed; *IRF7* showed a smaller late decrease, whereas *DDX41* followed an opposing relative trend. Together, these findings indicate that skin mucus can provide biological information beyond direct pathogen detection: early host-response shifts may help identify fish entering the infection process, whereas later *TNFα/IFNγ*-dominated profiles reflect progression toward systemic disease. Skin mucus provides two complementary layers of information during *P. salmonis* infection: direct detection of the pathogen and a measurable host-response signature. The temporal multigene expression profiling supports mucus as a biologically informative, non-lethal matrix for longitudinal surveillance and suggests that selected early-response markers could complement qPCR detection, particularly during the preclinical phase when pathogen burden may still be low or heterogeneous. The strong late *TNFα/IFNγ* response reflects progression toward established systemic disease, while earlier interferon- and sensing-associated changes represent candidate biomarkers for further validation. Combining pathogen detection with host-response profiling may therefore improve early SRS surveillance by adding information on the biological state of the fish, not only the presence of the agent. Further studies should validate the most informative early markers in longitudinal field cohorts.

Keywords: *Piscirickettsia salmonis*; Atlantic salmon; skin mucus; mucosal immunity; biomarkers.

Acknowledgments: The authors acknowledge the technical teams of ADL Diagnostic Chile and TEKBios Fish Trial Center. The immune-marker panel was supported in part by FONDECYT Regular Project 1240030.

INNBOOST®: BRINGING PRECISION MEDICINE TO AQUACULTURE, A NEW RECOMBINANT PROTEIN-BASED FEED ADDITIVE TO PREVENT SRS OUTBREAKS

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Abstract

Current solutions against Salmon Rickettsial Septicaemia (SRS) rely mainly on injectable vaccines and dietary immunostimulants such as phytogenics and β-glucans, none of which have translated into a sustained reduction of mortality in commercial settings. Commercial vaccines report 46–94% relative percent survival (RPS) under controlled trials, but under Chilean field conditions fail to significantly reduce total mortality or delay outbreak onset, as *Piscirickettsia salmonis* is a facultative intracellular pathogen that evades CD8+ T-cell-mediated immunity, with protection waning by approximately 1,800 degree-days post-vaccination, coinciding with the onset of outbreaks. Phytogenics and β-glucans act as non-specific innate-immunity stimulants with inconsistent, poorly characterized, dose- and species-dependent effects, and typically require several weeks of continuous supplementation (commonly 4–8 weeks in reported trials) before any protection is measurable — too slow to cover the short, critical risk windows of a production cycle. AQUIT approach utilizes native proteins from the animal as preventive treatment, bringing precision medicine to animal farming, starting with salmon. Our first product InnBoost® is based on a native recombinant protein of the salmon with the aim to improve immunity so fish are more resilient and are able to overcome bacterial outbreaks. InnBoost® is generated using *Pichia pastoris* and precision fermentation, and was first validated by injecting fish. After 10 days, a challenge against SRS and *Tenacibaculum* was performed by intraperitoneal (ip) injection. Fish that received the treatment achieved 50% and 83% RPS against SRS and *Tenacibaculum*, respectively. These results, together with the analysis of a comprehensive gene expression panel, validated the robust immune system activation and broad protection of the fish against two distinct bacterial pathogens. Following these proof-of-concept injection trials, AQUIT developed an oral formulation of the protein to be incorporated directly into commercial aquafeed as an additive, with the aim of using InnBoost® throughout the entire production cycle at critical, high-risk time points where disease outbreaks are most probable to occur. For the oral formulation, InnBoost® was incorporated directly into the feed raw materials during mixing prior to extrusion, utilizing standard industrial processing equipment (e.g., 75-liter bioreactor scale production) without requiring complex microencapsulation steps. The resulting feed was evaluated in a robust cohabitation challenge against SRS designed to mimic natural sea-site infection dynamics: fish received either InnBoost®-supplemented or control feed for a short seven-day period, after which shedder (“trojan”) fish were introduced into the tanks (DPC0) to trigger infection, and cumulative mortality was systematically recorded over the following weeks until day 60 (DPC60). Despite only seven days of dietary application prior to challenge, InnBoost®-fed fish reached a relative percent survival (RPS) of 67.3% against SRS at DPC60. This level of protection demonstrates superior efficacy compared to traditional immunomodulators and supports its strategic deployment as a short, targeted preventive intervention applied immediately ahead of high-risk operational and environmental windows in the production cycle, rather than as an expensive, continuous long-term dietary supplement. The technology developed by AQUIT has been recognized internationally by being selected into the 100 Deep Tech Pioneers of Hello Tomorrow (2026), the Foodtech 500 List (2026), winning the Responsible Seafood Innovation Award (2023) and the Sustainability Award of the Global Aquaculture Challenge (2021). Together, these results indicate that a native, recombinant salmon protein delivered through feed can activate innate immune protection against SRS within a markedly shorter application window than current vaccines or conventional immunostimulants, without relying on external antigens or broad-spectrum, non-specific compounds. By pairing a mechanism grounded in the fish’s own biology with a practical, feed-based delivery route, InnBoost® supports a precision-medicine approach to SRS prevention and warrants further field-scale validation across production stages, marine site conditions, and commercial scaling trials.

Keywords: SRS; *Piscirickettsia salmonis*; precision medicine; recombinant protein; *Pichia pastoris*; feed additive; innate immunity.

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A MORPHOLINO-BASED APPROACH TO INVESTIGATE TRIM33L, A CANDIDATE GENE ASSOCIATED WITH SALMONID RICKETTSIAL SEPTICAEMIA RESISTANCE IN ATLANTIC SALMON

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Abstract

Morpholino antisense oligonucleotides constitute a valuable tool for functional genomics studies due to their relatively rapid and simple implementation compared with knockout approaches. Morpholinos are synthetic DNA analogs that bind to complementary sequences of a target mRNA, blocking translation or altering splicing. Their chemical backbone confers high stability and resistance to nuclease degradation, making them an attractive alternative for transient gene knockdown studies. *Piscirickettsia salmonis* is a facultative intracellular bacterium that causes Piscirickettsiosis, also known as Salmonid Rickettsial Septicaemia (SRS), one of the most important diseases affecting the Chilean salmon industry. Selective breeding represents a feasible strategy for improving resistance to disease. In Atlantic salmon, resistance to SRS is a polygenic trait with moderate to high heritability, and genome-wide association studies (GWAS) have identified several candidate genes potentially involved in host resistance. Notably, a previous gene-based meta-analysis of SRS resistance identified trim33l among the strongest candidate genes associated with both binary survival and time to death following *P. salmonis* challenge in Atlantic salmon. However, establishing causal relationships between candidate genes and disease resistance requires functional validation. In the present study, we are developing a morpholino-based approach to functionally investigate the role of trim33l in the response of Atlantic salmon cells to *P. salmonis* infection. As an initial proof of concept, we are evaluating the feasibility of morpholino-mediated knockdown of trim33l in the Atlantic salmon SHK-1 cell line using two delivery strategies: electroporation and the Endo-Porter delivery agent (Gene Tools). Following establishment of suitable knockdown conditions, trim33l-silenced cells will be challenged with *P. salmonis* to assess whether modulation of this candidate gene affects the cellular response to infection. Overall, this approach will provide a framework to move from genomic association to functional assessment of candidate genes involved in SRS resistance. Establishing the role of trim33l during *P. salmonis* infection may contribute to understanding the biological mechanisms underlying host resistance and support the functional prioritization of candidate genes identified through genomic studies in Atlantic salmon.

Keywords: Morpholino; knockdown; genomics; aquaculture; SRS.

Acknowledgements: The authors acknowledge the financial support provided by the National Agency for Research and Development (ANID), Fondecyt Regular No. 1251977, and the technical support provided by LABGEN, University of Chile.

DEVELOPMENT OF A RECOMBINANT BCG-BASED VACCINE EXPRESSING A CHIMERIC PROTEIN AGAINST *Piscirickettsia salmonis*

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Abstract

Despite more than 30 SAG-approved commercial vaccines against *Piscirickettsia salmonis* currently available in Chile, based on inactivated bacteria, attenuated strains, and recombinant proteins, piscirickettsiosis (SRS) remains a critical challenge for the aquaculture industry. These conventional formulations do not confer protection against both genogroups (LF-89 and EM-90), elicit short-lived humoral responses, and have limited capacity to activate cellular immunity, an essential component for controlling intracellular pathogens such as *P. salmonis*. In this context, *Mycobacterium bovis* BCG emerges as an ideal strategy, a low-cost, adjuvant-free platform capable of inducing robust cellular immunity that has already demonstrated efficacy in Japanese flounder and zebrafish against marine mycobacteria. In addition, our laboratory has developed recombinant BCG genetically modified to express heterologous antigens for mammalian vaccines; however, this platform has never been evaluated as a vaccine vector in salmonids. By integrating bioinformatic approaches to design multi-epitope chimeric proteins that overcome the limitations of traditional recombinant protein vaccines, this study aims to develop a recombinant BCG-based vaccine expressing a *P. salmonis* chimeric protein to confer protection against piscirickettsiosis. Candidate protective proteins against *P. salmonis* (strain LF-89) were selected to design a multi-epitope construct. Linear B-cell epitopes were predicted using IEDB, and T-cell epitopes were mapped using NetMHCpan with manually loaded *Salmo salar* MHC-I and MHC-II sequences from UniProt. Candidate epitopes were filtered for immunogenicity (VaxiJen v2.0), conservation (IEDB), allergenicity (AllerTOP v2.1), and toxicity (ToxinPred2). Selected MHC-I, MHC-II, and B-cell epitopes were joined with AAY, GPGPG, and KK linkers, respectively, and the epitope clusters were connected by a HEYGAEALERAG peptide. The 3D structure was modeled, validated, and analyzed for conformational B-cell epitopes via IEDB. Finally, the construct was codon-optimized for *M. bovis* BCG Danish 1331 and cloned *in silico* into the pMV361 vector using SnapGene. Eight candidate antigens were selected from various cellular locations of *P. salmonis*. Epitope selection yielded 9-aa MHC-I, 13-aa MHC-II, and 9–30-aa linear B-cell epitopes, all with immunogenicity scores well above the established threshold. The resulting chimeric protein demonstrated a favorable safety profile, showing non-allergenicity and non-toxicity. Codon optimization significantly improved the Codon Adaptation Index (CAI) for *Mycobacterium bovis* BCG, and the finalized construct was successfully assembled *in silico* into the pMV361 expression vector. An *in silico* approach was successfully developed to design a recombinant *M. bovis* BCG vaccine expressing a multi-epitope chimera targeting *P. salmonis*. This platform leverages BCG's intrinsic immunogenicity to induce long-lasting cellular, humoral, and trained immunity, potentially conferring cross-protection against heterologous pathogens. Future studies will focus on generating the recombinant BCG construct for *in vivo* validation in salmonids.

Keywords: Recombinant vaccine; Chimeric protein; BCG, cellular response; long-term protection.

Acknowledgments: This work was supported by the Millennium Institute on Immunology and Immunotherapy (ACE 210015), the CORFO Technological Consortium (25CTEC-307461), and the ANID National Doctoral Fellowship (21260667).

DEVELOPMENT AND VALIDATION OF A FUNCTIONAL FEED TO INCREASE RESILIENCE AGAINST *Piscirickettsia salmonis* IN ATLANTIC SALMON (*Salmo salar*)

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Abstract

Salmonid rickettsial septicemia (SRS) or Piscirickettsiosis, caused by *Piscirickettsia salmonis*, remains one of the most significant infectious diseases affecting Atlantic salmon production, particularly in Chile. Despite advances in vaccination, genetics, and husbandry practices, SRS continues to be associated with substantial mortality and inflammation, and remains the main driver of antimicrobial use in salmon aquaculture. Consequently, there is increasing interest in complementary strategies capable of enhancing host robustness and reducing disease-associated losses. Farmed salmon are routinely exposed to stressful conditions that increase the production of reactive oxygen species (ROS), leading to oxidative stress and disruption of cellular redox homeostasis. Prolonged redox imbalance has been associated with cellular damage, chronic inflammation, impaired immune cell function, and reduced ability to cope with disease challenges. Nutritional strategies that support redox balance may therefore improve the capacity of fish to cope with infectious diseases and enhance disease resilience. The present work summarizes the outcomes of a multi-year research program aimed at developing and validating a functional preventative feed that reinforces endogenous antioxidant network interactions to improve resilience against *P. salmonis* infection. Independent cohabitation challenge trials were conducted in Atlantic salmon over multiple years with different regional *P. salmonis* strains. Experimental diets were formulated to find the optimal levels of conventional antioxidant nutrients together with a novel vitamin-like antioxidant. Atlantic salmon post-smolts with an initial body weight ranging from 103 to 200 g were fed the experimental diets during a pre-challenge period before exposure to *P. salmonis* LF-89 or EM-90 through cohabitation with infected shedder fish. Survival, growth performance, and infection-related parameters were evaluated throughout each study. The trials included an initial efficacy study, a dose-optimization study, and a subsequent validation study designed to confirm repeatability of the observed effects. Survival analyses were performed using Kaplan-Meier methodology, and relative percent survival (RPS) were calculated relative to control-fed fish. The functional feed consistently reduced mortality associated with SRS across all trials. In the initial efficacy study, the relative percent survival improved by 39% compared with the control group. Subsequent optimization resulted in even better protection, achieving an RPS of 48.8%, while also maintaining normal growth performance. An independent validation trial confirmed the results, demonstrating reproducible reductions in mortality following *P. salmonis* challenge. Across studies, fish receiving the optimized dietary strategy also showed indications of reduced pathogen persistence relative to controls. These survival benefits align with earlier results showing lower oxidative damage and inflammatory responses in fish fed the dietary strategy under chronic stress. The results demonstrate that a functional dietary strategy based on an optimized immune control and antioxidant nutrition can consistently improve Atlantic salmon survival during experimental SRS challenges. Repeated validation across independent trials identified the optimal formulation, providing robust protection while avoiding adverse effects on growth performance. Overall, these results indicate that supporting redox balance and immune function through nutrition can improve disease resilience in Atlantic salmon during SRS challenge by reducing oxidative damage and promoting an effective physiological response to infection. They also highlight the potential of targeted nutritional strategies as complementary tools to strengthen robustness against intracellular bacterial infections in salmon aquaculture.

Keywords: Functional nutrition; Host robustness; Disease resilience.

NUTRITIONAL CONDITIONING TO ENHANCE BIORESILIENCE AGAINST SALMONID RICKETTSIAL SEPTICAEMIA

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Abstract

Salmonid rickettsial septicaemia (SRS), caused by the intracellular bacterium *Piscirickettsia salmonis*, remains a major health challenge for salmonid aquaculture. Nutritional strategies capable of preparing fish to respond more effectively to biological stressors may provide a complementary approach to conventional disease management. In this context, nutritional conditioning is proposed as a strategy to promote bioresilience, defined here as the capacity to mount a coordinated physiological response to a challenge while maintaining functional homeostasis. Futerpenol®, a functional micro-ingredient formulated from algal and botanical extracts, has previously demonstrated immunomodulatory activity and increased survival during *P. salmonis* challenge. This work integrates experimental evidence from controlled challenge and commercial farming conditions to examine whether these responses are consistent with a nutritional conditioning effect associated with enhanced bioresilience. Two complementary studies were considered. In a controlled study, rainbow trout (*Oncorhynchus mykiss*) were fed either a control diet or a diet supplemented with Futerpenol® at 1 kg ton^{-1} for 30 days before exposure to a virulent *P. salmonis* LF-89 strain using a cohabitation challenge model. Mortality was monitored for 80 days post-infection, and Relative Percent Survival (RPS) was determined. Complementary in vitro assays using salmon head-kidney SHK-1 cells assessed immune-regulatory responses and cellular protection against *P. salmonis*. In a subsequent commercial-scale study, Atlantic salmon (*Salmo salar*) post-smolts were fed a control diet or a diet containing 0.1% Futerpenol® for 90 days under seawater farming conditions. Immune-related gene expression was evaluated in head kidney, gills and spleen during functional feeding and 42 days after supplementation ended. During this latter period, fish were naturally exposed to an algal bloom, providing an opportunity to assess the persistence of the physiological response following nutritional conditioning. Pre-challenge dietary supplementation significantly improved survival following *P. salmonis* exposure. Cumulative mortality decreased from 35.0 ± 4.3% in control fish to 15.0 ± 6.0% in Futerpenol®-fed fish, corresponding to RPS values of 62.3% at 60 days and 57.1% at 80 days post-infection. *In vitro*, Futerpenol® induced dose- and time-dependent modulation of IL-12 and IFN-I and reduced the cytopathic effects associated with *P. salmonis* infection. Under commercial farming conditions, nutritional supplementation induced coordinated modulation of innate and adaptive immune-related biomarkers. After 34 days, responses involved cytokines and effector molecules associated with inflammatory regulation, antimicrobial defence and humoral immunity, including *ifng*, *il-10*, *il-12*, *tnfa*, *cath2*, *hepcidin*, *igm* and *igt*. Importantly, 42 days after functional feeding had ceased, previously supplemented fish retained a differentiated response during a natural environmental challenge. This response was particularly evident in the head kidney and involved *ifng*, *il-1b*, *il-10*, *il-12* and *tnfa*, antimicrobial effectors including *cath2*, *hepcidin* and *inos*, and markers related to antigen presentation and cellular immune coordination. During the algal bloom period, the previously supplemented group recorded 61% fewer dead fish than the control group. The integration of controlled SRS challenge and commercial-scale immunological evidence suggests that nutritional intervention with Futerpenol®, a functional micro-ingredient formulated from algal and botanical extracts, may induce more than transient immune stimulation. The coordinated modulation of inflammatory, antimicrobial, humoral and cellular responses, together with persistence of immune responsiveness after supplementation withdrawal and increased survival against *P. salmonis*, is consistent with a nutritional conditioning effect. These findings support bioresilience as a framework for understanding how functional nutrition may prepare salmonids to mount a coordinated physiological response to subsequent biological and environmental challenges while maintaining functional homeostasis.

MAJOR LOCUS AND CANDIDATE GENES CONTROLLING SURVIVAL AGAINST SRS IN COHO SALMON

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Abstract

Salmonid rickettsial septicaemia (SRS), caused by *Piscirickettsia salmonis*, remains a major health constraint for Chilean salmon farming. Previous studies have demonstrated that resistance to SRS in coho salmon is heritable, with high variation of mortality after infection across different families. Here, we tested a low-cost genotyping-and-imputation workflow to identify genomic regions and candidate genes associated with SRS resistance in a commercial population of coho salmon. A total of two thousand SRS challenged-fish were genotyped with an approximately 2.2K SNP panel designed for cost-efficient routine genomic selection. After quality control, 1,916 low-density SNPs allowed genotype imputation against an ancestry-matched, multigeneration reference dataset. We retained 37,755 high-quality imputed SNPs with expected accuracy >0.80, producing a reported final dataset of 39,672 markers for downstram Genome-wide association analyses (GWAS). Variance components for binary survival trait were estimated from the genomic relationship matrix, and association testing was performed by GCTA MLMA-LOCO using the current Okis_V2 coho reference assembly to position SNP markers. Candidate prioritization integrated three explicitly separated evidence layers: i) the identification of a highly significant quantitative trait locus (QTL) in chromosome 21 identified from GWAS using genotyped imputed from 2K to 40K; ii) A structural variant (SVs) catalog generated from animals of the same population using both short- and long-reads approaches (DNBseq MGI and PacBio Reviocell, respectively); iii) and external RefSeq annotation and functional literature. Binary-survival heritability was 0.28 (SE = 0.04), and MLMA-LOCO detected a genome-wide-significant QTL on chromosome 21 (Figure 1).

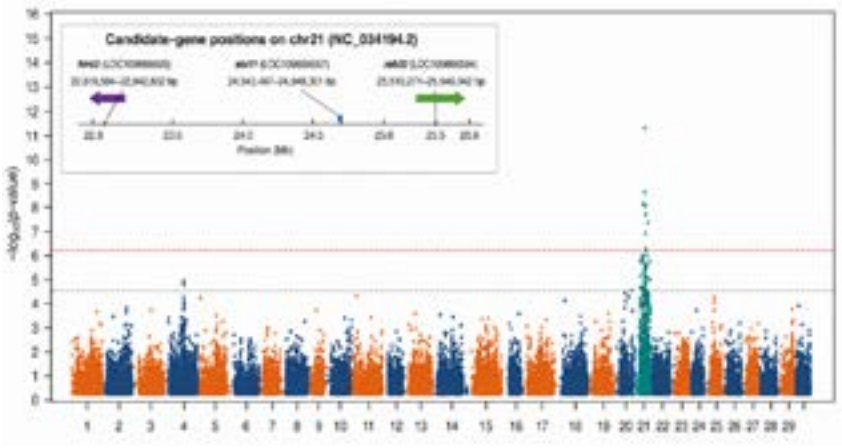


Figure 1. Genome-wide association analysis of binary survival following experimental *Piscirickettsia salmonis* challenge in a coho salmon population. The Manhattan plot shows a genome-wide-significant QTL on chromosome 21; dashed horizontal lines indicate the suggestive and genome-wide significance thresholds. The inset displays the annotated positions of the candidate genes *hint3*, *stx11*, and *rab32* on the coho salmon reference sequence NC_034194.2 within the chromosome 21 candidate region.

After the whole-genome re-sequencing we generated a catalogue of 65,738 high-quality structural variants (SVs), including 40,757 deletions, 10,162 insertions, 4,222 duplications, and 2,460 inversions. Integration of

SV positions with the broader chromosome 21 candidate landscape identified variants within or near *stx11*, *rab32*, and *hint3*. STX11 regulates macrophage endosomal trafficking and late-endosome–lysosome fusion, whereas RAB32 participates in cell-autonomous pathways that restrict intravacuolar bacteria, making both genes biologically relevant to infection by the intracellular pathogen *P. salmonis*. Although the function of HINT3 in fish immunity remains unknown, mammalian evidence links it to mitochondrial homeostasis, oxidative-stress regulation, and apoptosis, cellular processes potentially relevant to the host response to *P. salmonis*. SRS resistance is moderately heritable in the evaluated coho salmon population, and the detection of a genome-wide-significant QTL in chromosome 21 confirms this region as an important target for genetic improvement for SRS resistance in this species. Integrating association results with whole-genome structural-variant information and functional information we identified *stx11*, *rab32*, and *hint3* as biologically plausible candidates involved in intracellular pathogen restriction, endolysosomal trafficking, mitochondrial homeostasis, oxidative stress, and apoptosis. Although causality remains to be established, these findings prioritize candidate genes in chromosome 21 for functional validation by using *in vivo* or *in vitro* gene editing approaches. Combining low-density SNP genotyping and imputation with structural-variant discovery provides a cost-effective framework for genomic selection and for identifying potential targets for precise gene-editing experiments aimed at improving SRS resistance in coho salmon.

Keywords: *Piscirickettsia salmonis*; coho salmon; genotype imputation; QTL; genome editing.

Acknowledgments: This study was partially funded by National Agency for Research and Development (ANID) through the FONDECYT Regular Grant N°1251977. We also thank AquaChile for providing biological material and the phenotypic data used in the FONDECYT Grant.

REPEATED EXPOSURE TO SUB-INHIBITORY CONCENTRATIONS OF FLORFENICOL INDUCES THE EXPRESSION OF ANTIBIOTIC RESISTANCE-ASSOCIATED PROTEINS IN BIOFILM-EMBEDDED *Piscirickettsia salmonis*

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Abstract

Piscirickettsia salmonis is the etiologic agent of piscirickettsiosis, the most prevalent bacterial disease affecting farmed fish and causing substantial economic losses to the Chilean salmon farming industry. Importantly, despite vaccination and the intensive use of antibiotics in the Chilean aquaculture industry, high mortality rates in farmed fish have persisted over the years, mainly in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Biofilm facilitates and promotes the bacterial survival in hostile environments, including tolerance to antibiotics. Thus, an association between bacterial communities and antibiotic resistance in surface seawater could contribute to the higher prevalence of antibiotic-resistant bacteria in areas associated with salmon farming. Therefore, the aim of this study was to identify and quantify antibiotic resistance-associated proteins in biofilm-embedded *P. salmonis* following repeated exposure to sub-MIC concentrations of FLO. *P. salmonis* LF-89 and Ps007 were cultured in in 6-wells flat-bottom culture plates in 3 mL/well of a minimal medium supplemented with cysteine and ferric chloride, in the presence of sub-MIC concentrations of florfenicol (FLO) to evaluate its effects on biofilm-associated and planktonic bacteria. Biofilms were developed at 18°C for 7 days with moderate agitation (75 rpm) followed by 5 more days under static incubation. Thus, the experimental conditions included an untreated control, biofilm-associated bacteria after a single passage (P1) or four sequential passages (P4) under 0.0156 µg/mL FLO, and the corresponding planktonic fractions. Finally, both biofilm-derived and planktonic bacterial fractions were collected and separated for proteomic analyses.

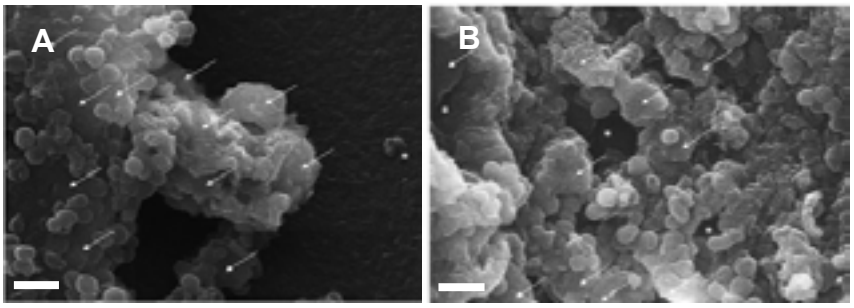


Figure 1. Biofilm formed by *P. salmonis* under sub-MIC concentration of FLO. *P. salmonis* LF-89^T (A) and EM-90-like strain Ps007 (B) were cultured under 0,0156 µg/mL (sub-MIC concentration) of FLO. White bars: 2 µm.

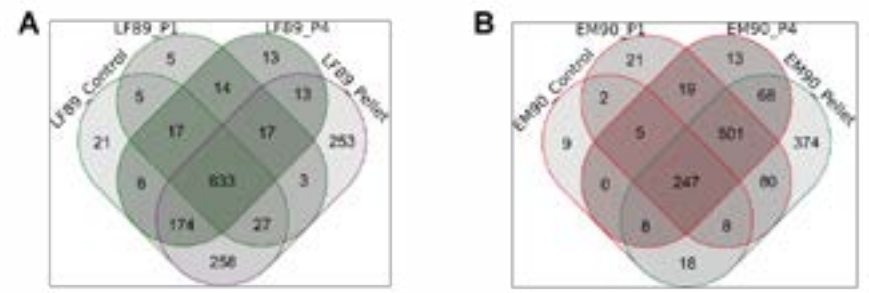


Figure 2. Venn diagrams showing the comparison of proteins identified under the different experimental conditions. (A) Comparison of proteins identified under the different conditions for the LF-89 genogroup. (B) Comparison of proteins identified under the different conditions for the EM-90 genogroup.

Proteomic analysis revealed a strain- and growth-state-dependent response to repeated exposure to sub-MIC florfenicol. Proteins associated with multidrug efflux, including TolC and RND-type efflux components, were detected, although their abundance and distribution differed between genogroups. LF-89 showed increased representation of MexH, AcrB and RarD, particularly in planktonic cells after repeated exposure, whereas EM-90 exhibited a distinct profile involving QseB/CpxA and efflux-associated proteins such as MexB and BepE. Repeated florfenicol exposure was also associated with changes in proteins involved in oxidative-stress response, proteostasis and translation, including peroxiredoxin/AhpC/TsaA, FtsH, TufA, FusA and ribosomal proteins. These changes were generally more pronounced after four passages than after a single passage, suggesting progressive physiological adaptation to florfenicol exposure. These findings indicate that repeated exposure to sub-MIC florfenicol induces a complex, genotype- and growth-state-dependent proteomic response in *P. salmonis*. Although no canonical florfenicol resistance determinants were identified, differential detection of efflux-, regulatory-, stress- and translation-associated proteins between LF-89 and EM-90 suggests that *P. salmonis* may employ distinct adaptive strategies during prolonged florfenicol exposure. These results support a model in which sub-MIC florfenicol exposure promotes physiological adaptation involving both biofilm-associated and planktonic populations.

Keywords: SRS; Piscirickettsiosis; biofilm; antibiotic resistance.

Acknowledgments: This work was made with support of grants: FONDECYT 11180994. The authors sincerely thank Dagoberto Boisier from the Unidad de Microscopía Electrónica (UME - UACh) for his valuable assistance.

INTEGRATIVE TRANSCRIPTOMICS UNCOVERS MECHANISM OF HINT3-MEDIATED SRS RESISTANCE IN COHO SALMON (*Oncorhynchus kisutch)*

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Abstract

In Coho salmon (*Oncorhynchus kisutch*), resistance to Salmonid Rickettsial Septicemia (SRS) caused by *Piscirickettsia salmonis* is significantly influenced by a major QTL on chromosome 21, driven by variants in the protein-coding gene and overexpression of *hint3* and the long non-coding RNA *LOC109866666*. To elucidate the downstream mechanisms of this locus, head-kidney RNA-seq profiling was performed on fish differing in *hint3* genotype (following a seawater cohabitation challenge at the peak of the mortality curve. Differential expression analysis identified 1,237 upregulated (including *hint3* and *LOC109866666*) and 276 downregulated genes in susceptible relative to resistant fish. Functional enrichment terms were embedded using a sentence transformer (*all-MiniLM-L6-v2*), cosine-similarity-distilled to reduce redundancy, UMAP-projected, and partitioned into distinct functional modules via K-Means clustering to further reduce term redundancy. This spatial semantic mapping revealed that host resistance involves tightly coordinated defense hubs: genome integrity, replication, and mitosis (*mki67*, *cdc20*, MCM complex) and vasculature development and wound healing (*kdr*, *pecam1b*, *egfl7*, VEGF pathway) to drive cellular replacement and lesion recovery. Re-establishment of organ architecture was further supported by cell-junction and cytoskeletal dynamics (ITGA*5*, tight/focal junctions) alongside upstream morphogenetic and stress signaling (Hippo and Notch pathways). Crucially, these structural repair mechanisms were underpinned by enzymatic signaling and energy-metabolism cascades (including kinase activity, ATP binding, and GTPase activation). As *hint3* plays a fundamental role in nucleotide turnover, energy homeostasis, and stress-induced signal transduction, these findings provide a mechanistic model directly linking allelic variation at the *hint3*/lncRNA locus to differential metabolic resilience, host-pathogen interactions, and survival under SRS challenge in salmonids.

Keywords: Disease resistance; Coho salmon; *P. salmonis;* disease resistance.

STRATEGIES AND BEST MANAGEMENT PRACTICES IN CHILEAN SALMON AQUACULTURE: EVIDENCE FROM THE PINCOY INITIATIVE

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Abstract

Chile is the second-largest producer of farmed Atlantic salmon in the world, where Salmon Rickettsial Syndrome (SRS), caused by *Piscirickettsia salmonis*, accounts for more than 50% of pathogen-related mortality at sea sites and remains the main driver of antibiotic use. Although a decreasing trend in antibiotic use has been observed over the years, antimicrobial treatments continue to be a major sustainability concern for the industry.
The Pincoy Initiative is a collaborative program encouraging strategies based on scientific evidence and data collection. Strategies that are based on a wide range of production practices, from genetics and functional and high-performing diets to smolt selection and vaccination, all aimed at reducing mortality and antibiotic use. To evaluate the effectiveness of these practices and strategies, the project has been collecting and analyzing data at cage and farm site levels over the years. A Gaussian process boost model (GPBoost), that combines tree boosting with Gaussian process and mixedeffects components, was applied to identify factors influencing SRS mortality. In parallel, a Cox proportional hazard mixed model quantified the drivers of the first SRS mortality event. Both models included key productive and sanitary variables such as bath treatments, generic origin and selection, vaccination strategies, freshwater origin and feed additives. The farmlevel random effects provided estimates of relative risk and predicted SRS mortality across sites. The analysis showed that productive practices, genetic origin, functional feeds, vaccine strategies, and environmental conditions significantly explained variation in SRS mortality. Farm effects accounted for 16.2% of total variance, revealing significant sitespecific heterogeneity beyond measured covariates. Identifying farms with high risk enables targeted evaluations and potential future corrective actions, while lowrisk farms offer insight into successful management practices. Overall, our findings highlight the importance of integrated and sustainable diseasemanagement strategies. The evidence demonstrates clear associations between production practices, therapeutic interventions, and SRS outcomes, highlighting the need for holistic approaches to enhance the longterm sustainability and resilience of Chilean salmon farming.

GWAS META-ANALYSIS OF RESISTANCE AGAINST PISCIRICKETTSIA SALMONIS IN COHO SALMON (*Oncorhynchus kisutch*)

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Abstract

Piscirickettsia salmonis, the causative agent of Salmonid Rickettsial Septicemia (SRS), is a major health threat to Chilean salmon farming. Because existing control measures provide limited long-term protection, genetic improvement represents a complementary strategy to reduce disease-related mortality. This study aimed to identify genomic regions and candidate genes associated with resistance to *P. salmonis* in coho salmon (*Oncorhynchus kisutch*) through a meta-analysis of GWAS conducted in five experimental cohorts using imputed genotypes. Five cohorts from three genetic lines of the AquaChile coho salmon breeding nucleus were analyzed. Overall, 12,527 fish from 111–140 families per cohort were subjected to controlled challenge tests with the LF-89 genotype of *P. salmonis*. Resistance was recorded as binary survival at the end of the challenge (1 = dead; 0 = survivor). A total of 7,005 phenotyped fish were genotyped with low-density panels containing approximately 2K SNPs. Genotypes were imputed to a panel based on the 70K Axiom Coho Salmon Genotyping Array using FImpute v3.0 and a reference population of 766 high-density-genotyped or whole-genome-sequenced individuals. Imputation accuracy was evaluated by 10-fold cross-validation, retaining SNPs with $r^2 \geq 0.80$. An independent GWAS was performed for each cohort using GCTA fastGWA-MLM-Binary. Cohort-specific covariates and a genomic relationship matrix were included to account for systematic effects, relatedness, family structure, and population stratification. Summary statistics were harmonized and combined using an inverse-variance meta-analysis implemented in METAL. Genome-wide and chromosome-wide significance thresholds were determined using Bonferroni correction. A gene-based association analysis was also performed with MAGMA v1.10. Cumulative mortality ranged from 28.9% to 67.9% across cohorts, while cohort sizes varied between 1,110 and 3,759 fish. After genotype imputation and quality control, 17,510–36,942 SNPs were available per cohort. Individual GWAS detected signals of varying magnitude, with significant associations on chromosome 21 in three of the five cohorts. The harmonized meta-analysis dataset comprised 45,497 SNPs. A total of 90 significant SNPs were concentrated within an approximately 21-Mb region on chromosome 21, spanning 14.3–38.8 Mb. The lead SNP, NC_034194.2_24404644, was located at 24,404,644 bp and showed an estimated effect of −0.7307, a standard error of 0.0711, and a p-value of 9.541×10^{-25}. Given the phenotype coding, its negative effect was associated with a lower probability of mortality, although this association does not demonstrate causality. The gene-based analysis prioritized 72 genes within the associated region or supported by the joint signal of their SNPs. The strongest evidence was observed for LOC109866665, annotated as histidine triad nucleotide-binding protein 3, utrn, and si:ch211-51e12.7. Integrating multiple cohorts increased the precision of the association estimates and identified a shared signal that was not significant in every individual GWAS. Nevertheless, differences in mortality, sample size, marker density, and imputation accuracy may have contributed to heterogeneity among cohorts. Furthermore, the broad associated interval and linkage disequilibrium limited the resolution required to identify causal variants. The meta-analysis identified a robust association between resistance to *P. salmonis* and a major QTL on chromosome 21 in coho salmon. The significant SNPs and prioritized genes provide candidates for fine mapping and functional validation but should not yet be regarded as causal. Integration with structural variants, gene-expression data, and independent populations will be required to determine the underlying biological mechanisms and assess their application in genetic selection programs.

Keywords: *Piscirickettsia salmonis*; *Oncorhynchus kisutch*; GWAS; meta-analysis; disease resistance.

SRS AND THE EVOLUTION OF AI: A VISION FROM ADJACENT AQUACULTURE DISEASES

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Abstract

AI and modern data infrastructure are quickly transforming how diseases are studied, monitored, and researched across many fields, both within aquaculture and, increasingly, in human health. This presentation explores several of those patterns, where they started, how they progressed, and where they stand today, as a way of examining how modern data science and AI could be applied to Piscirickettsiosis (SRS) in Atlantic salmon. Three threads are explored: how disease knowledge gets shared across a fragmented industry, how forecasting models have matured from basic statistics toward AI-driven early warning, and how data can be used to distinguish correlation from causation in noisy, non-randomized production data. Together, they offer a picture of how far AI has come in adjacent problems, and what a tailored solution for SRS may look like.

This is a synthesis of three vantage points on how AI has evolved in aquaculture disease management, drawn from Manolin's own experience and observations building this class of tooling within the aquaculture industry.The first is the Norwegian Fish Disease Library, a collaboratively maintained knowledge base supported by FHF project 910692 and operated with Manolin participation, which aggregates disease information across research institutions, producers, and industry bodies. It is an example of shared knowledge infrastructure built once an industry decides to invest in it together, improving knowledge dissemination and global collaboration. The second is the published development arc of Pancreas Disease (PD) forecasting models in Atlantic salmon, spanning more than a decade, which happens to track the broader evolution of AI itself. The third is the field's growing use of causal-inference methods, particularly techniques such as Probability Weighting, to separate genuine intervention effects from the confounding endemic to marine production data. Manolin has contributed to this line of work, including peer-reviewed analysis of production outcomes across 232.6 million fish and an IPWRA analysis of the same class of data presented at Aquaculture Europe 2025 (Le Gal et al., 2025). Together, these three threads represent complementary pieces of the same puzzle: understanding a disease, responding to it faster, and validating whether a given response actually worked, each of which is directly applicable to SRS. What these perspectives offer is a picture of where AI stands today in a closely related problem, and how it got there. A shared, collaboratively maintained knowledge makes information easier to find and makes gaps visible, which is often what motivates the next round of research, and it lowers the barrier for newcomers to contribute. This is important, as cross specialization between biological research and computer science can create an important ecosystem to develop new insights towards improving the action window in response to disease challenges. Table 1 is an example of this relationship, as in Norway multiple computational PD models have been developed over the last decade to assist in the understanding and response towards the disease. These models have evolved along a similar pattern as the field of epidemiology has evolved from classical statistics towards machine learning approaches as more data and compute have become available. Many of these approaches could also be adopted towards challenges like SRS as many of the infection vectors are similar. Although SRS has seen models attempted, most have not utilized modern machine learning types of approaches that we have seen in other areas.

Table 1. Notable Pancreas Disease (PD) models in Atlantic salmon, mapped to the broader evolution of AI, 2012–2024.

Model Name/Description	Type of Model	Organization	Reported Performance	Year	Stage in AI's Evolution
PD Risk Map Logistic Model (Tavornpanich et al., 2012) Predicts PD occurrence at farm sites using spatial clustering.	Bayesian logistic regression	Norwegian Veterinary Institute	AUC of ~0.76 in training, ~0.72 in validation.	2012	Classical statistics
Spatio-Temporal PD Spread Model (Aldrin et al., 2015) Simulates PD transmission within and between farms.	Stochastic SIR-based simulation	Norwegian Computing Center and Norwegian Veterinary Institute	Shows culling infected cohorts reduces outbreaks by ~57%. Simulated mandatory culling of infected cohorts reduced detected outbreaks by 57% (95% CI 24 to 90).	2015	Mechanistic simulation
Manolin Disease Forecasting Model. Machine learning model predicting PD and ISA outbreaks.	Proprietary ML ensemble	Manolin	About 93% accuracy in early PD prediction, internal validation. Alerts farmers to high-risk conditions.	2021	Early applied ML
Real-time PD Transmission Simulator (Bang Jensen et al., 2021). Simulates PD spread to neighboring farms in real-time.	Stochastic network simulation	Norwegian Veterinary Institute and Norwegian Computing Center	Stamping out within 30 days of detection reduced simulated outbreaks from 162 to 6 across the case studies.	2021	Mechanistic simulation, real-time
PD Syndromic Surveillance GLM (Oliveira et al., 2024). Flags abnormal mortality trends using autoregressive models.	Generalized linear model	Norwegian Veterinary Institute and SVA	Sensitivity from 80.5% to 87.4%, with false alarm rates from 45.3% to 53.2%.	2024	Classical statistics, refined
Zoetis / Pharmaq Analytiq Early Warning Model (Öhlschuster et al., 2024). Predicts SAV detection up to eight weeks ahead of a PD registration.	Stacked ML ensemble (XGBoost, AdaBoost, random forest, MLP)	Zoetis and Pharmaq Analytiq	Accuracy 94.4% and balanced accuracy 91.7% on a test set with 9.3% positives. Key features: wellboat traffic, seasonality, site proximity, environment.	2024	Modern ML/AI ensemble
Manolin Disease Forecasting Model. Machine learning model predicting 20+ diseases.	Proprietary ML classification model	Manolin	About 94.8% accuracy across disease classes, internal validation. Key features: biological performance metrics, environmental conditions, treatment history, production context.	2024	Modern ML/AI ensemble

Figures are reported as published and are not directly comparable across rows. Entries for proprietary models rest on validation reported by their developers.

Lastly, knowing the risk of a disease only matters if there are adequate solutions to combat the issue. In response to an outbreak or risk, the industry must have the tools and solutions to mitigate the challenges while preserving the fish and environmental health of the ecosystem. In order to do so, medications and other efforts need to be targeted within the optimum use case and understanding. This challenge lies within the field of causal analysis and understanding the true product performance of solutions available to the market. A major challenge in aquaculture is that many confounding factors can influence the outcome of a treatment, so it's hard to fully understand the true impact. AI's path in aquaculture disease management, covering shared knowledge infrastructure, increasingly capable forecasting models, more rigorous causal evaluation, is furthest along in diseases like PD, but the underlying ingredients (structured production data, environmental and treatment histories, a growing base of documented outbreaks) are increasingly available for SRS too. Where SRS goes from here is a question for this community; the hope is that seeing how far AI has already come next door gives that conversation a useful starting point.

Keywords: SRS; Piscirickettsiosis; disease forecasting; causal inference; knowledge infrastructure.

Acknowledgments: This work draws on the Norwegian Fish Disease Library project (FHF #910692), Manolin's Watershed data intelligence platform, and published research applying causal-inference methods to commercial Atlantic salmon data in works like Veramaris' EPA/DHA Studies and Merck's research into Slice.

NUTRIPATH: INTEGRATING IMMUNONUTRITION AND MULTI-LAYERED DIAGNOSTICS TO SUPPORT HEALTH AND WELFARE IN CHALLENGED SALMONIDS

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Abstract

Chilean salmonid aquaculture faces persistent health and welfare challenges that cause significant economic losses and threaten industry sustainability. For instance, stressful conditions during seawater transfer and grow-out phases, including environmental issues (e.g., rising sea temperatures, harmful algal blooms, and hypoxia) and bacterial diseases such as Salmonid Rickettsial Septicaemia (SRS) caused by *Piscirickettsia salmonis*, severely impact fish survival under commercial farming conditions. To address these challenges, immunonutritional strategies through diets containing bioactive ingredients and feed additives have gained momentum in recent decades, aiming to enhance fish responses to complex stressors. Immunonutritional approaches can modulate both innate and adaptive immune mechanisms in the gut, which are mediated by pro- and anti-inflammatory cytokines, effector molecules (e.g., antimicrobial peptides, enzymes, and immunoglobulins), and specific leukocyte subpopulations (myeloid- or lymphoid-derived cells). Furthermore, local humoral and cellular responses in the intestine can also influence systemic immunity, acting in coordination with organs such as the liver, head kidney, and spleen. Although several methods are available to assess the functional properties of aquafeeds (e.g., omics, welfare indicators, and specific immune-related parameters), their applicability in fish production, as well as the integration and interpretation of these heterogeneous datasets, remains a significant constraint for the industry, especially during pathogen outbreaks. Given that data integration is essential for achieving a comprehensive understanding of salmonid physiological responses to challenges, the NutriPath project uses a holistic framework to support overall health and welfare in vaccinated Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). This initiative focuses on seawater transfer and grow-out phases when fish are fed plant-based diets formulated to optimize energy use, the immune response, and fish robustness. Specifically, the project applies data integration for biomarker discovery using latent components, such as multi-block sparse partial least squares discriminant analysis (sPLS-DA). This ongoing workflow integrates results from both freshwater and seawater phases, covering host microbiota characterization, fish performance, and diagnostic packages across the intestine, liver, head kidney, and spleen. These packages were designed to evaluate key indicators, including histomorphometry, enzymatic activity, metabolic function, antioxidant capacity, detoxification, and immune responses. Preliminary data from individual datasets showed that fish fed increasing levels of polyunsaturated fatty acids (PUFAs) exhibited systemic immune activation. Driven by pro-inflammatory cytokines, this response potentially compromises the regulation of antioxidant defenses and the respiratory burst, two key innate immune processes for coping with *P. salmonis*. On the other hand, fish fed lower PUFA levels revealed improved epithelial barrier function in mucosal tissues, as well as a higher intestinal relative abundance of *Acetivibrio* spp. This bacterium has been described as a key producer of butyrate, a short-chain fatty acid used by epithelial cells as an energy source. In addition, butyrate has immunomodulatory properties that promote anti-inflammatory responses, while contributing to reduced mortality in bacterial-challenged salmonids fed diets containing soybean meal. Overall, the further integration of these results for biomarker selection has the potential to help predict salmonid robustness and resilience against challenges during farming.

Keywords: Immunonutrition; Salmonids; Multi-layer evaluation; Data integration.

Acknowledgments: This work was funded by PTEC-INVA Project (CORFO 21PTECIN-188130) from the Ministry of Economy, Development and Tourism of Chile.

DEVELOPMENT OF NEW LIVE ATTENUATED VACCINE FOR GLOBAL SRS CONTROL

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Abstract

Salmonid Rickettsial Septicemia (SRS), caused by *Piscirickettsia salmonis*, remains one of the most economically damaging diseases in salmon aquaculture. While historically concentrated in Chile, SRS is now emerging as a growing concern across the North Atlantic Hemisphere, with rising case reports in Ireland, Scotland, and Norway. This geographic expansion underscores the urgent need for control strategies with demonstrated cross-regional relevance. We present R&D data from a live attenuated vaccine platform designed against major circulating *P. salmonis* lineages. Controlled challenge trials demonstrated significant and long-lasting reduction in mortality among vaccinated fish, supported by robust safety profiles with no adverse effects observed. To assess the vaccine's relevance beyond its region of origin, we conducted comparative genomic analyses across strains from multiple production geographies, alongside molecular characterization of key genetic determinants underlying antigenic diversity. These analyses strongly suggest cross-protection against divergent isolates, providing early evidence of broad-spectrum efficacy potential. In parallel, compatibility studies evaluated the vaccine's interaction with existing products from current management programs, an essential consideration for real-world adoption. This presentation will discuss these R&D findings and outline potential pathways for international development, including considerations for adoption in other geographies. We aim to advance a scalable, genomically-informed solution to mitigate SRS outbreaks and support the long-term sustainability of global salmon production.

BEYOND SRS: LESSONS FROM EMERGING BACTERIAL DISEASES IN ATLANTIC SALMON

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Abstract

Bacterial diseases remain a major challenge to sustainable salmon production worldwide. While Piscirickettsiosis (SRS) continues to be of primary concern in Chile, the Norwegian aquaculture industry has in recent years faced increasing challenges associated with other bacterial pathogens, including *Moritella viscosa* and *Tenacibaculum finnmarkense*. Although these diseases differ substantially in pathology, epidemiology, and host-pathogen interactions, they share several features with SRS, including pathogen diversity, variable field performance of control measures, and the need for continuous improvement of preventive strategies. This presentation will provide an overview of ongoing work aimed at improving the understanding and control of *M. viscosa* and *T. finnmarkense* infections in Atlantic salmon. The work combines pathogen characterization, phylogenetic analysis, challenge model development, and vaccine evaluation to better understand factors influencing disease dynamics and protective immunity. Particular attention will be given to how genetic diversity within bacterial populations can impact vaccine design and disease management strategies. The presentation will discuss how integrating epidemiological knowledge, molecular characterization of isolates, and targeted vaccine development can contribute to more robust disease prevention programs. Drawing on experiences from Norway, we will reflect on whether elements of these approaches may also be relevant when addressing bacterial disease challenges in other salmon-producing regions, including those where SRS remains a dominant health concern. By comparing experiences across pathogens and production systems, the presentation aims to stimulate discussion on common principles for improving health management and reducing the impact of bacterial diseases in modern aquaculture.

EXPLAINABLE GNN DISEASE MODULE DETECTION IN THE *Salmo salar* MACROPHAGE RESPONSE TO *Piscirickettsia salmonis*

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Abstract

Intracellular bacterial pathogens reshape host macrophage transcription in ways that single-gene analyses capture only partially. Network-based approaches that combine protein-protein interaction data with expression profiles can recover coordinated gene modules underlying this response but require that both layers be mapped onto the same, correctly identified reference. We are building an approximate macrophage interactome for *Salmo salar* by relabelling the STRING v12 Atlantic salmon network to the current reference assembly and restricting it to genes expressed in a single-cell-defined mononuclear phagocyte population, correcting gene identifier and assembly mismatches identified in an earlier version of this mapping. This corrected network will serve as the graph structure for an explainable graph neural network (GNN-SubNet), adapted to a two-condition RNA-seq design of six samples, three unchallenged controls and three challenged with *Piscirickettsia salmonis*. The model will be trained to classify samples by condition using expression values as node features over the macrophage-restricted interactome, and its explainability module will be used to extract the subnetwork of genes and interactions most responsible for that classification. The expected output is a disease module, a set of genes together with their interactions, representing the host network most associated with the *P. salmonis* infection response. This approach is intended to complement conventional differential expression analysis by identifying coordinated, interaction-level signatures rather than genes considered individually.

SMOLTVISION CASE STUDY; POSITIVE SYNERGY WITH THE USE OF ALPHA JECT LIVAC SRS VACCINE

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Abstract

SmoltVision is the first commercially available test capable of differentiating between seawater and freshwater ATPase isoforms using real-time RT-PCR. The experience documented below corresponds to a comparison between SmoltVision and another tool used to verify the smoltification process, and to its synergistic use with the Alpha Ject LiVac SRS vaccine, in order to establish the health performance of different populations after seawater transfer. Na+/K+-ATPase activity (total NKA) has long been used as an indicator of seawater readiness in juvenile salmonids. Inadequate smoltification negatively affects marine survival, growth, and susceptibility to disease. Measurement of total NKA has been widely accepted worldwide as the "gold standard" method for assessing smoltification in the commercial salmon industry. SmoltVision, developed by PHARMAQ Analytiq, is a quantitative real-time RT-PCR test that differentiates between the ATPase α1a (freshwater) and α1b (seawater) isoforms, providing more precise information on the fish's smoltification status.A population of Salmo salar smolts from three different freshwater hatcheries was evaluated. Samples were processed using SmoltVision (RT-PCR) and total ATPase (enzymatic method), and the Alpha Ject LiVac SRS vaccine was administered at the time of transfer. Post-transfer follow-up was performed for 50 weeks, assessing mortalities due to maladaptation, mechanical damage, and SRS. SmoltVision provides gene-expression values for the ATPase α1a and α1b isoforms, calculating an α1b/α1a ratio that reflects the individual's level of smoltification. Tables 1 and 2 show SmoltVision results for each hatchery, including Ct values, efficiencies, and calculated ratios. Below are SmoltVision results for the different hatcheries evaluated, along with post-transfer mortality data. Adequate monitoring of the smoltification process during the freshwater stage, together with the use of tools that enable effective control of pathogenic agents, are key factors for achieving good production performance during the grow-out phase of salmonid species. SmoltVision made it possible to identify differences in smoltification level between populations, correlating with post-transfer health performance. Populations with better smoltification (higher α1b/α1a ratio) showed significantly lower mortalities due to maladaptation and SRS. The synergistic use of SmoltVision with the Alpha Ject LiVac SRS vaccine represents an effective strategy to improve post-transfer productive and health performance in Atlantic salmon.

Table 1. SmoltVision results. Hatchery A, (TK 208).

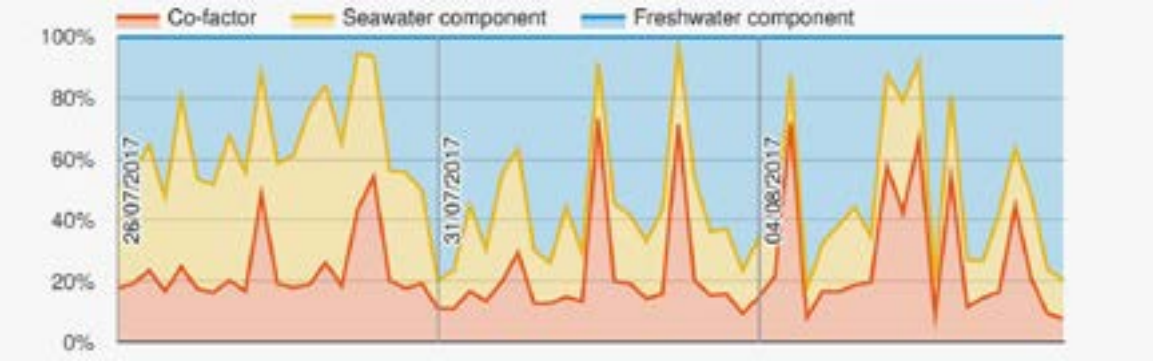


Table 2. SmoltVision results. Hatchery C (TK 305).

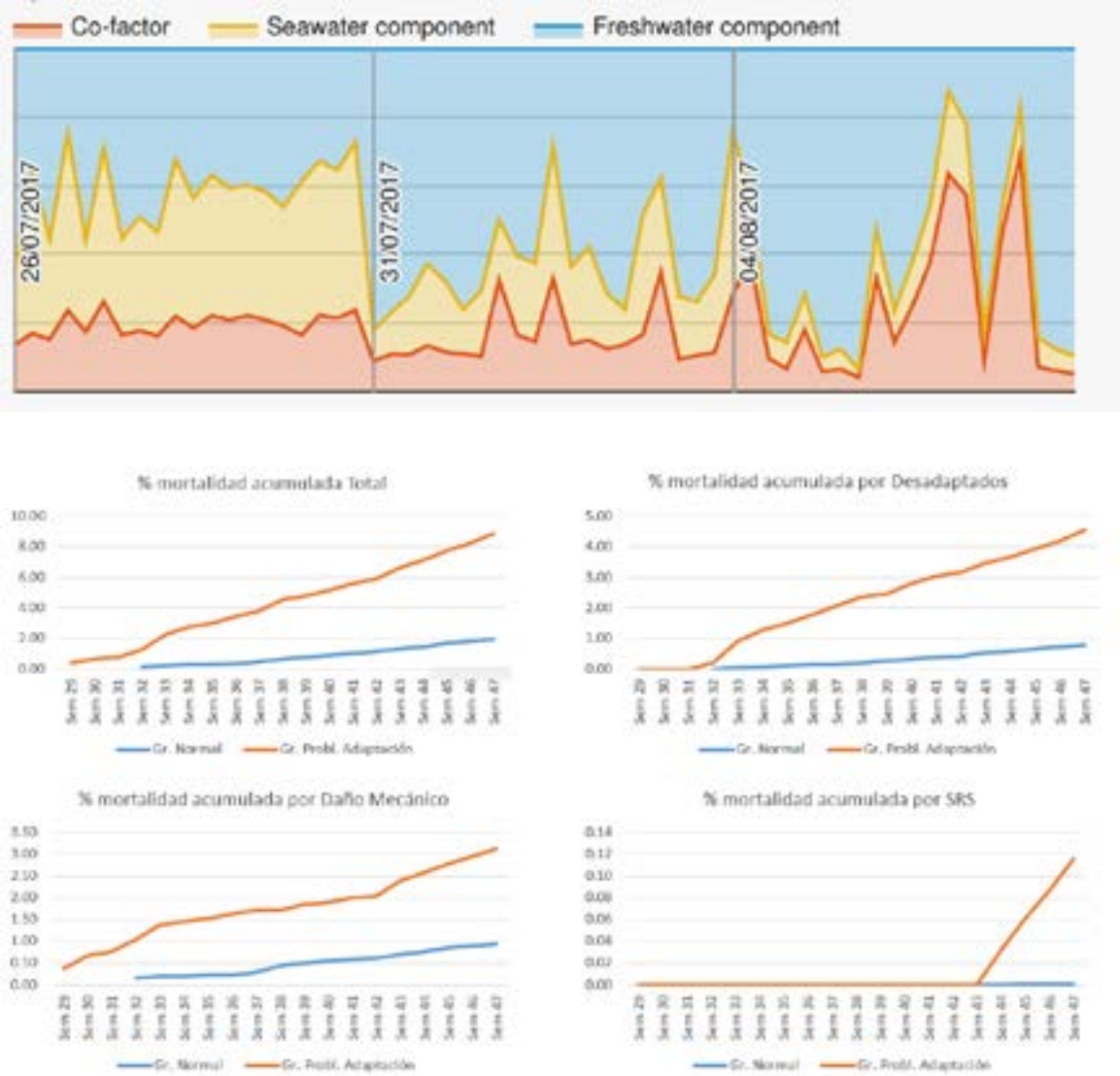


Figure 1. Plots of total cumulative mortalities, mechanical damage, and SRS (week 50).

THE FEWER, THE BETTER: THE ROLE OF TRANSCRIPTIONAL FRONTLOADING IN ATLANTIC SALMON RESISTANCE TO *Piscirickettsia salmonis*

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Abstract

Salmon rickettsial syndrome (SRS), caused by *Piscirickettsia salmonis*, remains a major infectious threat to Atlantic salmon aquaculture, yet the molecular mechanisms underlying host resistance remain incompletely understood. This study characterized basal and infection-induced transcriptional responses in the head kidney (HK) of Atlantic salmon (*Salmo salar*) with contrasting SRS phenotypes. Twelve fish were analysed, including six resistant (R) and six susceptible (S) individuals; within each phenotype, three fish were sampled under basal conditions and three following *P. salmonis* challenge. Total RNA was extracted using TRIzol Reagent (Ambion, USA), quantified using the Qubit RNA Broad Range Assay (Thermo Fisher Scientific, USA), and quality-assessed using a TapeStation 2200 system (Agilent Technologies, USA). TruSeq Stranded Total RNA libraries were prepared for mRNA profiling and analysed across phenotype and challenge-condition contrasts. Differential expression analyses identified candidate genes involved in innate immune recognition, inflammatory regulation, apoptosis, antimicrobial defence, cytoskeletal remodelling, intracellular trafficking, and water and ion homeostasis. These included members of the TRIM superfamily, *NLRC3, NLRC5, NK-lysin* genes, caspases, inflammatory mediators, aquaporins, and cytoskeleton-associated genes. Resistant fish displayed a basal transcriptional profile consistent with immune frontloading (the elevated pre-infection expression of a subset of genes that poises individuals for a rapid response upon exposure), whereas susceptible fish were comparatively enriched for pathways related to structural organization and cellular remodelling. Following challenge, resistant individuals mounted a more focused transcriptional response associated with immune modulation, cellular transport, and cytokine regulation. In contrast, susceptible fish showed a broader transcriptional shift characterized by inflammatory, reproductive, neuro-immune, and structural reprogramming. The magnitude of the transcriptional response was substantially greater in susceptible fish, with limited overlap between phenotype-specific differentially expressed genes, supporting the hypothesis that resistant and susceptible individuals resolve infection through distinct transcriptional strategies. Single-cell RNA sequencing was used to provide cellular context for TRIM gene expression across putative HK immune and hematopoietic cell populations, including granulocytes, mononuclear phagocytes, B cells, T cells, and NK-like cells, suggesting that phenotype-associated TRIM responses may be mediated by specific innate and adaptive immune cell compartments. TRIM-associated candidates were further evaluated through qRT-PCR expression analysis and de novo transcriptome validation based on sequence homology, RBCC domain architecture, and phylogenetic placement within TRIM classes using a curated *Salmoniformes* TRIM reference database. Overall, these results are consistent with a model in which resistance to *P. salmonis* is associated with transcriptional frontloading and a more constrained immune response, whereas susceptibility is linked to delayed and extensive transcriptional reprogramming. This work identifies molecular candidates relevant to SRS resilience and provides a foundation for functional validation and future selective-breeding strategies in Atlantic salmon.

Keywords: *Salmo salar*; *Piscirickettsia salmonis*; transcriptional frontloading; host resistance.

Acknowledgments: Financial support was provided by ANID INCAR2 CIA250009.

HBU: SOLUCIÓN NO FARMACOLÓGICA PARA MODULAR LA RESPUESTA INMUNOGÉNICA FRENTE A SRS EN ESPECIES SALMÓNIDAS

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Abstract

Las soluciones "No farmacológicas" como la Inmuno-nutrición, basada en extractos de antioxidantes de orujos de uva y aditivos alimentarios funcionales, están resultando una estrategia eficiente para reforzar la respuesta inmunogénica, bloqueando la colonización de bacterias patógenos, modulando el microbioma intestinal y la inmunidad mucosal, disminuyendo la respuesta inflamatoria crónica en el intestino, producida por Piscirickettsiosis a causa de *Piscirickettsia salmonis*. En este sentido, la harina de orujos de uva HBU, corresponde a un extracto fitogénico enriquecido en polifenoles que nace como una solución a partir de resultados de estudios *in vitro* e *in vivo*. Dentro de las moléculas bioactivas de HBU, cabe mencionar su perfil de polifenoles y aminoácidos presentes en la piel y semillas de la uva, donde destacan el ácido glutámico, el ácido aspártico, la arginina y la leucina. Por tal motivo, el objetivo de este estudio fue caracterizar *in vitro* las propiedades anti-*P.salmonis* de HBU y su efecto sobre parámetros productivos a través de la inclusión en la dieta de *Salmo salar*. La evaluación del efecto anti-*P. salmonis in vitro* se evaluó en células RTGutGC cultivadas a confluencia en medio L-15 suplementado con 10% de suero fetal bovino. El cultivo de las bacterias se realizó en caldo CASO y placas de agar Austral-TSHem. Para el desafío bacteriano *in vitro*, las células fueron tratadas con diferentes concentraciones de HBU y posterior infección con *P. salmonis* LF-89 por 14 días. En el caso del ensayo *in vivo*, se desarrollaron tres prototipos de dietas con incorporación de HBU a 1%, 3% y 5% y una dieta control para un ensayo de aceptabilidad en truchas arcoíris de 60 g de peso inicial durante 28 días. Finalmente, el estudio funcional *in vivo* comparó la dieta control con la dieta experimental de HBU al 5% por 60 días, recolectando muestras representativas que incluyeron plasma, histología del intestino medio y branquias, microbiota, páncreas y expresión génica. A partir de la preincubación de las células RTGutGC con 2.5, 100 y 250 µg/mL del extracto de orujo-HBU, se observó una disminución significativa de la lisis celular a los 7 días post-infección con *P. salmonis* LF-89 en comparación con las células control, infectadas y sin pre-tratamiento, evidenciando un efecto citoprotector hasta los 14 días post-infección. Por otro lado, los compuestos fenólicos en la dieta suplementada con 5% de HBU, resistieron el estrés térmico y mecánico de la extrusión, alcanzando 2086 mg GAE/100g y una actividad antioxidante superior a los 14.522 ORAC (µmol ET/100g). Las pruebas de aceptación de las dietas con HBU en trucha arcoíris, mostraron tasas de consumo promedio del 99,97%, destacando una ganancia de peso superior al 27% respecto al peso inicial y un factor de conversión (FCR) donde 1 Kg de dieta generó en promedio 1 Kg de biomasa. En conclusión y de acuerdo con las características del producto, el 79% de los compuestos fenólicos presentes en la HBU no son extraíbles, lo que asegura su biodisponibilidad en el tracto digestivo para la neutralización de especies reactivas durante la digestión. Asimismo, el extracto de HBU tiene la capacidad de mitigar el daño metabólico y la enteritis aguda provocada por dietas con alto contenido de harina de soya, protegiendo a los peces del estrés oxidativo. La inclusión de extracto de orujos de uva resultó en un FCR con 8% superior a la dieta control y es equivalente al uso de dietas con altos niveles de harina de pescado o insumos de origen animal.

Keywords: Ingrediente Funcional; Antimicrobiano; Antiinflamatorio; Inmunomodulador; Inmunogénico.

Acknowledgments: ANID INCAR[2] CIA250009. CORFO, Crea y Valida 24CVCS-255945.

IN SILICO IDENTIFICATION, TRANSCRIPTOMIC PROFILING, AND CHARACTERIZATION OF CELL WALL HYDROLASES FROM *Piscirickettsia salmonis* FOR THE DEVELOPMENT OF ANTIBIOFILM PLATFORMS

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Abstract

Piscirickettsia salmonis is a bacterial pathogen whose cell envelope and extracellular matrix components play important roles in adhesion, colonization, and biofilm formation and stability. Surface-associated components, such as lipopolysaccharides (LPS) and exopolysaccharides (EPS), can influence biofilm architecture, persistence, and host-pathogen interactions. Within the cell envelope, peptidoglycan remodeling is mediated by peptidoglycan hydrolases (PGHs), including amidases, glycosidases, and peptidases. These enzymes can exhibit modular architectures that combine catalytic domains with cell-wall-binding domains such as LysM domains, facilitating substrate recognition and localization at the bacterial cell wall. Given the conservation of LysM domains across Gram-negative and Gram-positive bacteria and their established role in cell wall recognition and binding, modular PGHs represent promising candidates for functional characterization and selection for future anti-biofilm applications. In this study, we conducted an *in silico* and transcriptomic analysis of *P. salmonis* peptidoglycan hydrolases (PGHs) to select candidates with functional potential to *P. salmonis* control. Comparative genomic and domain architecture analyses identified 13 hydrolase proteins, comprising 11 LysM domain-containing proteins (*Psal_LysM1–Psal_LysM11*) and two N-acetylmuramoyl-L-alanine amidases (*Psal_amidase1 and Psal_amidase2*). Subsequently, sequence conservation analysis, domain topology mapping, and three-dimensional structural modeling were used to characterize their molecular architecture and identify potential catalytic regions and peptidoglycan-interacting interfaces. RNA-seq transcriptomic profiling comparing *P. salmonis* growth under iron-overload and iron-limited conditions during an *in vitro* infection of SHK-1 salmon cells showed differences in the expression of two hydrolases: *Psal_LysM2 was* upregulated in iron overload, and *Psal_amidase2* was downregulated in the same condition. These results indicate that individual PGHs display distinct transcriptional profiles depending on the bacterial growth environment and interaction with host cells. Based on these transcriptomic profiles, we subsequently validated them by RT-qPCR. Overall, this integrated *in silico* and transcriptomic approach provides a rational framework for selecting bacterial cell-wall hydrolases with biotechnological potential as specific enzymatic tools for biofilm mitigation in aquaculture systems.

Keywords: *Piscirickettsia salmonis;* peptidoglycan hydrolases; LysM domain; amidases; biofilm control.

Acknowledgments: Financial support was provided by ANID INCAR2 CIA250009.

THE YELCHO ALLIANCE: A NEW MODEL OF PUBLIC-PRIVATE GOVERNANCE AND ENGAGEMENT WITH THE ANIMAL HEALTH SECTOR

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Abstract

The Yelcho Alliance is an unprecedented, permanent collaborative effort bringing together Chile's salmon farming industry through the trade associations SalmonChile, Consejo del Salmón, the Chilean Agricultural and Livestock Service (Servicio Agrícola y Ganadero, SAG) and the National Fisheries and Aquaculture Service (Servicio Nacional de Pesca y Acuicultura, Sernapesca), under the technical coordination of the consulting firm Aquabench. The Alliance brings together the companies Australis Seafoods, Blumar, Camanchaca, Cermaq Chile, Mowi Chile, Multi X, Salmones Austral, Marine Farm and Ventisqueros, which together represent approximately 73% of Atlantic salmon production in Chile. The Alliance's central objective is to seek, develop and implement immunological solutions primarily vaccines and boosters that effectively prevent piscirickettsiosis (SRS), caused by the bacterium *Piscirickettsia salmonis*, thereby reducing antibiotic use in salmon farming, in line with the guidelines of Chile's National Action Plan against Antimicrobial Resistance. Launched in 2024, Yelcho draws inspiration from Chile's strategy against COVID-19 and represents the Chilean salmon farming industry's commitment to a new way of working with pharmaceutical companies, fostering innovation and prototype vaccine testing, and supporting the fastest possible deployment of effective solutions for farmed salmon.

Keywords: public-private partnership; *Piscirickettsia salmonis*; vaccination; antimicrobial resistance; salmon industry.

Acknowledgments: This work was developed within the framework of the Yelcho Alliance, technically coordinated by Aquabench S.A., with the support of SalmonChile, Consejo del Salmón, SAG, Sernapesca and the founding producer companies.

YELCHO EXPERIMENTAL TRIAL: EVALUATION OF VACCINES AND VACCINATION STRATEGIES FOR IMPROVED IMMUNITY AGAINST SRS

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Abstract

The Yelcho Alliance launched, in 2025, an experimental trial to evaluate vaccines and vaccination strategies against Salmon Rickettsial Syndrome (SRS), an unprecedented initiative for the industry. The trial protocol was defined by the Alliance's Technical-Scientific Committee, with active participation from salmon-producing companies, the Chilean Agricultural and Livestock Service (Servicio Agrícola y Ganadero, SAG) and the National Fisheries and Aquaculture Service (Servicio Nacional de Pesca y Acuicultura, Sernapesca). The trial evaluated the efficacy and safety of different vaccine products against *Piscirickettsia salmonis* through intraperitoneal challenges with the EM-90 and LF-89 strains at two post-immunization time points: 650 and 1,800 Accumulated Thermal Units (ATU). The study was conducted at The Challenge Room experimental facility (Colaco, Chile) under controlled conditions, with replicated experimental groups, individual traceability through tagging, and environmental control. Evaluation included cumulative mortality, relative percent survival (RPS), vaccine safety (local adverse reactions), immunocompetence biomarkers, detection of *P. salmonis* by RT-qPCR, biometric growth and gross necropsy findings. A total of seven vaccination strategies were evaluated against an unvaccinated control group. Comparative results and conclusions across the products evaluated will be presented publicly for the first time during this international SRS conference. In addition to the main trial findings, two ongoing continuity studies conducted by the Alliance will be presented, together with a proposal to join a new experimental trial scheduled to begin in 2027, reflecting the Alliance's ongoing commitment to a recurring, progressively standardized framework for evaluating SRS vaccine efficacy.

Keywords: *Piscirickettsia salmonis*; SRS; vaccination strategies; relative percent survival; Atlantic salmon.

Acknowledgments: This trial was carried out under the public-private Yelcho Alliance, with technical coordination by Aquabench S.A. and co-financing from the participating vaccine companies.

COMPARATIVE USE OF AN INJECTABLE OXYTETRACYCLINE STRATEGY FOR SRS CONTROL IN ATLANTIC SALMON AND RAINBOW TROUT IN THE LOS LAGOS AND AYSÉN REGIONS OF CHILE

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Abstract

Piscirickettsiosis (SRS) is the bacterial disease responsible for the greatest mortality losses in the Chilean salmon farming industry, with substantial economic impact. This has led the Chilean government, through the National Fisheries and Aquaculture Service (Sernapesca), to implement surveillance and control programs for the disease. Despite numerous measures implemented through these programs and by individual companies, SRS remains the main driver of antimicrobial use in the industry. *Piscirickettsia salmonis* has demonstrated a remarkable capacity for adaptation, with pathogenicity and virulence mechanisms that contribute to its persistence and survival. Current SRS control relies largely on antimicrobial treatment, particularly oral florfenicol. Injected oxytetracycline represents a potentially effective alternative, as *P. salmonis* remains highly susceptible to it when used appropriately and with proper timing. Although producers have reported variable or unsatisfactory outcomes with oxytetracycline, treatment success may depend not only on the antimicrobial itself but also on the strategy and conditions under which it is administered. In this study, we evaluated different strategies for injectable oxytetracycline use in SRS control in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), assessing the influence of factors such as SRS mortality at the time of injection, fish weight, and treatment coverage on treatment outcomes. Treatment outcomes were strongly influenced by these factors, which drove important differences in treatment criteria, implementation, and outcomes between the two species. Overall, these findings indicate that injectable oxytetracycline can be a viable SRS control strategy, but its effectiveness depends on timely, strategically planned use rather than on the antimicrobial choice alone. The differences observed between Atlantic salmon and rainbow trout further underscore the importance of adapting treatment strategies to species-specific production and health conditions.

Keywords: *Piscirickettsia salmonis*; oxytetracycline; antimicrobial treatment; Atlantic salmon; rainbow trout.

RISK FACTORS ASSOCIATED WITH SRS MORTALITY AT MONTH 8 AND AT THE END OF THE PRODUCTION CYCLE IN ATLANTIC SALMON IN THE LOS LAGOS AND AYSÉN REGIONS OF CHILE

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Abstract

Salmon Rickettsial Syndrome (SRS) is the bacterial disease with the largest health impact in the Chilean salmon farming industry, accounting for approximately 2% of mortality and around 90% of antimicrobial use. Despite nearly 30 years since its emergence, the complexity and dynamics of this disease remain a major challenge, particularly in understanding its underlying mechanisms and the factors that predispose fish to its occurrence and severity. Because the epidemiology and impact of piscirickettsiosis can shift over time and across production systems, periodic reassessment of risk factors is needed. This study aimed to identify risk factors associated with SRS-related mortality in Atlantic salmon (*Salmo salar*) during 2024–2025 in the Los Lagos and Aysén regions of Chile. Production, health, and environmental variables were evaluated in relation to cumulative SRS mortality at month 8 of the production cycle and at harvest. Several factors were significantly associated with early SRS impact by month 8, and early SRS mortality was in turn associated with final SRS mortality outcomes. Potentially manageable production-related factors were also identified, highlighting opportunities for prevention and management strategies to reduce both the likelihood and impact of SRS throughout the production cycle. These findings contribute to a better understanding of piscirickettsiosis dynamics and offer practical insights to strengthen health and production management in the Chilean salmon farming industry.

Keywords: *Piscirickettsia salmonis*; risk factors; Atlantic salmon; mortality; antimicrobial use.

BAYESIAN BACK-CALCULATION OF THE ONSET AND TEMPORAL TRAJECTORY OF RECONSTRUCTED FATAL *Piscirickettsia salmonis* INFECTIONS FROM MORTALITY RECORDS IN TWO CHILEAN SALMON FARMS

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Abstract

Salmonid rickettsial septicaemia (SRS), caused by the bacterium *Piscirickettsia salmonis*, is a major infectious disease affecting farmed fish in southern Chile. Mortality is a delayed manifestation of infections acquired weeks earlier, so it does not directly reveal the onset of an outbreak or the timing of infection. We developed a Bayesian back-calculation approach to reconstruct the subset of infections that subsequently contributed to SRS-mortality in farmed Atlantic salmon (*Salmo salar*), while distinguishing this quantity from total infection prevalence and transmission intensity. We analyzed weekly SRS-attributed and all-cause fish mortality, the number of fish at risk and seawater temperature at two Atlantic salmon farms severely affected by SRS in the Los Lagos Region: Farm 1, near the mouth of Reñihué Fjord, and Farm 2, in Comau Fjord. Weekly deaths were represented using a negative binomial likelihood function comprising SRS mortality and a time-varying mortality background from other causes. Recorded SRS-attributed deaths were modelled conditionally on total mortality using a beta-binomial likelihood that allows for imperfect cause-of-death sensitivity and specificity, and additional binomial variation. The latent weekly fraction of reconstructed fatal SRS infections followed a Gaussian random walk and was convolved with a seawater temperature-dependent Gamma infection-to-death kernel (mean: 400 degree-days, SD: 80). Information on florfenicol treatments and quantitative polymerase chain reaction (qPCR) testing was excluded from the likelihood function because of insufficient temporal resolution but was retained for descriptive comparison with the reconstructed epidemic trajectory. Operational onset was defined as the first week in which the posterior median of the reconstructed fraction of fatal infections exceeded 0.001 and a kernel coverage was ≥0.50. Bayesian inference was implemented in Julia language using Turing.jl. Four Markov chains were run with the No-U-Turn Sampler, using 2,500 adaptation iterations followed by 2,500 posterior samples per chain. Posterior predictive checks were used to evaluate model fits. For each farm, six alternative sensitivity scenarios evaluated the robustness of the results to assumptions regarding the sensitivity and specificity of cause-of-death classification. Weekly mortality at Farm 1 was analyzed from August 27, 2018, to March 4, 2019. During this period, 107,803 total deaths and 77,947 SRS-attributed deaths were recorded. Registered SRS mortality peaked at 24,387 deaths during the week beginning December 10, 2018 (Fig. 1a). Under the baseline classification priors, operational onset was reconstructed on September 3, 2018. Reconstructed fatal SRS infections peaked on November 5, 2018, at 36,358 infections (95% credible intervals [CrI]: 20,272-52,871), 35 days (five weeks) before the registered SRS mortality peak. The cumulative reconstructed fatal SRS infections were estimated to be 81,769 (CrI: 68,451-99,753) and the cumulative true SRS mortality was estimated to be 79,471 deaths (95% CrI: 66,965-95,407). The model estimated 2,549 false negatives (95% CrI: 593-5,434) and 345 false positives SRS classifications (95% CrI: 50-1,040) (Fig. 1a). The primary model met all prespecified convergence criteria, with a maximum R-hat of 1.002, a minimum bulk effective sample size (ESS) of 2,264 and zero divergences. Weekly mortality at Farm 2 was analyzed from January 5, 2015, to April 18, 2016. During this period, a total of 207,874 deaths and 73,697 SRS-attributed deaths were recorded. Operational onset was reconstructed on September 14, 2015. The main reconstructed fatal-infection peak occurred on October 26, 2015, at 10,146 infections (95% CrI 2,920-19,286), five weeks before registered SRS mortality peaked at 7,581 deaths during the week beginning November 30, 2015 (Fig. 1b). Additional reconstructed infection pulses occurred on November 30, 2015, and February 29, 2016. Cumulative reconstructed fatal SRS infections were estimated at 82,487 (68,942-113,648) and cumulative true SRS mortality was estimated at 78,841 deaths (95% CrI: 66,753-94,719) (Fig. 1b). The model estimated 5,357 false negatives (95% CrI: 935-13,571) and 233 false positives SRS classifications (95% CrI: 33-821). The model met all prespecified convergence criteria (maximum R-hat: 1.004, minimum bulk ESS: 1,034, zero divergences). Across six alternative sensitivity and specificity

prior scenarios per farm, the reconstructed peak date remained unchanged. Relative to baseline, peak magnitude varied from -0.7% to +1.0% in Farm 1 and from -2.5% to +3.7% in Farm 2, while cumulative infections varied from -1.0% to +1.5% and from -3.4% to +4.3%, respectively. All 12 runs converged (maximum R-hat: 1.005, minimum bulk ESS: 944; zero divergences). Classification-error estimates were prior-sensitive but did not affect the reconstructed epidemic timing or overall infection burden.

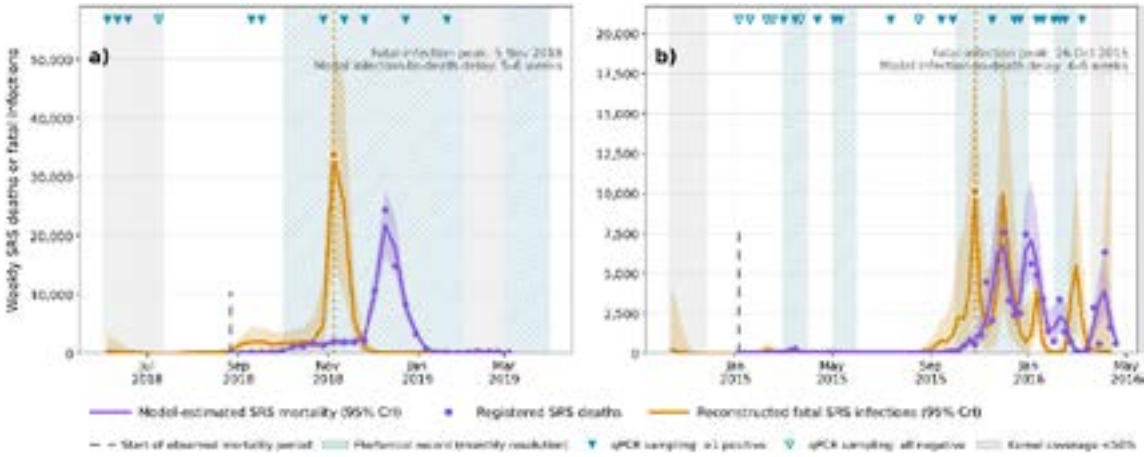


Figure 1. Reconstructed fatal SRS infections (orange), model-estimated SRS mortality (purple) and monthly florfenicol records (hatched areas) for a) Farm 1 and b) Farm 2. Shaded areas represent 95% credible intervals.

Bayesian back-calculation consistently reconstructed the temporal dynamics of fatal SRS infections across two salmon farms. In both farms, the main reconstructed infection peak preceded the registered SRS mortality peak by five weeks, while Farm 2 exhibited multiple infection pulses. Peak timing and overall reconstructed infection burden were robust to alternative assumptions regarding cause-of-death sensitivity and specificity. By translating routinely collected mortality records into estimates of otherwise infection timing, this framework can help identify infection windows and better align diagnostic sampling, outbreaks investigation, and control planning with the underlying epidemic process rather than its delayed mortality signal. Weekly qPCR observations and monthly florfenicol records provided complimentary temporal information. Future access to week-specific treatment timing and duration, together with validated estimates of qPCR diagnostic sensitivity and specificity, would enable these complementary data sources to be formally incorporated into the model and strengthen inference on infection dynamics and treatment-associated changes. Although the consisted findings across both farms support the feasibility of this approach, the reconstructed trajectories remain conditional on the infection-to-death kernel and mortality-classification assumptions. Further validation across many salmon farms with contrasting oceanographic and epidemiological conditions is therefore required.

Keywords: *Piscirickettsia salmonis*; Bayesian deconvolution; back-calculation; mortality surveillance.

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SEAWATER IMMERSION BOOSTER VACCINATION AS A STRATEGY TO EXTEND SRS PROTECTION UNDER COMMERCIAL ATLANTIC SALMON FARMING CONDITIONS

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Abstract

Salmonid rickettsial septicaemia (SRS), caused by *Piscirickettsia salmonis*, remains a major health challenge for Atlantic salmon production in Chile and an important driver of antimicrobial interventions. Protection elicited by freshwater vaccination may be insufficient to cover the entire seawater production cycle. We evaluated an inactivated *P. salmonis* vaccine administered as an immersion booster during the seawater phase, with the aim of assessing its potential to extend protection under controlled and large-scale commercial conditions. The development program combined immunogenicity and controlled challenge studies with prospective commercial field evaluations at two Atlantic salmon seawater sites in southern Chile. All fish had received the farm standard pre-transfer vaccination program. Booster groups subsequently received a standardized seawater immersion vaccination protocol, while contemporaneous non-boosted cages served as controls. SRS-associated mortality was monitored at cage level, with antimicrobial use and anti-*P. salmonis* IgM responses evaluated as complementary outcomes. A controlled challenge performed during the expected post-booster protection period was used to assess biological efficacy against *P. salmonis*. Detailed study design parameters and quantitative outcomes are intentionally not disclosed in this abstract because a patent application covering aspects of this development is currently being prepared. Across the development program, the seawater immersion booster generated a consistent biological and field signal compatible with additional protection against SRS compared with the baseline vaccination program alone. Findings from controlled challenge and independent commercial field evaluations were directionally concordant, and complementary immunological and production-health observations supported further evaluation of the approach. The available evidence supports seawater immersion boosting as a promising and operationally scalable strategy to extend SRS protection beyond pre-transfer vaccination in Atlantic salmon. Results obtained under both controlled and commercial conditions justify continued multi-site evaluation of the approach and its potential impact on SRS-associated mortality and antimicrobial requirements. Full quantitative results will be disclosed at the conference once the ongoing patent-protection process allows their public presentation.

Keywords: *Piscirickettsia salmonis;* SRS; Atlantic salmon; immersion vaccination; booster vaccination.

Acknowledgments. This work was supported by Veterquímica S.A. The authors thank the participating salmon farming teams, veterinary personnel and laboratory staff involved in field implementation, health monitoring and sample analysis.

HISTOPATHOLOGICAL CHARACTERIZATION OF GRANULOMATOUS LESIONS ASSOCIATED WITH *Piscirickettsia salmonis* IN ATLANTIC SALMON (*Salmo salar*) FROM SEAWATER FARMING SITES

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Abstract

Piscirickettsia salmonis is a significant pathogen in salmonid aquaculture; it is the most prevalent infectious disease in the Chilean salmon farming industry and a major cause of mortality and antibiotic use in Atlantic salmon (*Salmo salar*). This systemic pathogen induces granuloma formation in visceral organs such as the liver and kidney; these granulomas are key lesions in host-pathogen interactions. Although granulomatous lesions are frequently described in affected fish, their histopathological characteristics and organ-specific presentation under field conditions require more detailed characterization. The aim of this study was to characterize (at both morphological and cellular levels) the granulomas induced by *P. salmonis* in the liver and kidney of Atlantic salmon from marine aquaculture sites in southern Chile. Twenty-one Atlantic salmon (*Salmo salar*) were evaluated from two targeted samplings performed at seawater farming sites with confirmed Piscirickettsiosis outbreaks. Fish were selected based on lethargy, moribund condition, skin ulcerations and epidemiological history. Paired liver and kidney samples were collected, resulting in 42 tissue samples. Samples were fixed in 10% buffered formalin, processed for histopathological examination and stained with hematoxylin and eosin. The presence of *P. salmonis* was evaluated by immunohistochemistry using a polyclonal anti-*P. salmonis* antibody. Lesions were characterized according to their distribution, intensity, affected tissues, inflammatory cell composition and association with bacterial immunoreactivity. Granulomatous inflammation was identified in the liver of 16 of the 21 fish (76.2%) and in the kidney of 13 of the 21 (61.9%). Hepatic lesions ranged from small, concentric inflammatory foci in the early stage to extensive, coalescing granulomatous formations. These lesions were associated with hepatocellular vacuolar degeneration (47.6%), apoptosis (28.6%), congestion (66.7%), necrosis (14.3%), and occasional cholangiohepatitis (38.1%). Immunohistochemical staining demonstrated the presence of *P. salmonis*-positive material within granulomatous foci, macrophages or mononuclear inflammatory cells, hepatic sinusoids, vascular lumens, and the hepatic parenchyma. Renal lesions included interstitial inflammation with a multifocal to diffuse distribution pattern (100%), granulomatous nephritis (61,9%), hematopoietic tissue hyperplasia (42.9%), congestion (19%), and tubular alterations (28,9%). *P. salmonis*-positive material was detected via immunohistochemistry in the renal interstitium, capsule, blood vessels, and reticuloendothelial or hematopoietic compartments. In several fish, the simultaneous presence of bacterial antigen in the circulation, parenchymal tissue, and inflammatory foci suggested active systemic dissemination. The intensity and distribution of lesions varied among fish and farming sites, indicating different stages or patterns of disease development; furthermore, immunohistochemistry for *P. salmonis* yielded positive results in both the liver and kidney in 19 of the 21 fish (90.5%). Preliminary results indicate that *P.salmonis*-associated lesions in seawater-farmed Atlantic salmon are characterized by a spectrum of granulomatous response in liver and kidney, with varying degrees of bacterial antigen distribution and tissue damage. The high frequency of immunoreactivity against *P. salmonis* supports the association of these lesions with the pathogen and lays the groundwork for future research into granuloma organization and host-pathogen interactions in field cases. Furthermore, the combined use of conventional histopathology and immunohistochemistry enabled the identification of granulomatous lesions at early, established, and extensive stages, as well as the detection of signs consistent with systemic bacterial dissemination. This approach could improve the pathological characterization of piscirickettsiosis and facilitate the interpretation of diagnostic and surveillance samples in salmon farming.

Keywords: *Piscirickettsia salmonis;* granulomatous inflammation; histopathological characteristics.

Acknowledgments: ANID INCAR[2] CIA250009 and MULTIX.

HIPOXIA EXPOSURE DURATION AND THE EFFECTIVENESS OF ORAL ANTIMICROBIALS FOR SRS CONTROL IN ATLANTIC SALMON

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Abstract

Low dissolved oxygen is a growing constraint on Chilean salmon production, driven by the interaction of local oceanographic conditions with broader atmospheric and climatic variability. This is particularly relevant to the management of salmonid rickettsial syndrome (SRS), where the effectiveness of oral antimicrobial therapy depends on feed intake and host physiological status. Despite widespread use of continuous oxygen monitoring at marine sites, the relationship between measured hypoxic exposure and treatment outcomes has received limited quantitative attention. We conducted a longitudinal study linking continuous multi-sensor oxygen records from marine salmon farms in southern Chile to weekly pen-level mortality around oral antibiotic treatment events. Oxygen data were aggregated into farm-week exposure metrics capturing the proportion of time spent below welfare-relevant thresholds, using temperature and salinity corrected saturation. Post-treatment SRS mortality was modeled using negative binomial mixed models with nested farm and pen random effects, adjusting for fish weight, pre-treatment baseline mortality, and seasonality. Hypoxic exposure and fish weight were both significantly associated with post-treatment SRS mortality. These findings indicate that oxygen conditions at the time of oral antimicrobial treatment influence therapeutic outcomes. Incorporating oxygen monitoring into treatment planning, alongside attention to treatment timing and fish size, is recommended as a precautionary measure to safeguard antimicrobial efficacy.

TREATMENT MANAGEMENT DETERMINANT OF ANTIBIOTIC USE AGAINST SALMON RICKETTSIAL SYNDROME

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Abstract

Salmonid rickettsial syndrome (SRS) is the most impactful infectious disease affecting Chilean salmon farming, and antibiotic treatment remains the primary measure for controlling SRS-associated mortality. Most research has focused on prevention and mitigation strategies to reduce overall antibiotic consumption, with comparatively little attention paid to how the management and application of antibiotic treatment affects consumption directly. In this study, we used data from 7,053 pens across 590 Atlantic salmon farms in the Los Lagos and Aysén regions, harvested between 2022 and 2026. We fitted a generalized linear mixed model with a log link to evaluate how antibiotic consumption was explained by treatment-management variables, including weight at first treatment, antibiotic class, delivery route, time between treatments, treatment extension or overlap, dosage, and duration, while controlling for harvest year, season, and production cycle. Treatment-management variables were significant predictors of antibiotic consumption, and farm-cycle clustering accounted for a substantial share of the remaining variance, indicating both a meaningful management signal and considerable unexplained heterogeneity across farms. These results show that *how* antibiotic treatments are administered, not merely whether or when to treat, is a significant and actionable determinant of overall antibiotic consumption, and that treatment-management indicators could be used by farmers and veterinarians to inform stewardship efforts.

ACUTE MORTALITY AND DOWNSTREAM SRS RISK FOLLOWING WELLBOAT DELOUSING IN ATLANTIC SALMON

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Abstract

Outbreaks of salmonid rickettsial syndrome (SRS) and *Caligus rogercresseyi* frequently overlap during the warmer months of the production cycle in Chile. Wellboat-based delousing is becoming increasingly popular, as it provides a controlled microenvironment that ensures consistent and higher treatment efficacy, but it can also be stressful due to the additional handling required, such as hauling and pumping of fish. In this study, we analyzed the short-term consequences of sea lice treatments performed in wellboats. We used a longitudinal, retrospective epidemiological analysis to assess the impact of wellboat delousing on the subsequent risk of SRS outbreaks and antibiotic usage. Wellboat delousing was associated with higher mortality than tarpaulin-based treatments, including an approximately four-fold increase in mechanical mortality relative to pre-treatment baseline levels. Although most treatments resulted in limited short-term mortality, approximately 10% were characterized by substantially higher-than-expected mortality. These excessive-mortality events were followed by increased SRS-associated mortality in subsequent weeks. However, this increased health burden did not translate into earlier therapeutic intervention, as treatment timing following excessive- and routine-mortality events did not differ significantly. In conclusion, this study indicates that wellboat delousing carries a short-term mortality cost that can predispose fish to a subsequent rise in SRS mortality, yet current management practices do not consistently respond to this early warning signal with earlier antibiotic intervention. These findings highlight an opportunity to improve post-delousing monitoring and treatment decision-making to reduce both SRS-related mortality and antibiotic use.

CONTEMPORARY *Piscirickettsia salmonis* FIELD ISOLATES DEMAND UPDATED CHALLENGE MODELS: A STANDARDISED PLATFORM BASED ON LF-89 AND EM-90 GENOGROUPS

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Abstract

Chile is the second-largest producer of farmed Atlantic salmon in the world, where Salmonid Rickettsial Syndrome (SRS), caused by *Piscirickettsia salmonis*, remains one of the most significant constraints on the sustainability and productivity of the industry. In recent years, farms have reported that *P. salmonis* isolates are changing and showing differences in virulence, antimicrobial sensitivity, and genogroup distribution. This constant change is reducing the predictive value of historical laboratory strains and challenging current healthmanagement strategies. Recent outbreaks have documented major transitions in genogroups, accompanied by marked differences in virulence and antibiotic sensitivity. These traits directly affect the performance of vaccines, functional feeds, genetic resistance programs, and treatments. Consequently, updated and biologically relevant challenge models that incorporate contemporary *P. salmonis* isolates are urgently needed. This need is not restricted to Chile: SRS has recently been reported in other salmon-producing regions, including Europe, where it has caused mortality in farmed fish. To address this need, we developed standardized fish challenge models using newly obtained field isolates to better represent the current strains of *P. salmonis*. From a large laboratory library, we selected more than 30 candidates based on outbreak date and location, genogroup, MIC profile, and their ability to grow consistently under laboratory conditions for inoculum preparation. This multicriteria selection ensured that only epidemiologically relevant and experimentally robust isolates were advanced for model development. We established reproducible intraperitoneal and cohabitation challenge models with three isolates belonging to the LF89 and EM90 genogroups, all originating from recent outbreaks in Chile's main salmonfarming regions. Both models produced consistent and dose-dependent cumulative mortality. These models, built with current farm isolates and standardized wetlab procedures, provide a reliable platform for evaluating new diseasecontrol strategies, product and feedadditive development, treatments, and for supporting strategies aimed at reducing antimicrobial use, thereby strengthening the resilience of aquaculture suppliers and farmers.

PRISMA-SRS: AN EVIDENCE-BASED DECISION-SUPPORT CALCULATOR FOR PISCIRICKETTSIOSIS RISK AND INTERVENTION PRIORITISATION AT THE FARM LEVEL IN CHILEAN SALMON AQUACULTURE

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Abstract

Piscirickettsiosis (SRS), caused by *Piscirickettsia salmonis*, remains the leading infectious cause of mortality and antimicrobial use in Chilean salmon farming. Over the last decade, field epidemiology has produced a substantial body of quantitative evidence on SRS risk factors, time to first outbreak, treatment timing and the relative value of control measures, yet this evidence is scattered across studies and is rarely available to farm health managers at the moment decisions are made. We developed PRISMA-SRS (Predictive Risk & Intervention Support Model for Aquaculture), a farm-level decision-support calculator that consolidates this evidence into a single transparent risk score and links it to an evidence-ranked intervention checklist and antibiotic-timing guidance. PRISMA-SRS was built as a lightweight web application in three layers. (i) A weighted risk score (0–100) combining eleven inputs across production context (species, week of seawater cycle, stocking season), environment (sea temperature), spatial exposure (neighbourhood status, active farms within 5 km), parasite load (egg-laying female *Caligus* per fish, number of antiparasitic baths), husbandry stressors (net fouling, sea-lion pressure, recent acute stressors), disease history (prior SRS event in the cycle) and the weekly SRS-mortality threshold at which antibiotic treatment was last initiated. Weights and thresholds were derived from four sources: a retrospective cohort of 652 farms and 1,546 production cycles (SIFA, 2014–2021) modelled with mixed-effects negative binomial and Cox models; a Weibull accelerated-failure-time analysis of 571 Atlantic salmon cycles (2013–2019); a structured expert elicitation of risk and protective factors; and a field-based systematic review of 70 studies (1997–2025) graded by strength of evidence. Species-specific hazard curves (median time to first outbreak ≈48 weeks for Atlantic salmon, ≈33 weeks for rainbow trout, >50 % of coho cycles never affected) generate a projected cumulative outbreak probability over the cycle. (ii) An intervention module listing 67 SRS control measures scored for effectiveness and consensus by nine head fish-health managers covering ≈59 % of Chilean Atlantic salmon and ≈65 % of rainbow trout production; measures are tiered and, when toggled as implemented, return a residual risk. (iii) A treatment-timing module that flags the association between delayed antibiotic initiation and earlier recurrence. Scores are classified as low (<35), moderate (35–64) or high (≥65) and mapped to recommended actions. Table 1 summarises the factor weights and the evidence anchoring them. Spatial exposure (0.18), *Caligus* load (0.14), sea temperature (0.12) and the antibiotic-initiation threshold (0.11) together account for more than half of the maximum score, consistent with the ranking of predictors in the underlying cohort models. In illustrative scenarios, an isolated Atlantic salmon farm at week 20, 12 °C, with <0.5 egg-laying females per fish and early treatment initiation scores in the low tier, whereas the same farm downstream of an infected neighbour, at 16 °C, with >1 egg-laying female per fish, two or more baths and a prior outbreak in the cycle moves to the high tier, with neighbourhood exposure and *Caligus* load as the two largest contributors. Tier 1 measures (net cleanliness, shorter sea cycles, removal of moribund fish, necropsy-based mortality classification, effective *Caligus* control and smolt-quality programmes) reduce the score more than Tier 2 measures; therapeutic and vaccination measures were rated lower and with markedly higher inter-expert variability, in line with field evidence that vaccination delays rather than prevents outbreaks. The prototype makes explicit that current weights are literature-derived starting points and that the outbreak curve is illustrative rather than a fitted survival model.

Table 1. Risk-factor weights in PRISMA-SRS v2 and their evidence base.

Risk factor (domain)	Weight	Evidence anchoring the weight
Neighbourhood exposure (spatial)	0.18	Between-farm connectivity; expert-elicited threshold of ≈2 active farms within 5 km (AUC 0.82)
Egg-laying female *Caligus* per fish (parasitic)	0.14	Strongest actionable predictor of weekly SRS mortality and time to first outbreak (SIFA cohort)
Sea temperature (environmental)	0.12	Summer SRS mortality ≈10× winter median
Antibiotic-initiation threshold (treatment)	0.11	Initiation at ≥0.03 % weekly mortality vs. <0.01 %: HR 1.37 for next outbreak (Weibull AFT, 571 cycles)
Antiparasitic bath treatments (management)	0.09	Risk rises above ≈2 baths per cycle (treatment-associated stress)
Net fouling (management)	0.08	Net cleanliness ranked Tier 1 by expert panel
Weeks in seawater; prior SRS event this cycle	0.07 each	Species-specific hazard curve; post-outbreak weeks carry markedly higher risk
Stocking season; sea-lion pressure	0.05 each	Autumn vs. spring HR 1.15; sea-lion exclusion ranked Tier 2
Recent acute stressor (management)	0.04	Handling, transport, hypoxia in previous 2 weeks

PRISMA-SRS translates a decade of Chilean field epidemiology on *P. salmonis* into an operational, auditable tool that ranks the drivers of risk at a given farm and the interventions most likely to reduce it. Its main limitation is that the score is a weighted index rather than a model-based probability; the next development step, now under way with a producing company, replaces the index with company-calibrated GLMM and AFT-with-frailty models, adds within-farm cage-to-cage outbreak dynamics and confidence intervals, and recalibrates the tool prospectively. We argue that decision-support tools of this kind are a necessary complement to surveillance and treatment guidelines if the evidence on SRS is to change practice at the farm level.

Keywords: *Piscirickettsia salmonis*; risk score; decision support; PRISMA-SRS; antimicrobial stewardship.

Acknowledgements: The evidence base underlying the calculator was generated with the support of Chilean salmon producers and fish-health managers who contributed data and expert judgement to the cohort, elicitation and review studies.

ANTIMICROBIAL SUSCEPTIBILITY SURVEILLANCE OF *Piscirickettsia salmonis* IN CHILE: REGIONAL, GENOGROUP, AND TEMPORAL TRENDS (2017–2025)

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Abstract

Piscirickettsia salmonis is the causative agent of salmonid rickettsial septicemia (SRS), or piscirickettsiosis, which is the leading cause of mortality due to infectious diseases and antimicrobial use in Chilean salmon aquaculture. This, together with the limited availability of therapeutic alternatives for disease control, makes this pathogen one of the major threats to salmonid production in Chile. In this context, antimicrobial susceptibility surveillance is a relevant tool for characterizing changes in bacterial populations and supporting fish health decision-making. Over the past decade, the Fisheries Development Institute (Instituto de Fomento Pesquero; IFOP) has implemented a surveillance program to monitor changes in susceptibility to antimicrobials used for the treatment of SRS, as well as variations in the genogroup distribution of this pathogen. The aim of this study was to characterize the antimicrobial susceptibility of *P. salmonis* to florfenicol (FFC) and oxytetracycline (OT), evaluating its distribution according to region, genogroup, and surveillance period from 2017 to 2025. A total of 143 *P. salmonis* isolates obtained from the Los Lagos Region (n = 89) and the Aysén Region (n = 46), were analyzed and classified according to genogroup (LF-89, n = 79; EM-90, n = 59). For susceptibility to FFC and OT, minimum inhibitory concentrations (MIC) were determined according to the LABD/NT4 technical standard. MIC data were analyzed by region, genogroup, and three-year surveillance periods (2017–2019, 2020–2022 and 2023–2025). Overall trends were assessed based on the proportion of isolates classified as wild type (WT) or non-wild type (NWT), according to the epidemiological cutoff value (ECOFF) established for each antimicrobial (FFC = 0.25 µg/mL and OT = 0.5 µg/mL). Additionally, descriptive statistics were calculated, including mode, MIC_{50}, MIC_{90}, geometric mean MIC (GMMIC), and geometric mean MIC of the WT population (WTGMMIC). Descriptive analysis showed that isolates from Los Lagos exhibited higher MIC values than those from Aysén for both antimicrobials, particularly for FFC (mode: 1 vs. 0.25 µg/mL; NWT proportion: 65.2% vs. 50.0%; GMMIC: 0.54 vs. 0.39 µg/mL; and MIC_{50}: 0.5 vs. 0.25 µg/mL). At the genogroup level, LF-89 exhibited higher MIC values than EM-90 for both antimicrobials, particularly for FFC (mode: 2 vs. 0.25 µg/mL; NWT proportion: 70.9% vs. 45.85%; GMMIC: 0.59 vs. 0.36 µg/mL; MIC_{50}: 1 vs. 0.25 µg/mL; and MIC_{90}: 2 vs. 1 µg/mL). Temporal analysis revealed contrasting trends between antimicrobials. For FFC, MIC_{50} increased progressively from 0.25 to 1 µg/mL, whereas MIC_{90} remained constant at 2 µg/mL. An increase was also observed in GMMIC (from 0.39 to 0.76 µg/mL), mode (from 0.25 to 2 µg/mL), and the proportion of NWT isolates (from 49.0% to 82.86%), indicating a shift in the central portion of the MIC distribution toward higher values. Conversely, WTGMMIC progressively decreased, reflecting changes within the WT population toward lower MIC values. For OT, a pattern different from that observed for FFC was identified, MIC_{50} fluctuated across surveillance periods, whereas MIC_{90} remained stable at 1 µg/mL, and the proportion of NWT isolates decreased from 17.65% to 11.43%. Overall, these results demonstrate differences in the distribution of antimicrobial susceptibility in *P. salmonis* associated with regional and genogroup variation. Isolates from Los Lagos showed higher MIC values for both antimicrobials and a higher proportion of NWT isolates compared with those from Aysén. Likewise, the LF-89 genogroup displayed a susceptibility profile shifted toward higher MIC values than EM-90, a pattern particularly evident for FFC. Temporal analysis also revealed contrasting patterns between antimicrobials. For FFC, a progressive shift of the MIC distribution toward higher values was observed, as reflected by increases in MIC_{50}, GMMIC, mode, and the proportion of NWT isolates. In contrast, OT did not show a pattern comparable to that of FFC, with fluctuating MIC_{50} values and a decrease in the proportion of NWT isolates. These findings highlight the importance of continued surveillance of MIC distributions through the monitoring program implemented by IFOP, providing a framework for detecting changes in the antimicrobial susceptibility of *P. salmonis* populations.

Keywords: *Piscirickettsia salmonis;* antimicrobial susceptibility; antimicrobial resistance surveillance; florfenicol; oxytetracycline.

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A MAJOR QTL FOR RESISTANCE AGAINST SALMONID RICKETTSIAL SEPTICAEMIA IN COHO SALMON (*Oncorhynchus kisutch*) MAPS TO A NARROW REGION ON CHROMOSOME 21, IMPLICATING TWO CANDIDATE GENES

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Abstract

The facultative intracellular bacterium *Piscirickettsia salmonis* causes Salmon Rickettsial Syndrome (SRS) in coho salmon, Atlantic salmon, and other salmonids. In this study we identified a major QTL for resistance to SRS in coho salmon as well as putative candidate genes underlying the QTL. Heritabilities across datasets were moderate to high for days-post-challenge (DPC) and binary survival in both shedders and cohabitants. The major QTL on chromosome 21 explained 82% and 72% of the genetic variation in DPC within two distinct year-class populations. Two SNPs were more strongly associated with SRS-resistance compared to other SNPs and in complete linkage disequilibrium with each other. The resistance allele was found to be dominant over the susceptibility allele at these SNPs. One of the two SNPs was located within the first exon of two genes which are transcribed in opposite directions: a histidine triad nucleotide-binding protein 3 (*HINT3*) gene and a gene (LOC109866666) encoding a long non-coding RNA (lncRNA). Genotypes at the SNP were correlated with expression levels at both *HINT3* and the lncRNA gene, and the differential expression was manifested in both SRS-challenged and non-challenged fish. The exonic SNP is located 3 base pairs upstream of the start codon of the *HINT3* gene, at a position which is crucial for effective translation according to the rules of Kozak. The resistance allele was associated with increased expression of *HINT3* and is predicted to increase translational efficiency through a more favourable Kozak sequence, although the latter remains to be experimentally validated.

Keywords: selective breeding; *Piscirickettsia salmonis;* SRS; coho; QTL.

Acknowledgement: This work was co-funded by CORFO.

GENOMIC SELECTION FOR SRS RESISTANCE INCREASES SURVIVAL IN THE FIELD

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Abstract

Controlled challenge tests representing the EM-90 and LF-89 genogroups were conducted across multiple year classes using fish from AquaGen's Atlantic salmon breeding nucleus. Challenge-tested fish were phenotyped and genotyped using a custom SNP array, and genomic prediction models were evaluated by 10-fold cross-validation. Field relevance was assessed using survivors and mortalities sampled from commercial SRS outbreaks. SNP effects derived from challenge tests were used to calculate GBVs, and their association with field survival was evaluated using logistic regression. Resistance to SRS showed a polygenic pattern and moderate heritability. Genetic correlations among challenge tests within genogroups were high, supporting the reproducibility of the resistance phenotypes. Correlations across genogroups were lower, indicating that resistance to EM-90 and LF-89 represents partly distinct traits. Nevertheless, GBVs derived from EM-90 challenge tests predicted survival during LF-89 field outbreaks. These results demonstrate that integrating genomic and phenotypic information from controlled challenge tests provides a robust basis for improving resistance to SRS caused by both major *P. salmonis* genogroups.

Keywords: selective breeding; genomic selection; *Piscirickettsia salmonis;* EM-90; LF-89.

Acknowledgements: This work was co-funded by CORFO.

UNRAVELING THE GENOMIC BASIS AND CONVERGENT EVOLUTION OF RESISTANCE TO *Piscirickettsia salmonis* IN ATLANTIC SALMON (*Salmo salar*) AND COHO SALMON (*Oncorhynchus kisutch*)

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Abstract

Salmonid Rickettsial Septicemia (SRS), caused by the intracellular bacterium *Piscirickettsia salmonis*, remains one of the most critical health challenges facing commercial salmon aquaculture. The pathogen's ability to invade, replicate within, and disseminate through host macrophages relies heavily on subverting host actin cytoskeleton dynamics. Deciphering the underlying genomic architecture driving host resistance is essential for developing target-driven molecular breeding programs and gene-editing strategies. To map the mechanisms of host resistance, we integrated transcriptomic and population-genomics datasets across Atlantic (*Salmo salar*) and Coho (*Oncorhynchus kisutch*) salmon. To detect signatures of selection and local adaptation, we implemented cross-population extended haplotype homozygosity (XP-EHH) tests by leveraging whole-genome sequencing (WGS) and high-density SNP array (220K) datasets, contrasting populations originating from the USA and Norway. RNA seq data were used within commercial populations from contrasting phenotypes associated with mortality after disease challenge. Our results demonstrate that host resistance rely on restricting the host cytoskeleton remodeling that *P. salmonis* hijacks for cellular entry and spread. Resistant *S. salar* families exhibited sustained expression of *ARPC4* and *WASP* (N-WASP), limiting bacterial interaction of actin dynamics and accelerating pathogen clearance. We also identified key *cis*-regulatory lncRNAs modulating cytoskeletal assembly, mucosal and systemic immunoglobulins *IgT*, and selenoproteins *SEPN1, SEPP1*. Cross-population genomic scans confirmed strong, parallel selection pressure acting on the NWASP–Arp2/3 complex pathway in both *S. salar* and *O. kisutch*, showing high pathway-level concordance (R^2=0.9222) across focal adhesion, calcium signaling, MAPK cascades, and clathrin-mediated endocytosis. Additionally, resistant fish mitigated fatal tissue damage and hemorrhaging by suppressing hyper-inflammatory responses via plasmin *PLG* downregulation. By bridging functional transcriptomics in resistant families with evolutionary footprints derived from cross-population analyses between USA and Norway cohorts, this work highlights vesicular entry and cytoskeletal remodeling as the principal axis of host adaptation to *P. salmonis*. These insights offer validated targets to increase the accuracy of genomic selection (GS) models and guide future CRISPR-based gene editing strategies to better understand the causal genes explained *P. salmonis* in aquaculture.

INTEGRATIVE TRANSCRIPTOMICS UNCOVERS THE MECHANISMS OF *HINT3*-MEDIATED SRS RESISTANCE IN COHO SALMON (*Oncorhynchus kisutch*)

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Abstract

In Coho Salmon (*Oncorhynchus kisutch*Septicaemia (SRS) caused by the intracellular bacterium *Piscirickettsia salmonis* is strongly influenced by a major QTL on chromosome 21. The most significant variants at this locus lie within two genes transcribed in opposite directions: the protein-coding gene *HINT3* and a long non-coding RNA (LOC109866666). Genotypes at these variants correlate with expression differences of both transcripts and with a substantial reduction in mortality (Moen et al. 2025). To elucidate the downstream mechanisms of this locus, head-kidney RNA-seq profiling was performed on 20 fish differing in *HINT3* genotype (following a seawater cohabitation challenge at the peak of the mortality curve. Differential expression analysis identified 1,237 upregulated (including *HINT3* and LOC109866666) and 276 downregulated genes in susceptible relative to resistant fish. Functional enrichment terms were embedded using a sentence transformer (all-MiniLM-L6-v2), cosine-similarity-distilled to reduce redundancy, UMAP-projected, and partitioned into distinct functional modules via K-Means clustering to further reduce term redundancy. This spatial semantic mapping revealed that host resistance involves tightly coordinated defense hubs: genome integrity, replication, and mitosis (*MKI67*, *CDC20*, MCM complex) and vasculature development and wound healing (*KDR, PECAM1B, EGFL7,* VEGF pathway) to drive cellular replacement and lesion recovery. Re-establishment of organ architecture was further supported by cell-junction and cytoskeletal dynamics (*ITGA5*, tight/focal junctions) alongside upstream morphogenetic and stress signaling (Hippo and Notch pathways). Crucially, these structural repair mechanisms were underpinned by enzymatic signaling and energy-metabolism cascades (including kinase activity, ATP binding, and GTPase activation). As HINT3 plays a fundamental role in nucleotide turnover, energy homeostasis, and stress-induced signal transduction, these findings provide a mechanistic model directly linking allelic variation at the *HINT3*/lncRNA locus to differential metabolic resilience, host-pathogen interactions, and survival under SRS challenge in salmonids., *PECAM1B, EGFL7,* VEGF pathway) to drive cellular replacement and lesion recovery. Re-establishment of organ architecture was further supported by cell-junction and cytoskeletal dynamics (*ITGA5*, tight/focal junctions) alongside upstream morphogenetic and stress signaling (Hippo and Notch pathways). Crucially, these structural repair mechanisms were underpinned by enzymatic signaling and energy-metabolism cascades (including kinase activity, ATP binding, and GTPase activation). As hint3 plays a fundamental role in nucleotide turnover, energy homeostasis, and stress-induced signal transduction, these findings provide a mechanistic model directly linking allelic variation at the hint3/lncRNA locus to differential metabolic resilience, host-pathogen interactions, and survival under SRS challenge in salmonids., pecam1b, egfl7, VEGF pathway) to drive cellular replacement and lesion recovery. Re-establishment of organ architecture was further supported by cell-junction and cytoskeletal dynamics (ITGA5, tight/focal junctions) alongside upstream morphogenetic and stress signaling (Hippo and Notch pathways). Crucially, these structural repair mechanisms were underpinned by enzymatic signaling and energy-metabolism cascades (including kinase activity, ATP binding, and GTPase activation). As hint3 plays a fundamental role in nucleotide turnover, energy homeostasis, and stress-induced signal transduction, these findings provide a mechanistic model directly linking allelic variation at the hint3/lncRNA locus to differential metabolic resilience, host-pathogen interactions, and survival under SRS challenge in salmonids., pecam1b, egfl7, VEGF pathway) to drive cellular replacement and lesion recovery. Re-establishment of organ architecture was further supported by cell-junction and cytoskeletal dynamics (ITGA5, tight/focal junctions) alongside upstream morphogenetic and stress signaling (Hippo and Notch pathways). Crucially, these structural repair mechanisms were underpinned by enzymatic signaling and energy-metabolism cascades (including kinase activity, ATP binding, and GTPase activation). As hint3 plays a fundamental role in nucleotide turnover, energy homeostasis, and stress-induced signal transduction, these findings provide a mechanistic model directly linking allelic variation at the hint3/lncRNA locus to differential metabolic resilience, host-pathogen interactions, and survival under SRS challenge in salmonids.

Keywords: disease resistance; Coho salmon; *Piscirickettsia salmonis*.

THE PANGENOME OF *Piscirickettsia salmonis* REVEAL, DIFFERENT EVOLUTIONARY TRAJECTORIES GIVEN HOST-PATHOGEN INTERACTION AND ENVIRONMENTAL PRODUCTION FACTORS

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Abstract

Piscirickettsia salmonis, a marine gammaproteobacterium, is the causative agent of Salmonid Rickettsial Septicaemia (SRS) and remains the most economically impactful pathogen in Chilean salmon aquaculture since its description over 30 years ago. Despite documented host-specific differences in susceptibility and host-pathogen interactions between Atlantic salmon (*Salmo salar*) and Coho salmon (*Oncorhynchus kisutch*), the long-term genomic drivers and speciation patterns of *P. salmonis* across hosts remain poorly understood. We aimed to reconstruct the evolutionary dynamics and pangenome fluidity of *P. salmonis* across time. We analyzed a comprehensive dataset of isolates collected in Chile between 1989 and 2025 across the LF-89 and EM-90 genogroups. Combining a core-genome molecular clock framework with temporal models of accessory gene dynamics, we evaluated rates of gene gain/loss and lineage diversification relative to host species. Pangenome analyses revealed marked contrast in gene acquisition dynamics between hosts. Isolates from *S. salar* exhibited double the rate of pangenome fluidity (+32.27 genes/year) compared to those from *O. kisutch* (+16.86 genes/year). In Atlantic salmon, this rapid expansion is driven by an open, highly fluid pangenome sustained by active horizontal gene transfer (*HGT*) under intensive farming conditions, considering the production system of *Salmo salar* mediated largely by mobile transposable cassettes (e.g., co-segregating IS30 and IS6/ISPsa2 family transposases). Conversely, in Coho salmon, phylogenetic and molecular clock analyses uncovered an ancient divergence into two distinct clades: one inheriting a conserved block of accessory genes and another clade with a sparse accessory profile. Consequently, the apparent annual gene gain in *O. kisutch* reflects the successful clonal expansion and decades-long persistence of a pre-existing, gene-rich lineage rather than continuous, active *HGT*. This may be explained by the relatively small production of Coho salmon in Chile. Functional annotation of the *Piscirickettsia salmonis* accessory genome of *Salmo Salar* reveals a specialized genetic reservoir dominated by mobile elements, virulence traits, and resistance mechanisms that collectively fuel genomic plasticity and intracellular survival. This dynamic accessory pool is fundamentally underpinned by a massive expansion of insertion sequences across diverse transposase families; including *IS1, IS3, IS4, IS5, IS6, IS30, IS481, IS630, IS701, IS91*, and *IS982*, which together with group II introns, conjugative mating-pair proteins, and the ComE natural transformation operon, facilitate ongoing horizontal gene transfer and structural chromosomal rearrangements. Beyond genetic mobility, the accessory genome encodes robust fitness and defense modules: toxin-antitoxin addiction systems (HicB, HigA, MazE, VapB, YefM) and BREX phage exclusion mechanisms preserve plasmid stability and immunity, while petrobactin siderophore synthesis, Catalase A, and surface-remodeling enzymes enable iron scavenging, oxidative stress detoxification within host macrophages, and immune evasion. Additionally, the presence of multidrug efflux machinery, heavy metal (mercuric) resistance operons, and specific antimicrobial resistance determinants (against chloramphenicol, bicyclomycin, and aminoglycosides) reflects strong anthropogenic selection in aquaculture environments. Our findings demonstrate two distinct evolutionary strategies in *P. salmonis*: rapid, transposon-driven accessory genome adaptation in high-density *S. salar* farming environments, versus historical diversification followed by stable lineage persistence in *O. kisutch*.

AUTOMATED PHENOTYPING STRENGTHENS GENETIC EVALUATION OF GROWTH AND SRS RESISTANCE IN SALMON BREEDING PROGRAMS

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Abstract

Genetic selection in salmonid breeding programs depends on accurate and representative phenotypic records to estimate breeding values (BV), genetic parameters and expected genetic gain. Growth (harvest weight) is commonly incorporated into a multi-trait selection index together with health-related traits, including resistance to Salmonid Rickettsial Septicemia (SRS). However, conventional phenotyping based on manual sampling is labor-intensive, subject to measurement error and typically represents only a small fraction of the population, limiting the precision of individual and family evaluations. We developed an automated robotic phenotyping and sorting system as a high-throughput alternative for measuring salmon growth-related traits at production scale. The central premise was that increasing the number and quality of phenotypic records, while reducing measurement error, can strengthen genetic evaluation and improve the estimation of relationships between growth and disease-resistance traits. The robotic system, IDE+SELECTOR®, combines industrial machine vision, geometric image analysis, automated biometric estimation and real-time fish sorting, with individual traceability through Pit-tags when available. Rainbow trout (*Oncorhynchus mykiss*) were evaluated at Centro Pucheguín Chico, Región de Los Lagos, Chile, between March and April 2026. The system recorded 32,262 fish from seven origin cages. Accuracy was assessed using 12,978 paired observations obtained from automated and trained-manual measurements of length, width and weight. Agreement was quantified using Lin's concordance correlation coefficient (CCC), R^2, mean absolute percentage error (MAPE), bias and Bland–Altman limits of agreement. Repeatability was assessed from 400 consecutive measurements of a reference fish. The resulting phenotypic dataset was evaluated as a basis for integration into genetic evaluations, considering growth as a component of a multi-trait selection index jointly with SRS resistance. The robotic system showed high agreement with manual measurements for length (CCC = 0.998; R^2 = 0.996; MAPE = 1.64%), width (CCC = 0.994; R^2 = 0.988; MAPE = 2.54%) and weight (CCC = 0.996; R^2 = 0.993; MAPE = 4.32%). Overall, 92.7% of fish were estimated within 10% of manual weight and 67.4% within 5%. Repeatability testing showed less than 1% variation in length and weight across 400 consecutive passes. Importantly, the system substantially increased phenotypic coverage relative to conventional manual sampling, generating a large and structured dataset suitable for individual-level evaluation. The use of automated measurements resulted in higher estimates of harvest-weight heritability (h^2 = 0.40 vs. 0.34 using trained-manual measurements) and a stronger estimated genetic correlation between growth and SRS resistance (r_g = 0.18 vs. 0.14). These results indicate that improved phenotypic coverage and measurement consistency can strengthen the genetic information underlying selection for both productive and disease-resistance traits. Automated robotic phenotyping provides accurate, repeatable and scalable measurements of salmonid growth-related traits, overcoming a major bottleneck of manual phenotyping: limited population coverage and measurement error. Its value for genetic improvement extends beyond automation itself. By generating denser and more reliable phenotypic records, the technology can strengthen genetic evaluation and the multi-trait selection index combining growth with SRS resistance and other economically relevant traits. This creates an opportunity to connect industrial-scale phenotyping with precision genetic selection, enabling more reliable identification of superior animals and potentially increasing realized genetic gain while maintaining selection for disease resistance.

Keywords: automated phenotyping; machine vision; salmon breeding; genetic evaluation; selection index; SRS resistance; genetic gain.

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FRESHWATER HEALTH HISTORY AND HOST PHYSIOLOGICAL-INMUNOLOGICAL CONDITION AS POTENTIAL DETERMINANTS OF SRS SUSCEPTIBILITY AFTER SEAWATER TRANSFER: PRELIMINARY EVIDENCE.

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Abstract

Piscirickettsiosis (SRS) remains one of the major bacterial challenges during the seawater phase of Atlantic salmon production. Susceptibility to SRS may not depend exclusively on marine exposure, because fish can enter seawater with different levels of physiological reserve and immune competence resulting from previous health events in freshwater. This preliminary study explored whether a history of bacterial disease and physiological-immunological disturbance before seawater transfer could be associated with subsequent fish condition, performance, and severity of SRS in sea water phase. A retrospective, integrative case analysis was performed using information from two farming sites. Freshwater health history was reviewed together with seawater production performance and cage-level diagnostic information. The analysis integrated previous bacterial disease events, antimicrobial treatments, vaccination and smoltification history, selected freshwater environmental stressors, seawater mortality, detection of bacterial and viral agents, histopathology, hematological findings, and physiological, immunological and oxidative-stress profiles. Patterns were interpreted across life stages and between cages as hypothesis-generating associations rather than evidence of causality. Both cases included diagnosis of *Aeromona salmonicida atypical* which required antimicrobial treatment in freshwater phase, together with handling and changes in environmental factors that could have increased physiological demand during smolt development. In seawater phase, *Piscirickettsia salmonis* was widely detected and SRS was the principal infectious condition associated with mortality and marked cage-to-cage heterogeneity according to origin. The most affected cages showed combinations of anemia/hemolysis, metabolic-hepatic or nutritional compromise, persistent inflammation, and sustained metabolic stress, whereas other cages showed comparatively greater immune reactivity. Histopathology showed generalized irritative gill changes and inflammatory renal/splenic lesions, while the most compromised group displayed pronounced hematological disturbance. Across both cases, poorer seawater performance was consistently observed where hematological and metabolic disruption coexisted with inflammatory or immune alterations, suggesting that immune activation alone was not equivalent to effective protection. Our results reinforce the hypothesis that presence of *Aeromona salmonicida atypical* in fresh water may be correlated with higher mortality in fish that were exposed to this bacterium versus those that were not or had a much lower prevalence. The farming site cases support a working model in which bacterial disease and cumulative physiological stress during freshwater may reduce the physiological reserve available after seawater transfer, potentially contributing to heterogeneous susceptibility and clinical expression of SRS. The observed association appears multifactorial and is likely modulated by coinfections, gill condition, environmental exposure, and cage-specific host responses. These preliminary findings justify expanding the analysis to additional production sites and formally testing whether freshwater physiological, hematological and immune biomarkers can predict subsequent SRS risk and seawater performance.

Keywords: *Piscirickettsia salmonis*; freshwater health; physiological condition; immune status; seawater performance.

Acknowledgments: Fish Health & Nutrition area Mowi Chile, Mowi Laboratory.

GLOBAL DEVELOPMENTS IN VACCINATION AGAINST SRS

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Abstract

Salmon Rickettsial Septicemia (SRS), caused by *Piscirickettsia salmonis*, remains the most important infectious disease challenge in the Chilean salmon industry. Vaccination has become a key tool for reducing antibiotic use associated with SRS, particularly following the introduction of a live attenuated vaccine strategy in 2015. In recent years, SRS has also become an increasing concern outside Chile (especially in Ireland/UK, Tasmania, and New Zealand) while countries such as Canada and Norway report more sporadic SRS challenges. In autumn 2025, the first commercial use of a live attenuated SRS vaccine outside Chile began in Scotland, and interest in SRS vaccination is growing across other affected regions. We present the current status of SRS across salmon-producing markets, with a focus on vaccination as a potential mitigation measure. The discussion is framed by key lessons from three decades of Chilean experience: (i) the realistically expected level of protection achievable through vaccination, (ii) laboratory evidence for cross-protection against local diversity of *Piscirickettsia* strains, and (iii) likely future developments in SRS vaccination from an industry perspective.

Keywords: vaccination; industry status.

COMPARATIVE GUT MICROBIOME AND METABOLOMIC PROFILING OF ATLANTIC SALMON FAMILIES WITH CONTRASTING RESISTANCE TO *Piscirickettsia salmonis* ACROSS FRESHWATER AND SEAWATER STAGES

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Abstract

Piscirickettsia salmonis, the etiological agent of piscirickettsiosis, is a major bacterial pathogen affecting Atlantic salmon aquaculture. Characterizing gut microbiome composition and metabolic profiles associated with contrasting host resistance phenotypes may help identify biological patterns associated with resistance to this pathogen. This study aimed to characterize and compare gut microbial communities, predicted functional profiles, and metabolomic patterns in Atlantic salmon families classified as resistant (R) or susceptible (S) to *P. salmonis* across freshwater (FW) and seawater (SW) stages. Gut samples from five R and five S fish were analyzed at each stage, yielding 20 samples in total. Microbial community composition was characterized using 16S rRNA gene sequencing, and microbial functional potential was inferred from the resulting taxonomic profiles. Metabolomic profiles were characterized using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Microbiome analyses revealed no significant differences in diversity, richness, or evenness indices between R and S groups in FW. In contrast, greater microbial diversity was observed in the R group in SW, alongside differences in microbial diversity between FW and SW within the R group. Predicted functional profiles in SW also revealed differences between R and S groups in pathways associated with carbohydrate transport. Untargeted metabolomic analysis detected 792 features in FW and 879 in SW, corresponding to 95 and 114 unique putatively annotated compounds, respectively. Preliminary comparisons suggested phenotype-associated metabolic patterns in FW, as well as potential differences in SW involving lipid-related signals and antioxidant-associated metabolism. Overall, these findings provide an exploratory framework for investigating associations between gut microbial communities, their predicted functional potential, and metabolomic profiles in Atlantic salmon families with contrasting resistance phenotypes. As the fish were not experimentally challenged with the pathogen in this study, the observed patterns should be interpreted as associations with family-level resistance classification rather than direct responses to infection or evidence of specific resistance mechanisms.

Keywords: *Piscirickettsia salmonis*; gut microbiome; 16S rRNA gene sequencing; functional prediction; metabolomics.

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