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BOOK OF ABSTRACTS
SEA LICE CONFERENCE 2025

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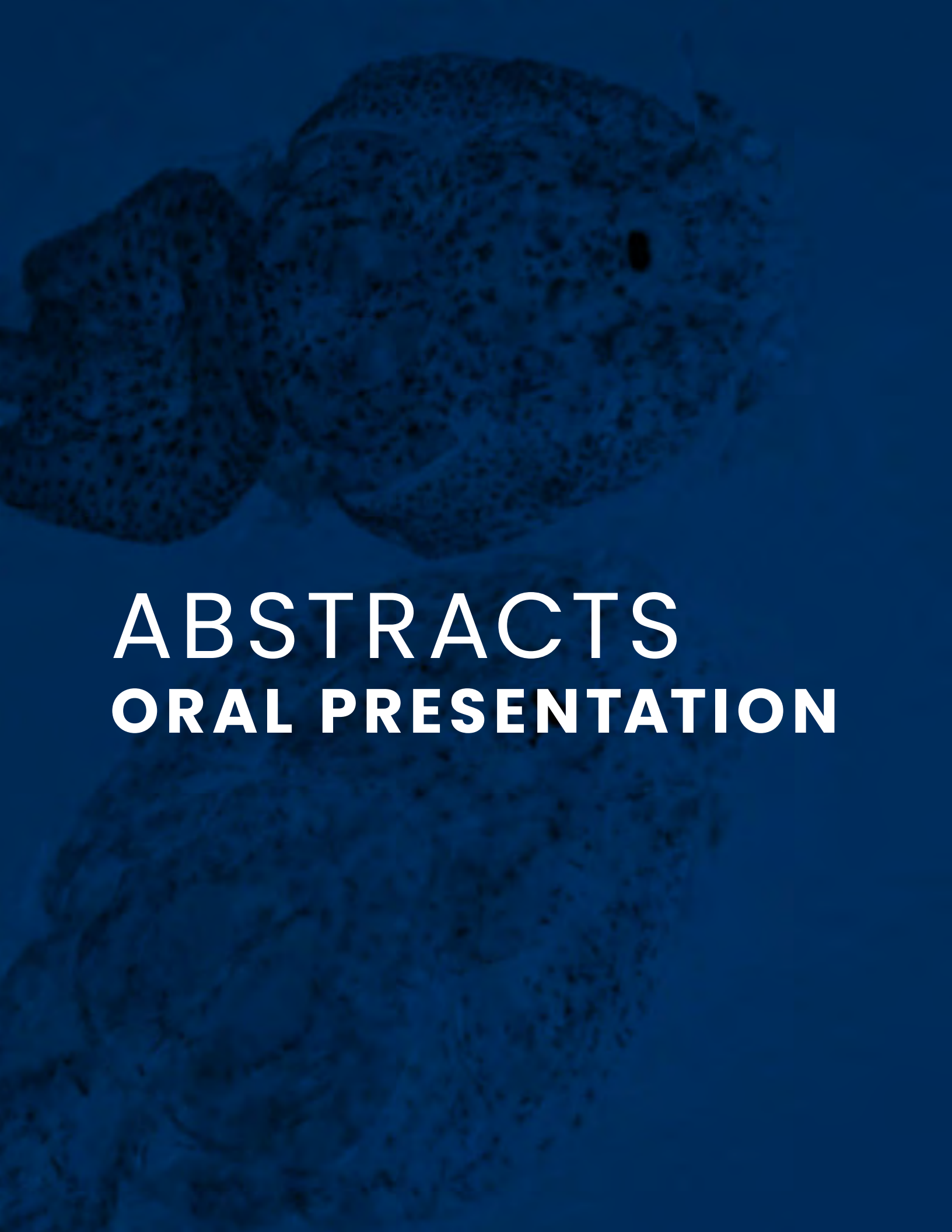

ABSTRACTS
ORAL PRESENTATION

FISH WELFARE

EFFECTS OF PARASITE CO-INFECTION ON LICE ACQUISITION AND BODY CONDITION

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Juvenile Atlantic salmon acquire a range of parasite species during their development in both freshwater and marine environments, including sea lice *(Lepeophtheirus salmonis & Caligus elongatus).* However, we currently lack an understanding of how the likelihood of acquiring lice infestations is influenced by the presence of other naturally occurring parasite species. For instance, are sea lice more likely to successfully establish on fish with high endoparasite burdens, or are individual fish which have already been challenged with a range of parasites during their development more likely to resist sea lice infestations? Furthermore, is the condition of the fish correlated to overall parasite burdens, rather than just sea lice infestations? By utilising a unique 10+ year archive of salmon post-smolts from fjords spanning the Norwegian coastline, this study aimed to test the potential relationship between sea lice infestations and overall parasite burdens in wild Atlantic salmon populations. Preliminary results suggest that the number of sea lice acquired by salmon post-smolts may be positively correlated with endoparasite biomass, though this relationship is fjord-specific. This study provides novel insights into the coinfection dynamics experienced by juvenile Atlantic salmon.

FISH WELFARE

DIFFERENTIAL EFFECTS OF SEA LICE TREATMENTS AT DIFFERENT TEMPERATURES ON ATLANTIC SALMON SKIN INDICATORS

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Using an underwater fish monitoring camera (Aquabyte), we assessed the effects of two different sea lice treatments on the skin health of Atlantic salmon. The evaluation was performed in the region of Nordland, Norway. The camera was strategically positioned in four different pens for six months each, from July 2024 to January 2025. Throughout this period, we monitored the daily number of adult and adult female of *Lepeophtheirus salmonis* attached to fish, as well as skin condition indicators such as scale loss and skin wounds. These skin injuries were classified according to the Laksvel recommendations with a scoring system, ranging from 0 (no damage) to 3 (severe damage). Our findings indicate that all treatments negatively impact the skin integrity of otherwise healthy salmon, but not all treatment affect the skin with similar level of damage. Another factor to consider was the temperature of the water during the delousing treatments. For the first two treatments the temperature was about 10°C higher than for the last treatments. Our results suggest that lower temperatures play a role in the degree of skin damage after delousing.

These results show that underwater cameras are an effective tool for non-invasive monitoring of salmon external health indicators, providing valuable insights into the impact of various sea lice control strategies on the fish welfare.

IMPROVING FISH WELFARE DURING LICE TREATMENTS

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What can be done to improve fish welfare during lice treatments? Several studies were conducted to evaluate the effectiveness of sedation in improving welfare during transport and fish handling, with data focused on the results during sea lice treatments. These results set a new standard for the industry, and help farmers connect welfare and ROI.
Two independent studies with Manolin and LetSea were conducted across more than 100 Norwegian farms from 2015 to 2023. The Manolin study analyzed 11,000 treatments comparing mechanical sea lice treatments with and without sedation. The LetSea studies assessed the effects of sedation on lice management in two field experiments; one without treatment and another with lice treatment, measuring lice counts and key welfare indicators. The results of the studies will be presented, demonstrating the benefits of sedation in improved lice clearance rates and investigating the impact on welfare and methods to improve lice clearance during thermal treatments. These findings underscore the significant benefits of sedation in aquaculture, highlighting its role in stress reduction, improved health management, and increased production efficiency. By integrating sedation into routine practices, aquaculture operations can enhance fish welfare and optimize treatment intervals, ultimately leading to more sustainable fish farming practices.

FISH WELFARE

PHYTOGENIC FEED ADDITIVE L-END MODULATES MUCOSAL IMMUNITY AND REDUCES *Caligus rogercresseyi* BURDEN IN ATLANTIC SALMON

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This study evaluated the effects of the patented (PFA) phytogenic feed additive L-END, developed by Acuanativa, on *Caligus rogercresseyi* infestation and mucosal immunity in Atlantic salmon under both experimental and field conditions. In the experimental trial, fish were infested with *Caligus* copepods and fed a diet supplemented with L-END (1 kg/ton) for 14 days. The treated group showed a significant reduction in parasite load (5.7 lice per fish) compared to the control group (12.8 lice per fish), achieving 55.9% efficacy at 20 days post-infestation. Skin samples revealed upregulation of immune markers such as IgT, IgM, muc5ac2, muc5b, and IL-12, indicating enhanced mucosal immunity. Additionally, L-END affected the expression of parasite genes related to molting and reproduction (chit-2, cpd, lpr), and reduced the thickness of the egg capsule in ovigerous females. In field trials, L-END was incorporated into feed at the same dosage and administered over 12 weeks. Compared to the control group, this strategy led to a 51% reduction in juvenile lice loads and a 40–70% decrease in the need for antiparasitic baths. These outcomes also contributed to improved fish welfare by reducing the frequency of handling interventions. Overall, the integration of L-END into feeding strategies presents a sustainable, flexible, and cost-effective alternative to reduce synthetic antiparasitics, supporting both parasite control and fish health in salmon aquaculture.

WILD FISH INTERACTION

TRENDS IN ABUNDANCE OF SEA LICE *Lepeophtheirus salmonis* AND *Caligus clemensi* ON JUVENILE WILD PACIFIC SALMON UNCHANGED FOLLOWING CESSATION OF SALMON AQUACULTURE IN TWO REGIONS OF COASTAL BRITISH COLUMBIA, CANADA

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Phased removals of net-pen salmon aquaculture facilities in two regions (Discovery Islands and Broughton Archipelago) of coastal British Columbia, Canada, has recently occurred. The removals have been in response to concerns that salmon aquaculture adversely impacts the conservation of wild salmon. Harm to locally migrating juvenile wild salmon caused by elevated risk of exposure to sea lice derived from infestations on the cultured salmon is one hypothesized impact mechanism. In BC, although cultured salmon are sources of sea lice infestations the relative magnitude of these sources is uncertain because of a paucity of information on the natural infestations on reservoir species. The removal of Atlantic salmon production in these two regions of British Columbia has, for the first time, provided an opportunity compare sea lice infestations of *Lepeophtheirus salmonis* and *Caligus clemensi* on juvenile wild both with and without aquaculture operations. Two papers have been published recently presenting long term datasets for each of the areas where net pen aquaculture has been removed. Both articles conclude that the level of sea lice infestation on juvenile wild salmon did not change with the removal of finfish aquaculture from these regions. The results from both articles will be discussed in this presentation.

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WILD FISH INTERACTION

QUANTIFYING THE IMPACT OF SALMON LICE ON WILD ATLANTIC SALMON SEA SURVIVAL: IN SITU EXPERIMENTAL INFESTATION TRIALS IN NORWAY

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The wild Atlantic salmon *(Salmo salar)* is in decline across its range, with parasitic salmon lice *(Lepeophtheirus salmonis)* identified as a key driver in intensively farmed coastal areas. Understanding tolerance thresholds to lice infestation is essential for effective management, yet current thresholds are largely based on laboratory studies with limited ecological relevance. This study addresses this critical knowledge gap by experimentally infesting wild-caught salmon smolts in a controlled in situ setting, tracking their marine survival using passive integrated transponder (PIT) tags. In 2024 and 2025, over 1900 wild smolts were captured in the Nidelva river (southern Norway), PIT-tagged, and exposed to graded salmon lice infestation levels. Fish were released at the river mouth and will be monitored for return using PIT antennas. This is the first large-scale study using wild smolts and direct infestation to assess dose-response relationships between lice load and return rates. Infestation levels across both years covered a wide range of intensities (0 - > 0.5 lice/g), with 30% of fish exposed to 0.2 - 0.5 lice/g, a level previously associated with increased mortality. Preliminary survival data from returning one-sea-winter adults (2024 cohort) will be presented at the conference, providing the first robust estimates of lice-induced mortality under natural conditions. This innovative approach represents a new gold standard in assessing ecological impacts of salmon lice and will directly inform thresholds used in Norway's Traffic Light System for aquaculture regulation.

WILD FISH INTERACTION

IN SITU EXPERIMENTS REVEAL EFFECTS OF SALMON LICE ON ALL WILD SALMONID SPECIES IN NORWAY

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The proliferation of salmon farming in Norway has led to significant increases in host availability for the parasitic salmon louse *(Lepeophtheirus salmonis),* posing a severe threat to wild salmonid populations, including Atlantic salmon *(Salmo salar),* sea trout *(Salmo trutta)* and Arctic char *(Salvelinus alpinus)*. We have investigated mortality as well as physiological and behavioral effects of salmon lice on these species in environments impacted by aquaculture. Our *in situ* experiments document that the risk ratio of dying for Atlantic salmon can increase 55 times at high lice densities. Sea trout responded in a dose-response manner to lice by returning to the river prematurely, losing nearly 50 % of its expected time at sea. Arctic char post smolts also showed a preliminary return to the river, but at even lower infestations than for sea trout. Veteran Arctic char were affected with reduced growth and condition factor but also changes in plasma fatty acids and ion concentrations. Effects on veteran char were found even from the small chalimus stages of the salmon louse and from very low burdens of all louse stages combined (<0.03 lice per gram fish). Our findings underscore the acute risks posed by salmon lice to all wild anadromous salmonid species in Norway, suggesting that current aquaculture practices are not sustainable. Effective management strategies, including stricter lice control measures and habitat protection, are essential to mitigate harmful interactions between farmed and wild salmonids and to safeguard the ecological integrity of coastal marine ecosystems.

WILD FISH INTERACTION

INTERACTIVE EFFECTS OF TEMPERATURE AND *Leophtheirus salmonis* INFECTION ON GROWTH AND HEALTH METRICS IN WILD-CAUGHT POST-SMOLT ATLANTIC SALMON

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The impact of *Lepeophtheirus salmonis* on wild post-smolt Atlantic salmon under controlled conditions remains poorly understood. This study investigated host-parasite dynamics at three ecologically relevant water temperatures (7, 10, 13°C), representing conditions during outmigration. 321 wild salmon (mean weight: 17.28 g; condition factor: 0.87) were captured in the Etne river during the 2025 outmigration and transferred to the Institute of Marine Research facility in Matre (Norway). Following acclimation and saltwater transfer, fish were exposed to L. salmonis (mean infection intensity at chalimus 1: 0.83 lice g^{-1}) and, at chalimus 1 stage, redistributed into group tanks (16 fish per tank) and individual tanks. Mortality onset paralleled the development of female preadult 2 lice at 10 and 13°C but was delayed at 7°C until adult female lice were present. Overall mortality varied by temperature, highest at 10°C (26.53%) and lowest at 7°C (14.49%). Lice loss was high across all temperatures. Linear models revealed a significant negative effect of infection intensity on SGR, though this effect did not differ between temperatures. No impact on condition was observed from infection intensity, but the best overall condition was observed at 7°C, likely due to a longer trial period. Cardiosomatic indices were unaffected by temperature or infection. Liver dry weight percentage (%DWL) was lowest at 7°C and highest at 13°C, with infection intensity significantly reducing %DWL at 7°C. The findings of this study support a relationship between temperature and mortality timing, whereas infection severity in wild hosts appears less influenced by temperature within this thermal range.

SEA LICE BIOLOGY AND ECOLOGY

TEMPERATURE PREDICTS DEVELOPMENT IN A SIMILAR FASHION IN *Leophtheirus salmonis* and *Caligus elongatus*

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The sea lice *Lepeophtheirus salmonis, Caligus elongatus and C. rogercresseyi,* all belong to the family of *Caligidae.* Despite differences in host specificity and life strategy, they all pose major challenges to sustainable salmonid aquaculture worldwide. These ectothermic ectoparasites have similar morphology and life cycles, although *L. salmonis* is notably larger. Given their physiological similarities, temperature is expected to influence their development in comparable ways. We investigated the effects of water temperature in the relevant environmental range (6–15 °C) on the development rate and egg production of *L. salmonis and C. elongatus* in separate species-specific experiments. From these data, we developed a temperature-dependent model to predict progression through the lifecycle. Notably, we found that the C. elongatus development model can be derived from that of *L. salmonis by applying* a constant scaling factor, *with C. elongatus* reaching adulthood in approximately two-thirds the time. Additionally, we qualitatively compared our results with published data on *C. rogercresseyi* to explore interspecies differences in life-history traits and epidemiological dynamics. This study presents a novel experimental framework for modeling caligid development and highlights how life-history parameters can be scaled across species using temperature-independent constants to better understand and compare the biology and epidemiology of sea lice.

SEA LICE BIOLOGY AND ECOLOGY

SEX-BIASED EPIGENETIC SIGNATURES OF CHROMOSOME 6 IN THE SEA LOUSE *Caligus rogercresseyi*

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Caligus rogercresseyi, a copepod ectoparasite of major concern in Chilean salmon aquaculture, poses significant production and environmental challenges due to the widespread use of delousing chemotherapeutics. Vaccination has emerged as a sustainable alternative, although its efficacy appears to be influenced by sex-specific gene expression responses in lice exposed to immunized hosts. Recent studies suggest a ZW sex-determination system in sea lice species, supported by the discovery of a female-specific W chromosome. The *C. rogercresseyi* genome comprises 21 chromosomes, including over 23,000 protein-coding genes and a broad repertoire of non-coding RNAs expressed throughout developmental stages. Using Hi-C chromatin conformation data, we identified a centromeric inversion on chromosome 6 (Chr6) in females, indicating a structural feature associated with sex differentiation. In this study, we integrated transcriptomic data with DNA methylation profiles and RNA modification analyses to investigate the epigenetic landscape associated with Chr6. Comparative methylome analyses between male and female lice revealed sex-dependent differentially methylated regions (DMRs), including both hyper- and hypomethylated loci in females, spanning protein-coding genes and regulatory non-coding elements. These epigenetic marks were closely associated with sex-biased gene expression patterns, indicating a regulatory role of Chr6 in female-specific transcriptional activity. Our findings support the hypothesis that Chr6 functions as the female W chromosome in *C. rogercresseyi,* providing novel insights into the epigenetic mechanisms underlying sexual dimorphism and adaptation in this parasitic copepod.

SEA LICE BIOLOGY AND ECOLOGY

SURVIVAL, REINFESTATION AND BEHAVIOUR IN PARASITIC STAGES OF SALMON LOUSE *Leophtheirus salmonis* AND *Caligus elongatus* WITHOUT HOST

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Sea lice infestation remains a challenge for the salmonid aquaculture industry. Crowding of infested salmonids, e.g. during delousing treatments, releases some of the mobile (preadult and adult) stages of sea lice to the water masses. Herein, the aim is to investigate the survival of preadult and adult salmon lice *Lepeophtheirus salmonis* without host, and the ability to successfully reinfest after losing a host, as well as investigating the swimming behaviour of these stages. Survival and swimming behaviour were also examined in the generalist sea lice *Caligus elongatus.*

L. salmonis were placed in a flow-through incubator and checked daily for survival. A subset was placed on Atlantic salmon after 0, 3 and 7 days of starvation (off host), and the ability to successfully reinfest was examined 7 and 28 days after reinfection. To investigate behavioural response to stimuli over time, tethered *L. salmonis* were given light stimuli daily for up to 19 days.

L. salmonis had a mean survival of 8 (max 10) and 5 (max 10) days without host for adult female and male *L. salmonis,* respectively. Infestation success 28 days after reinfestation was similar for adult female *L. salmonis* that had been 0 and 3 days without host (68% and 62%, respectively), but markedly lower for 7 days without host (6%). Female and male C. elongatus lived longer without host than *L. salmonis,* with a mean survival of 18 (max 33) and 17 (max 31), respectively, and demonstrated a higher activity level compared to *L. salmonis.*

METAGENOMIC AND METATRANSCRIPTOMIC ANALYSIS OF BACTERIAL MICROBIOME OF *Caligus rogercresseyi* ACCORDING TO SEXAND ONTOGENIC DEVELOPMENT

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Salmon farming in Chile faces various challenges, the most significant being the sea louse *Caligus rogercresseyi,* the causative agent of Caligidosis. In this context, the microbiota of *C. rogercresseyi* has garnered interest due to its potential influence on the parasite physiology. This study aimed to characterize the microbiota of *C. rogercresseyi* throughout its ontogenetic development to better understand its biological and functional roles. Specimens were collected from salmon farming sites in southern Chile, and a metagenomic approach based on 16S rRNA gene sequencing (Oxford Nanopore) was used to profile bacterial communities. Additionally, metatranscriptomic analyses were performed using RNA-seq data from different developmental stages, as well as metagenomic data derived from proximity ligation sequencing (Hi-C). The results revealed significant differences in microbial composition, abundance, and diversity during ontogenetic development, potentially linked to specific biological functions. For example, females exhibited higher bacterial richness and diversity, with a notable prevalence of the genus *Aliivibrio*, whereas Tenacibaculum dominated the male microbiota; both genera are closely associated with recurrent diseases in salmonids. Functional analysis further indicated that the microbiota modulates parasite metabolic pathways, such as amino acid metabolism and sugar degradation, in a stage-dependent manner, suggesting a relevant interaction between microbial communities and host physiological traits. These findings provide key insights into the microbiome of *Caligus rogercresseyi* and highlight the integral role of bacteria in the biology of this parasitic copepod.

Funding: *FONDAP #1523A0007*

SEA LICE BIOLOGY AND ECOLOGY

COMPARATIVE MICROBIOME ANALYSIS OF THE SALMON LOUSE, *Lepeophtheirus salmonis*, USING ILLUMINA AND NANOPORE SEQUENCING TECHNOLOGIES

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Crustacean ectoparasites have been identified as potential vectors of pathogens, notably obligate species like Aeromonas *salmonicida,* which survive poorly in seawater. The salmon louse *(Lepeophtheirus salmonis)* is a major concern in salmon aquaculture across the northern hemisphere, primarily due to its feeding on host mucus, blood, and tissue - activities that cause dermal tissue damage and may facilitate secondary infections. While the physical effects of sea lice are well documented, their potential role as vectors in pathogen transmission remains poorly understood. We analysed the gut microbiome of *L. salmonis* collected over a four-month period (February – May, 2022) as part of the Irish National Sea Lice Monitoring Programme at a commercial Atlantic salmon farm. Microbiome profiling was conducted using two sequencing platforms: Illumina MiSeq and Oxford Nanopore Technologies (ONT) PromethION. The use of both sequencing platforms enabled direct comparison of microbiome profiles and assessment of each method's effectiveness for rapid pathogen detection. The analysis revealed the presence of 15 and 24 genera of known fish pathogens using Illumina and Nanopore sequencing, respectively. The most prevalent pathogenic genera included Arcobacter, *Tenacibaculum,* and Vibrio, while others such as Aeromonas, *Moritella,* and Pseudomonas were detected in lower abundance. These findings provide a comprehensive microbiome characterisation of *L. salmonis* in a commercial aquaculture setting and highlight the potential of sea lice to act as vectors or reservoirs of pathogens. These findings have important implications for pathogen surveillance, management, and prevention strategies in salmon aquaculture.

DEMOGRAPHIC ALLELE EFFECT IN *Lepeophtheirus salmonis*: IMPLICATIONS FOR REPRODUCTIVE SUCCESS AND MANAGEMENT

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Salmon lice *(Lepeophtheirus salmonis)* are ectoparasitic copepods that represent a persistent and economically significant threat to salmon aquaculture. These parasites can cause extensive damage to host fish, compromising welfare and leading to substantial financial losses across the industry. While existing management approaches primarily focus on reducing infestation levels, the potential influence of density-dependent reproductive constraints, particularly the Allee effect, has received comparatively little attention.

This study aimed to explore the reproductive dynamics of *L. salmonis* under varying density conditions, with a specific focus on indicators that could reveal evidence of a demographic Allee effect. To this end, key reproductive parameters were measured, including spermatophore count, cementation (used as a proxy for successful mating), and gonad size.

A total of 1,653 adult female lice were sampled from 30 salmon aquaculture sites across the Faroe Islands. The analysis revealed that the presence of cementation increased with parasite density, supporting the hypothesis that successful mating is constrained at lower population levels, a hallmark of the Allee effect.

Overall, the results suggest that maintaining salmon lice populations below certain density thresholds may not only suppress overall abundance but also impair reproductive success, thereby enhancing the long-term effectiveness of management efforts. Recognizing and leveraging these reproductive constraints could provide a valuable complement to existing control strategies, contributing to more sustainable and ecologically informed lice management in aquaculture systems.

SEA LICE BIOLOGY AND ECOLOGY

CHARACTERIZATION OF ABUNDANCE PATTERNS OF *Caligus rogercresseyi* LARVAE IN DIFFERENT GEOGRAPHICAL ZONES OF THE LOS LAGOS AND AYSÉN REGIONS

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Caligus rogercresseyi is one of the pathogens that has become a biological and economic problem for the salmon industry in Chile. Infections from one farming site to another can occur through the dispersal of planktonic larvae. There is scare knowledge on the abundance and distribution of these larvae around the farming sites in Chile. Thus, it is necessary to know their spatial and temporal distribution to have a better understanding of the sources of these larvae to develop strategies and solutions that can reduce their impact. The aim of this study was to characterize the abundance patterns of planktonic larvae of *C. rogercresseyi* in different geographical areas in the south of Chile. Data were collected from the zooplankton monitoring program conducted by the Mowi Chile Laboratory as part of health surveillance programs. The analysis included data from 2019 to 2025 in Los Lagos and Aysén region. The results suggest that planktonic stages are observed and remain close to the farming sites, and it can generate a larvae network among areas. Differences in abundance over time were observed among the different zones, with some areas showing abundances above 50 larvae per m³ during the study period and others with not more than 1 larvae per m³. These results suggest that would exist areas in which larvae abundances are higher, constituting probable hot spots, which may be associated with salmon production, currents and retention of water masses, among others, which may impact the salmon farms immersed in these areas.

(MIS)UNDERSTANDING SALMON LICE LARVAE: MEASURING THEIR OBJECTIVE DISTRIBUTION HAS IN DIFFERENT OUTCOMES THAN LAB AND MESOCOSM TRIALS

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Extensive investment in depth-based lice prevention technology is occurring due to assumptions that salmon lice copepodites only inhabit the upper water column. Laboratory and mesocosm experiments have observed preferences for certain environmental conditions, with copepodites generally favoring surface habitat. Regardless of these findings, avoidance measures such as submerged cages, snorkel cages, and lice skirts don't always work. To investigate these discrepancies, we collected large volumes of plankton at depths of 2-18m and distances of 0-4km from a salmon farm in Storfjorden, Norway during an active and fallow period. We measured the number of lice larvae in the samples using ddPCR and AI microscopic ID to examine where they are found in the water column.

During the production period, the highest concentrations of larvae (nauplii + copepodites) were found at sampling locations closest to and down current from the farm, with more lice found at depths of 6-18m compared to 2m. However, lice larvae were found abundantly at all depths and distances with patchy frequency. During the fallow period, copepodites were observed more frequently at 2m than 9m or 18m but were detected at all depths with no diurnal pattern.

We conclude that salmon lice copepodites inhabit a greater depth range than previously observed in Norway and that the vertical distribution of larvae is affected by additional factors beyond pressure, salinity, temperature, or light regime.

SEA LICE BIOLOGY AND ECOLOGY

FRESHWATER PREFERENCE IN SALMON LOUSE COPEPODIDS IS HERITABLY AFFECTED BY EXPOSURE AND DOMESTICATION

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Freshwater treatments are commonly used to remove salmon lice from farmed salmonids, offering an effective delousing method without major negative effects on fish welfare and the surrounding environment. However, repeated exposure to freshwater may induce adaptive responses in salmon lice, potentially leading to copepodids with increased infectivity in brackish fjord environments. This could diminish natural refuges for wild salmonids.

To investigate potential adaptive effects, salmon lice settled on fish were exposed to freshwater for two hours, three times per generation, repeated over five generations. Parallel control groups remained untreated and kept at 34 ppt continuously. We assessed the salinity preference of unexposed offspring copepodids by presenting them with a choice between seawater at 14, 24, and 34 ppt.

Results showed that copepodids from freshwater-exposed lineages exhibited a statistically significant, though modest, shift in preference toward lower salinity compared to controls. Interestingly, the results also showed that copepodids from newly collected lice showed a much stronger preference for low-salinity conditions than their domesticated counterparts kept at 34 ppt, even when the latter had also been exposed to the above-mentioned freshwater treatment regime for five generations.

These findings suggest that natural environmental pressures present along the Norwegian coast exert a greater influence on freshwater adaptation in salmon lice than the experimental freshwater treatments applied in this study. The results underscore the importance of carefully selecting representative populations when designing experiments to assess adaptive responses in aquatic parasites.

NOVEL INSIGHTS INTO FRONTAL FILAMENT FORMATION IN SALMON LOUSE CHALIMUS LARVAE

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After entering the host *Lepeophtheirus salmonis* develops through 5 stages before becoming adult. This involves development through the parasitic phase of the copepodid stage, two chalimus stages and two preadult stages. A frontal filament secures the louse to the host during each of the chalimus stages, and later, short term filaments secure the louse during the remaining molts until adult. The prolonged period of attachment during the chalimus stages makes the filament a vulnerable construct that can be rejected by the host immune system, thus representing a potentially important target for vaccines.

While the structure of the filament has previously been described, its specific compounds have remained unidentified. By correlating transcriptomic data of developing lice with filament enriched proteomic data, we have managed to identify possible genes encoding filament proteins. *In situ* hybridization was applied to localize gene expression of the most relevant candidates, and their functions were explored in a pilot study using RNA interference.

We identified seven genes without any annotation, active in cells responsible for filament production, and two resilin genes that potentially give elasticity to the cuticle lining the frontal pocket in which the filament is formed. In a pilot knock-down study simultaneously targeting multiple paralogous genes, the basal plate at the distal end of the filament in contact with the host appeared to be reduced. These lice seemed to have a reduced strength in the host attachment at sampling, indicating that at least one of the genes is involved in basal plate formation.

SEA LICE BIOLOGY AND ECOLOGY

Lepeophtheirus salmonis LARVAE ORIGINATING FROM SUBMERGED AND SURFACE SALMON FARMS: BEHAVIOURAL RESPONSES TO LIGHT, SALINITY AND PRESSURE STIMULI

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Lepeophtheirus salmonis infestation is a major challenge for the salmon aquaculture industry, and submerged farming (cages below 20 m depth) has emerged as a promising preventive measure against the parasite. Although *L. salmonis* infestation levels are strongly reduced, some infestation still occur, and infestation mechanisms are poorly known. This study aims to investigate how environmental parameters influence the swimming behaviour of *L. salmonis* planktonic larvae originating from surface and submerged farms.

L. salmonis egg strings were collected from salmon in full-scale surface and submerged cages and hatched in the laboratory. Swimming behaviour of individual larvae was video recorded in a vertical setup where light (dark, light from above, light from below), salinity (brackish layer from 16 to 30 ppt), and pressure (0, 2.5 and 5 bar) could be manipulated simultaneously.

Nauplius 2 and copepodites displayed negative barotaxis, with a stronger upwards swimming response with increasing pressure (0 to 5 bar). Light stimuli attracted the larvae to some extent, but pressure seemed to be a stronger mediator of behaviour. This indicates that larvae may swim upwards in the water column, even though artificial lights are present in submerged cages. Copepodites originating from submerged cages were more sensitive to salinity than those from surface cages, and avoided moving into 28, 26 and 24 ppt salinities even though pressure increased. Copepodites from surface cages were more affected by pressure and moved upward into these brackish layers. The results indicate that light, salinity and pressure mediate behaviour differently depending on *L. salmonis* origin.

GENETICS AND GENOMICS

THE CELLULAR AND GENETIC RESPONSE TO SEA LICE INFECTION: HOW MIGHT WE USE THIS KNOWLEDGE TO IMPROVE ATLANTIC SALMON HOST-RESISTANCE TO SEA LICE?

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Selective breeding for Atlantic salmon host-resistance has been looked upon as a promising and cost-effective solution for reducing the lice problem. The trait under selection has been the number of sessile lice per fish. However, recent work has shown that the potential for group-level protection is lower than originally expected casting doubt on the reliance on lice count as an indicator trait. Lice counts are heavily influenced by the initial rate of copepodid attachment success and would not be very sensitive to subtle underlying differences in the immune response of the fish against the lice. So, if our aim is to improve the immune response of Atlantic salmon against sea lice, we will need cost effective indicator phenotypes that reflect the strength of the underlying genetic variation in the response against the lice. This is where the knowledge we have gained about the effective immune responses mounted by other Pacific salmonid species in the FHF funded *CrispResist* project (*Harnessing cross-species variation in sea lice resistance* 901631) could be applied. Here we show how the genetic and cellular response after sea lice infection differs between more susceptible and more resistant salmon species, and how this might allow us to test and select Atlantic salmon for a Pacific-like immune response to directly reduce the lice burden and reduce the need for delousing.

GENETICS AND GENOMICS

METAGENOMIC AND METABOLOMIC SIGNATURES ASSOCIATED WITH THE TOLERANCE OF ATLANTIC SALMON TO SEA LICE INFESTATION

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The mucosa is the first line of defense in aquatic organisms, were epidermal cell, mucus, glycoproteins, antimicrobial molecules, microorganism and metabolites converge to shape the structure and functioning of the mucosal environment. While multiple studies have shown protective proprieties of the mucosa in marine organisms to bacterial and viral infections, its role in the resistance of fish against ectoparasite remains less understood. Ectoparasite, like the sea lice *(Caligus rogercresseyi),* have become one of the most challenging issues for salmon aquaculture worldwide. Although delousing drugs are currently used to control the parasite, these treatments threat the sustainable development of salmon aquaculture. Thus, understanding the importance of the mucosa in salmon resistance to sea lice could lead to the development of novel tools and strategies to manage this disease. To evaluate this, a metagenomic and metabolomic approach was used to identify differences in the microbial communities and metabolites in the skin and gut mucosa of Atlantic salmons with contrasting resistance to sea lice. The results evidenced that the most significant changes in the mucosa between resistant and susceptible individuals were found in the skin, and in lesser extent in gut. While no significant differences were found in the microbial diversity of gut mucosa, the alpha diversity of the skin mucosa was significantly higher ($p < 0.05$) in resistant salmonids compared with susceptible ones (Fig. 1A). A Linear discriminant analysis Effect Size (LEfSe) analysis evidenced that the skin mucosa of susceptible individuals were significantly associated with bacteria from the Comamonas and Vibrio genus, while the resistant phenotype was significantly associate with bacteria from the Pseudomonas, Novosphingobium and Labrys genus (Fig 1B). Regarding metabolome analysis, a t-test was used to identify metabolites with significant differences in gut and skin mucosa from resistant and susceptible salmons. The results evidenced a higher number of metabolites with differential abundance ($FC > 4$ and $p < 0.01$) in the skin that in the gut mucosa. Differences were also found in the distribution of this metabolites between resistant and susceptible salmonids. While the largest number of differentially abundant metabolites in gut mucosa were associated with susceptible individuals, the skin mucosa evidence a higher number of metabolites significantly associated with the resistant individuals (Fig. 1C). Overall, our results provide evidence about the differences in the metagenomic and metabolomic profiles between Atlantic salmon with contrasting resistance to sea lice infestation. These results open new perspectives about the role of the mucosal environment in the resistance of salmonids to sea lice that could be further explore for the development of novel and sustainable strategies to deal with sea lice infestation in salmon aquaculture.

SEA LICE UNDER PRESSURE: HOW HOST RESISTANCE SHAPES THE TRANSCRIPTOME AND MICROBIOTA OF *Caligus rogercresseyi*

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Selective breeding programs that enhance salmon resistance to sea lice represent a sustainable strategy for ectoparasite control, reducing reliance on antiparasitic treatments. This study investigates transcriptomic modulation and bacterial community shifts in *Caligus rogercresseyi* when exposed to genetically improved salmon strains. Sea lice were collected from three *Salmo salar* families categorized as susceptible (S), acquired lice resistant (ALR), and resistant (R), following three successive infections. Transcriptomic profiles were generated using Illumina RNA sequencing, and bacterial communities were characterized via 16S rRNA gene sequencing with Oxford Nanopore technology. Differential gene expression analysis revealed distinct patterns among lice from the different host phenotypes: lice from ALR and R families predominantly exhibited downregulated transcripts, whereas lice from the S group showed upregulation of genes, particularly those related to cuticle processes, validated by qPCR. KEGG pathway analysis further indicated downregulation of metabolic, signal transduction, and digestive pathways in ALR lice, while R lice displayed suppression of signal transduction, immune, digestive, and excretory pathways, accompanied by selective activation of secretory enzyme genes. Microbiome analyses revealed higher microbial diversity in the S group, dominated by *Proteobacteria* and *Bacteroidetes,* whereas ALR and R groups were over 88% *Proteobacteria.* Core microbiome and functional prediction analyses highlighted significant differences among host groups. These findings provide novel insights into *C. rogercresseyi*–host interactions, offering new perspectives for developing sustainable control strategies in salmon aquaculture.

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GENETICS AND GENOMICS

EFFECT OF SEASONAL TEMPERATURE ON THE SKIN TRANSCRIPTOME OF ATLANTIC SALMON NATURALLY INFESTED WITH *Caligus rogercresseyi* IN OPEN-OCEAN CAGES

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In Chilean salmon farming, infestation by the ectoparasite *Caligus rogercresseyi* represents a recurring challenge, which increases during autumn and spring. This study evaluated the effect of seasonal temperature on the transcriptomic response of the skin of Atlantic salmon *(Salmo salar)* naturally infested with *C. rogercresseyi* under marine culture conditions in the Aysén Region (Chile). For this purpose, an RNA-Seq-based transcriptomic analysis was performed on the skin of infested and non-infested fish during autumn and spring. Differentially expressed genes (DEGs) were identified, and the analysis was performed using functional networks from the STRING database. In addition, plasmatic cortisol was measured using an ELISA kit. In autumn, processes associated with the activation of integrated stress receptors (ISR), a downregulation of T cell proliferation, M2 macrophage polarization, tissue repair, and an increased response to oxidative stress were identified. This could be associated with the high levels of plasmatic cortisol compared to non-infested fish. On the other hand, in spring, we observed the activation of the immune response, highlighting an upregulation of processes associated with ribosomal biogenesis, complement system activation, granulocyte chemotaxis, and antigen processing and presentation. However, some of these molecules could have an unwanted side effect on the development of protective mechanisms to counteract infestation by *C. rogercresseyi*. Taken together, we suggest that seasonal temperature could differentially impact the skin response to *C. rogercresseyi* infestation under a real-world scenario, reinforcing the need to increase efforts to understand the response dynamics of the skin of Atlantic salmon where the problem currently takes place.

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GENOMIC MARKERS ASSOCIATED WITH TOLERANCE TO EMAMECTIN BENZOATE IN *Lepeophtheirus salmonis* FROM BRITISH COLUMBIA, CANADA

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The salmon louse *(Lepeophtheirus salmonis)* is an important ectoparasite of both wild and farmed salmon, with infestations contributing to significant economic losses in marine aquaculture. In British Columbia (BC), sea lice have been managed using a combination of chemotherapeutic and mechanical treatments. However, recent treatment outcomes and bioassay results have suggested variability in the efficacy of emamectin benzoate (EMB).

Previous research has identified a QTL region on chromosome 5 of L. *salmonis* associated with EMB resistance in Atlantic lice subspecies, and genotypes linked to EMB treatment outcomes in Pacific lice subspecies. However, the use of the Atlantic reference genome and the limited geographic scope of the samples restrict the generalizability of these findings. To investigate whether Pacific lice populations share similar underlying genetic mechanisms, we conducted a genome-wide association study (GWAS) using the newly published Pacific reference genome and lice collected between 2020 and 2024 from all active salmon farming regions in BC.

Phenotypic resistance to EMB was determined through EC50 bioassays, with lice classified as susceptible (dead at <250ppb EMB) or tolerant (survived at >1000ppb EMB) following 24-hour exposure. Genomic DNA from individual lice were sequenced using Illumina NovaSeq, and population structure was assessed to evaluate potential stratification between collection locations.

Our GWAS revealed several candidate markers on louse chromosome 5 significantly associated with EMB tolerance, supporting previous findings and suggesting a conserved mechanism of resistance across subspecies. Ongoing work aims to validate these markers and develop a SNP-based genotyping tool to support field monitoring and resistance management strategies.

GENETICS AND GENOMICS

UNCOVERING THE IMPORTANT GENETIC DEFENSES AGAINST SEA LICE IN COHO SALMON VIA CRISPR-MEDIATED GENE EDITING

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Enhanced resistance to sea lice infestation remains one of the most important goals to ensure sustainable Atlantic salmon farming in the marine environment. Some Pacific salmon, notably Coho salmon, exhibit natural resistance to both *Caligus rogercresseyi* (Chile) and *Lepeophtheirus salmonis* (Northern Hemisphere). Previous RNA-seq data identified several Coho salmon genes that could be responsible for this difference. To determine whether certain genes played a functional role in sea lice resistance, CRISPR-Cas9 was used to knockout three different regions (R1 – R3) in each one of four selected immune genes (GE1 – GE4) alongside a phenotypic marker gene (*slc*45a) that produces an albino phenotype in successful knockouts. Coho salmon embryos (n=5633) were injected with one of the four CRISPR constructs alongside slc45a, resulting in 9.3-13.8% of alevins presenting an albino/mosaic phenotype. For wildtype (WT) controls, a subset of embryos did not receive injections. Following development into smolts (~15 g), fish received a lice infestation challenge (100 copepodids/fish) to assess the impact of each gene disruption on parasite susceptibility. Significantly higher lice infestations were observed in fish with GE2 and GE4 knockouts compared to WT controls, suggesting these genes contribute to resistance. Fin tissue from challenged fish was sequenced (Illumina NextSeq), confirming high editing efficiency in the GE4 target, particularly at R2. R3 edits were strongly associated with the pigment marker. Ongoing RNA-seq analysis will evaluate how these gene disruptions affect broader expression patterns. Uncovering the genetic basis of sea lice resistance could help develop sustainable strategies to improve Atlantic salmon aquaculture.

GENETICS AND GENOMICS

IPATH® VACCINE REDUCES SEA LICE INFESTATION AND ENHANCES THE TRANSCRIPTOME RESPONSES OF ATLANTIC SALMON TO HYPOXIA

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Caligus rogercresseyi represents one of the major challenges for the sustainability of salmon aquaculture in Chile, affecting fish welfare, growth, and farm productivity. In parallel, environmental stressors such as hypoxia exacerbate host susceptibility to parasitic and bacterial infections. To address these dual stressors, we evaluated the performance of the ferritin/transferrin-based recombinant vaccine IPath®, designed to modulate iron metabolism and nutritional immunity in Atlantic salmon. Previous studies have shown that IPath® provides more than 70% protection against *C. rogercresseyi.* In this study, we investigated its potential to improve host resilience under hypoxic condition. Atlantic salmon were vaccinated with IPath® or injected with PBS (control), exposed to intermittent hypoxia cycles, and sampled for physiological and molecular analyses. Blood parameters and histological evaluations of head kidney and gill tissues were performed, while transcriptomic profiling (RNA-seq) of the head kidney was conducted to assess gene expression changes. Phenotypic and histopathological results revealed that IPath®-vaccinated fish exhibited enhanced hypoxia tolerance, as evidenced by higher erythrocyte and hemoglobin levels, reduced gill and head kidney tissue damage, and improved immune condition. Transcriptomic profiling indicated that IPath® modulated key genes of the HIF-1 signaling pathway, iron homeostasis, and immune response, particularly under hypoxic stress. These findings demonstrate that IPath® enhances both antiparasitic and hypoxia-adaptive mechanisms, highlighting its potential as a biotechnological strategy to strengthen fish resilience and mitigate production losses in salmon farming under changing environmental conditions.

GENETICS AND GENOMICS

MINING *Lepeophtheirus salmonis* TRANSCRIPTOMES UNEARTHS KEYS TO STAGE-SPECIFIC QUANTIFICATION OF PARASITIC LARVAE

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The salmon louse, *Lepeophtheirus salmonis*, is an ectoparasitic copepod which has devastating effects on both wild and farmed salmonids in the North Atlantic region. To better assess risk to wild salmonid populations there is an urgent need to individually quantify the different planktonic life stages of *L. salmonis.* Current molecular methods show great promise reliably identifying and counting L. *salmonis* individuals but are unable to discern between the infective and non-infective larval stage. In the present study, comparative transcriptomics of nauplii and copepodid larval stages was used to identify transcript candidates for stage-specific quantification of *L. salmonis larvae.* We demonstrate that approximately two thirds of the L. salmonis transcriptome is differentially expressed between the two planktonic life stages. We present 11 candidate quantitative PCR assays targeting RNA transcripts expressed solely in one of these two life stages, of which four were tested in vitro. All four candidates successfully amplified target life stage RNAs with high detection rates, of which three also showed capability as assays for separating the life stages of *L. salmonis* and measuring their densities using quantitative PCR. Applying these assays to well established eDNA collection and processing pipelines could pave the way to significantly upscale lice monitoring practices in regions with high infestation pressure or at-risk salmonid populations.

GENETICS AND GENOMICS

COMMERCIAL VACCINES DO NOT IMPACT THE EFFICACY OF THE IPATH SEA LICE VACCINE

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Vaccine-based methods represent a sustainable approach for pathogen control in aquaculture. For sea lice control, our group developed an Ipath vaccine, which is a recombinant vaccine with metal-chelating properties that reduces the *Caligus rogercresseyi* burden in Atlantic salmon. This study aimed to determine the Ipath performance in salmon vaccinated with Chilean mandatory vaccines and their effects on the Atlantic salmon transcriptome profile. Atlantic salmon were immunized with a pentavalent vaccine (P), a bacterial live attenuated vaccine (A), and Ipath. Four experimental groups were immunized: Ipath, P+A, P+A+Ipath, and PBS as a control. Following 400 ATUs, vaccinated salmons were infested with 35 copepodids per fish. Sea lice attachment was evaluated 25-days post-infestation. Also, head kidney tissue samples were collected for mRNA Illumina sequencing. Functional analyses were conducted using genes that were differentially expressed. Fish vaccinated with Ipath and P+A+Ipath showed 73.7% and 69.8% efficacy in lice reduction, respectively. Transcriptome analyses indicated a vaccine-dependent gene modulation. The highest number of DEGs was observed in the P+A group, with 3784 genes, while Ipath induced 612 genes. Notably, the combination P+A+Ipath group upregulated key immune-related genes, such as *cathelicidin, major histocompatibility complex class-I, and interferon regulatory factor,* suggesting an innate and adaptive response. Fish vaccinated with P+A meanly upregulated metabolic and structural genes. All three vaccinated groups enriched 11 immune response pathways, while P+A showed 6 additional unique pathways. This study demonstrates a synergy between Ipath and commercial vaccines, particularly at the immune response level, and offers a new strategy for integrated sea lice control in salmon aquaculture.

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PHARMACOLOGICAL TREATMENTS

EVOLUTION OF *Caligus rogercresseyi* MANAGEMENT STRATEGIES IN CHILEAN AQUACULTURE

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Caligidosis, caused by the parasitic sea louse *Caligus rogercresseyi*, remains a critical threat to Chilean aquaculture, compromising salmonid health and industry sustainability. Historically, control relied on chemical treatments, including oral and bath-administered chemotherapeutants, but their effectiveness has diminished due to increasing parasite resistance. This study chronicles the evolution of caligidosis management in Chile, highlighting the transition from chemical reliance to integrated, sustainable strategies. In the early 2000s, emamectin benzoate was the cornerstone of control, succeeded by pyrethroids (2007–2010), azamethiphos (2013–2016), and chitin inhibitors such as lufenuron and hexaflumuron (post-2016). Since 2016, the industry has increasingly adopted eco-friendly alternatives, including freshwater baths, hydrogen peroxide, and essential oil-based treatments, with the latter comprising approximately 25% of control measures in recent years. This shift reflects a broader commitment to balancing parasiticide efficacy with environmental responsibility and fish welfare, marking a transformative era in Chilean aquaculture.

PHARMACOLOGICAL TREATMENTS

A NEW SENSITIVITY TEST OF SALMON LICE LARVAE ENABLES MORE SUSTAINABLE USE OF SEA LICE MEDICINES

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One of the most used medications against sea lice in the aquaculture industry is SLICE®, an in-feed medicine containing the active substance emamectin benzoate (EMB). After 25 years of use its effectiveness has decreased due to reduced sensitivity.
Sensitivity tests for sea lice medicines have previously been developed for mobile lice stages to predict treatment outcome. These tests involve collecting sea lice from the aquaculture facility planning to treat and are conducted by exposing the lice to various concentrations of the medicine in baths. In the case of EMB, after 24 hours of exposure the proportion of moribund/dead lice at each concentration is examined, and the effective dose can be calculated. Such tests have frequently been used prior to prescribing medications against sea lice.
In Norway, EMB is effective on the infective copepodid stage , and is prescribed as an anti-resettlement treatment. There has been no protocol for sensitivity testing of larval stages to EMB until now. We have conducted studies which give new insights regarding lice copepodid sensitivity to EMB. Such tests are tools which can be used to predict the treatment outcome. By conducting bioassays in the planning phase of a lice treatment strategy, it is possible to limit the use of medicines to treatments where we can expect good effect.
The results of sensitivity testing of different lice strains at various exposure times will be shown. The opportunity to test the sensitivity of copepodids represents a significant advance which will enhance treatment strategies.

PHARMACOLOGICAL TREATMENTS

METHODS TO INCREASE DNA VACCINE EFFICIENCY AGAINST A SALMON LOUSE LABIAL GLAND PROTEIN

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Developing vaccines against ectoparasites presents significant challenges due to the limited exposure of these parasites to the host immune system. Effective immunization strategies must rely on immune effectors that impact the attachment site, hinder development, and/or alter the parasite's behavior during feeding. Potential antigen for a vaccine against the ectoparasite salmon louse *(Lepeophtheirus salmonis)* is immunomodulatory proteins, which are deposited on the skin and modulate the immune response of Atlantic salmon. Such a vaccine would not directly target the lice but would induce neutralizing antibodies against these immune-dampening proteins, thereby enabling the host to mount a more robust immune response towards the salmon louse. DNA vaccine technology is particularly suitable for this purpose, as such a vaccine must include several antigens. Given that DNA vaccines typically induce low antibody responses, additional measures were implemented to enhance their efficacy. Variations of synthetic hybrid constructs, combining a labial gland protein, a linker sequence, and flagellin from Moritella viscosa, were tested to evaluate the generation of specific antibodies against a labial gland protein.

Two common-garden in vivo vaccination experiments were conducted on Atlantic salmon parr. In both experiments, the fish were vaccinated with a 50 µl dose of different prototype vaccines, each containing 2.5 x 10^{12} plasmid copies, administered intramuscularly. The vaccinated fish were maintained in freshwater at 12°C. Muscle, blood, and mucus samples were collected at 2 weeks and 8 weeks post-vaccination and analyzed using RT-qPCR, histology, and ELISA. In the second experiment, different electroporation regimes were tested to optimize DNA vaccine administration.

Preliminary testing showed that DNA vaccination with unmodified labial gland proteins as antigens does not stimulate a strong immune response. Considerably higher antibody responses were observed in vaccine prototypes that included the fused labial gland protein sequence with flagellin and constructs that had an increased size of the antigen by linking the labial gland protein sequence together with multiple repeats. Histology revealed an influx of immune cells at the injection site 2 weeks post vaccination. The prototype DNA vaccines were safe for Atlantic salmon parr, with no mortalities and good K-factors observed 8 weeks post-vaccination. Intramuscular injections combined with electroporation resulted in increased antibody responses against the labial gland protein. The combination of synthetic hybrid constructs and electroporation shows potential to increase DNA vaccine efficiency and antibody generation against a target antigen in Atlantic salmon.

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PHARMACOLOGICAL TREATMENTS

DEFINING NICOTINIC ACETYLCHOLINE RECEPTOR STOICHIOMETRY IN *Lepeophtheirus salmonis* TO ADVANCE TARGETED PESTICIDE DEVELOPMENT

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Nicotinic acetylcholine receptors (nAChRs) are essential components of the arthropod nervous system and well-established targets for several classes of insecticides, including neonicotinoids (e.g., imidacloprid) and pore-blocking agents. Despite their proven importance in terrestrial pest control, the subunit composition of nAChRs in the salmon louse *Lepeophtheirus salmonis* has remained unresolved, limiting their potential as selective pesticide targets in aquaculture.

Two functional nAChRs have been identified in L. *salmonis*: Lsa-nAChR1, composed of α1, α2, β1, and β2 subunits, and Lsa-nAChR2, composed of α3, β1, and β2 subunits. However, the exact subunit arrangement within these pentameric receptors remained unknown. Here, we aimed to determine the precise subunit composition and order for both receptors.

We employed a dual approach, using AlphaFold2 Multimer to predict subunit arrangement, followed by experimental validation via expression of concatenated subunits in *Xenopus laevis* oocytes. This strategy revealed challenges with the gold-standard for experimental testing and shows that *in silico* modelling is a crucial tool in interpreting experimental results. Nonetheless, we resolved the complete stoichiometries of Lsa-nAChR1 and Lsa-nAChR2.

These findings fill a critical gap in the pharmacology of *L. salmonis* and provide a structural framework for future structural protein studies. By enabling the design of compounds that target louse nAChRs with high specificity, this work could potentially play a role in reducing off-target effects on non-pest crustaceans and minimizing environmental impact. Given the growing issue of resistance to existing treatments, the identification and characterization of novel molecular targets remain essential for sustainable sea lice management.

PHARMACOLOGICAL TREATMENTS

INTEGRATED EVALUATION OF EMAMECTIN USE: FROM FIELD EFFICACY AND FISH MUSCLE CONCENTRATION TO LABORATORY SENSITIVITY ASSESMENT

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C. rogercresseyi is one of the main ectoparasites affecting Chilean salmon farming. Several antiparasitics are used for its control, among them emamectin benzoate (EMA). Applied since 2000, EMA became a key tool for controlling this parasitosis during the early ongrowing phase at sea. However, increasing evidence of reduced parasite sensitivity threatens the sustainability of this strategy, making surveillance critical for disease management. Data from 2022–2025 from 20 salmon farms in Los Lagos and Aysén regions were analyzed. Adults EC50 values, EMA concentrations in fish muscle (ppb) from follow-up of oral treatments, sea lice abundance, and treatment timelines were considered. EC50 values showed a sustained increase since 2014, with peaks of lower sensitivity in 2023 and a historical maximum in early 2024; most assays then required >400 ppb to eliminate 100% of parasites. On average across years, the highest median muscle concentrations were detected in pulses 2 and 3 (≈180 and 174 ppb), with marked inter-site variability. Means were notably elevated in salmon administrative areas (ACS) 2 and 9a of Los Lagos. Time to the first bath treatment decreased from >50 weeks post-stocking at YC2022–YC2023 to only 7 weeks at YC2024, SNA 9c. In some farms, EMA delayed infestation onset until the fourth pulse, while in others no effect was evident. No linear correlation was found between fish weight, EMA muscle concentration, and treatment efficacy. These results show that local conditions and management practices are decisive, and that reduced sensitivity demands optimization of control strategies and continuous surveillance.

PHARMACOLOGICAL TREATMENTS

MUCOADHESIVE CHITOSAN-ALGINATE MICROPARTICLES LOADED WITH AZAMETHIPHOS FOR TARGETED CONTROL OF *Caligus rogercresseyi* IN SALMON AQUACULTURE

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The ectoparasite *Caligus rogercresseyi* represents a significant national health concern in salmon aquaculture, affecting fish health and causing substantial production losses. The aim of this study was to develop mucoadhesive particles loaded with azamethiphos, capable of adhering to the mucosal surfaces of salmonid epithelium and releasing the drug in close proximity to the parasite. The particles were formulated through the formation of a polyelectrolyte complex of alginate and chitosan using the ionic gelation technique. The active compound was incorporated into the particles during their fabrication. System characterization included hydrodynamic diameter determination (Dynamic Light Scattering), zeta potential (Laser Doppler Electrophoresis), morphology and size distribution (Scanning Electron Microscopy), drug loading efficiency, and release profile (HPLC-UV/Vis). Mucoadhesive capacity was assessed by evaluating particle–mucin interactions. Antiparasitic efficacy was determined in vitro by exposing parasites to the particles and assessing lethality at 24 h. The particles exhibited a hydrodynamic diameter of approximately 500 µm, monodisperse size distribution, predominantly spherical morphology, and a positive surface charge. Drug loading efficiency exceeded 80%, with over 70% release achieved within 24 h. Additionally, the system demonstrated mucoadhesive properties and in vitro antiparasitic activity against *C. rogercresseyi.* In conclusion, the developed system shows favorable physicochemical characteristics, sustained drug release capacity, and in vitro antiparasitic efficacy, representing a potential alternative for targeted control of *C. rogercresseyi* in salmon aquaculture.

PHARMACOLOGICAL TREATMENTS

EFFECTS OF CALIGUS BATH TREATMENTS ON SALMON PRODUCTION: MORTALITY, GROWTH AND FEED CONVERSION RATIO IN CHILEAN SALMON AQUACULTURE (2020 – 2025)

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Caligidosis, caused by the parasitic copepod *Caligus rogercresseyi,* poses a major challenge to the Chilean aquaculture industry, significantly impacting salmon production. Traditional control methods, relying on oral and bath-administered chemotherapeutants, have seen reduced efficacy due to increasing parasite resistance, necessitating frequent treatment adjustments. Since 2023, a sharp increase in the number of required baths has further exacerbated the issue, negatively impacting key production parameters such as mortality, growth, and feed conversion, while also raising operational costs.

To address these challenges, we conducted a retrospective study analyzing over 15,000 bath treatments administered between 2020 and 2025, focusing on the three dominant chemotherapeutants—essential oils, azamethiphos, and hydrogen peroxide—which account for over 85% of treatments. We assessed their effects on mortality, growth performance, feed conversion ratio, production yield.

HAPLOTYPES ASSOCIATED WITH DELTAMETHRIN-SENSITIVITY IN SALMON LICE COLLECTED FROM FISH FARMS ALONG THE NORWEGIAN COAST

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The salmon louse *Lepeophtheirus salmonis* has become increasingly resistant to medicinal compounds, including pyrethroids which act on voltage gated sodium channels (VGSC), effectively paralyzing the parasites. The common assumption is that resistance derives from a target-site mutation, as this is true for multiple other arthropods. So far, no conclusive evidence exists for this in salmon lice. Instead, several studies have suggested that four single nucleotide polymorphisms (SNP) in the mitochondrial DNA (mtDNA) may play a central role.

The aim of this study was to assess deltamethrin sensitivity and to investigate the occurrence and association of the mitochondrial SNPs to the bioassay results in salmon lice populations from different parts of Norway. To achieve this, we collected gravid adult females from production sites and hatched the egg strings to copepodites. DNA was extracted from the adult females and sequenced for the four SNPs in the mtDNA, which are associated with decreased sensitivity, and the copepodites were used in bioassays. As mtDNA is passed down maternally, the offspring of an adult female salmon lice will have the same mtDNA, as well as the same mutations. Thus, the EC_{50}-value could be directly associated with the sequencing data from the sequenced female.

Preliminary results show that the pyrethroid sensitivity in salmon lice along the Norwegian coast is variable. Furthermore, an association between copepodite bioassay outcomes and the presence of SNPs in mtDNA in the mother individual was indicated. This supports the hypothesis that mitochondrial mutations contribute to pyrethroid resistance in *L. salmonis.*

PHARMACOLOGICAL TREATMENTS

ION CHANNELS FROM SLAMON LICE IN FROG OOCYTES – NEW KNOWLEDGE ON PHARMACOLOGICAL PROPERTIES

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Many of the substances used against parasites and arthropod pests act via ion channels in the nervous system. This also applies to agents used against salmon lice *(Lepeophtheirus salmonis)*, such as emamectin benzoate on chloride channels and imidacloprid on nicotinic acetyl choline receptors. Although they both activate their respective ion channels when they bind, they have opposite effects in the nervous system – emamectin leads to reduced nerve activity, while imidacloprid activates the system. The ion channels that these two substances target have been tested in a model system with frog *(Xenious laevis)* oocytes. Gene sequences from salmon lice were injected into the oocytes, where the salmon lice ion channels were then expressed on the cell surface. By exposing the oocytes to different substances, the effects were assessed by measuring the electrical current that passes across the membranes, more specifically through the ion channels. Different classes of substances were tested, and differences in response to different substances within the same group of compounds were also identified. The pharmacological properties are important to know, but become even more relevant when we also look at the effect of the same substances on ion channels from the salmon host. The comparison tells us something about how good safety margin a substance against sea lice will have at the receptor level. The results from electrophysiological testing on ion channels from both salmon lice and salmon after exposure to different substance classes will be presented, including effects of emamectin benzoate on chloride channels.

PHARMACOLOGICAL TREATMENTS

EFFICACY OF A NOVEL INSECTICIDAL FORMULATION FOR THE CONTROL OF THE SALMON LICE *Leophtheirus salmonis* AND *Caligus rogercresseyi* IN ATLANTIC SALMON

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Global aquaculture reached 185 million tonnes in 2022, with salmon farming central in Norway and Chile, the world's leading producers. Both industries face a shared challenge: sea lice infestations Lepeophtheirus salmonis in Norway and *Caligus rogercresseyi* in Chile which cause major health and economic impacts. Conventional control strategies are increasingly limited by resistance and environmental concerns, threatening the sustainability of salmon aquaculture. Being a priority to find more alternatives of control.
In this study, we investigated the efficacy of a novel insecticidal formulation against sea lice.
The parasites were evaluated in vitro and exposed to 25 different concentrations of this insecticidal formulation for *Caligus rogercresseyi* and at 5 different concentrations for Lepeophtheirus salmonis.
Results showed that the new formulation was able to affect the parasites at all developmental stages. These findings suggest promising potential for controlling *Lepeophtheirus salmonis* and *Caligus Rogercresseyi* infestations in salmon. However, further studies are required to elucidate the biological responses of the different developmental stages, during the oral treatment in Atlantic salmon *for Lepeophtheirus* salmonis to improve our understanding of the underlying mechanisms of action, and to determine the optimal concentrations needed for effective parasite control.

PHARMACOLOGICAL TREATMENTS

A NEW MOLECULAR APPROACH FOR MONITORING PESTICIDE RESISTANCE IN THE SEA LICE *(Caligus rogercresseyi)* BASED ON GENE COPY NUMBER VARIANTS (CNVs)

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The control of *(Caligus rogercresseyi)* in Chilean salmon farming is largely dependent on pharmacological treatments, including pyrethroids, organophosphates, and chitin synthesis inhibitors. However, these treatments pose economic, environmental, and social concerns, and resistance to multiple antiparasitic compounds has been documented, challenging sustainable management of infestations. Currently, drug efficacy monitoring relies mainly on laboratory bioassays, which are limited by reproducibility issues, high sample requirements, and operator-dependent variability. Here, we suggest a new molecular approach that could be applied to monitor drug sensitivity in C. rogercresseyi, that could overcome the limitations of classical bioassays. The system is based on the absolute quantification of gene copy number variations (CNVs) in the genome of C. rogercresseyi, particularly in genes previously related with drug response. To test this, we collected adult sea lice from populations with contrasting efficacy against Azamethiphos and Hexaflumuron to identify possible differences in CNVs in genes including Cuticle, MDRP, CytP450 and GST. Our results evidence that this type of structural variation is widespread through the genome of *C. rogercresseyi,* with genes like GST presenting high degree of variation across individuals. Regarding treatment efficacy, we found significant differences in the number of genes copies present in sea lice genome for cuticle and MDRP when comparing populations from sites with high and low treatment efficacy. Our results evidence that the individuals from low efficacy populations tend to have less copies of Cut and MDRP, suggesting a possible relationship between CNVs and sea lice resistance against delousing drugs. These findings suggest the potential of CNV-based qPCR as promising tool for monitoring sea lice resistance, that could lead to earlier detection of reduced drug sensitivity in salmon aquaculture and contributing to more effective sea lice management strategies.

PHARMACOLOGICAL TREATMENTS

NEW ORAL ANTIPARASITIC FORMULATION USAGE FOR SEA LICE AS AN ALTERNATIVE TO THE TRADITIONAL TREATMENTS

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The parasitic copepod *Caligus rogercresseyi* is considered as a relevant disease during the marine phase of Atlantic salmon *(Salmo salar)* and a potential vector of other bacterial pathologies. Different chemical compounds, including both in-feed and bath treatments have been used to prevent and control sea lice outbreaks. Commonly used antiparasitic chemicals include Lufenuron and emamectin benzoate (in-feed), deltamethrin, and azamethiphos (both bath treatments). Hydrogen peroxide is also used as a bath treatment. These chemicals target different stages of the sea lice, some inhibit chitin synthesis while others affect nerve transmission.

The aquaculture industry demands new effective strategies for controlling sea lice therefore Farmacología en Aquacultura Veterinaria (FAV) has developed an oral parasitic formulation for sea lice control as an alternative to actual treatments, containing a new active ingredient family not previously used in the salmon industry.

The antiparasitic has proven to be palatable, safe and 100% effective in all *Caligus rogercresseyi* life stages on various trials performed under controlled conditions. The product is environmentally friendly since it is non-toxic and does not persist or bioaccumulate. The product formulation is safe for use in salmonids species as much as ten times the recommended doses.

This oral alternative against *Caligus rogercresseyi* aims to complement newly available commercial molecules for rotational use, with the foal of extending their efficacy and useful life in the salmon industry.

NON-PHARMACOLOGICAL TREATMENTS

TRADE-OFFS IN SEA LICE CONTROL: ASSESSING MECHANICAL/THERMAL TREATMENTS AND BACTERIAL OUTBREAK RISKS IN CHILEAN SALMON AQUACULTURE

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While sea lice control strategies in the northern hemisphere have largely replaced chemotherapeutants with mechanical and thermal alternatives, their implementation in Chilean salmon aquaculture has faced challenges—despite increasing parasite resistance to traditional chemotherapeutants. In this study, we evaluated the effectiveness and short-term impacts of 79 mechanical and thermal treatments performed between 2015 and 2025, comparing them to conventional bath treatments (freshwater, azamethiphos, hydrogen peroxide, and essential oils). Our findings reveal that mechanical and thermal approaches, under specific environmental and production conditions, are associated with an elevated risk of *Piscirickettsiosis* (SRS) outbreaks, the primary driver of infectious mortality and antimicrobial use in Chile. Given recent reports of similar bacterial outbreaks in the northern hemisphere, our analysis provides critical insights into the risks of non-chemotherapeutant delousing methods. We offer practical guidelines to optimize their application, balancing sea lice control efficacy with mitigation of secondary bacterial disease impacts.

HARBOR FENCE; INNOVATIVE PULSE TECHNOLOGY AS A MEASURE AGAINST SEA LICE

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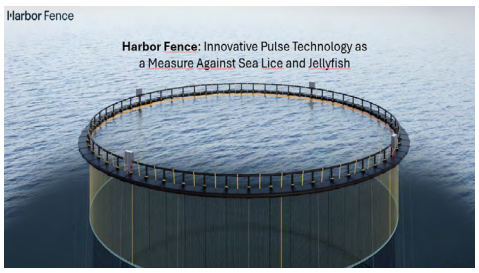
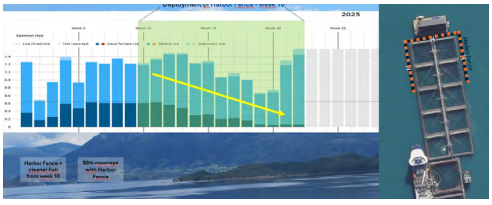
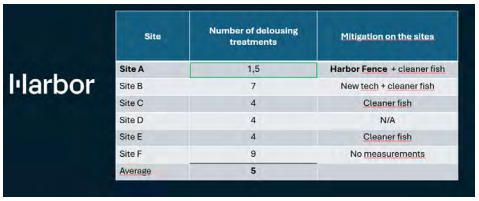
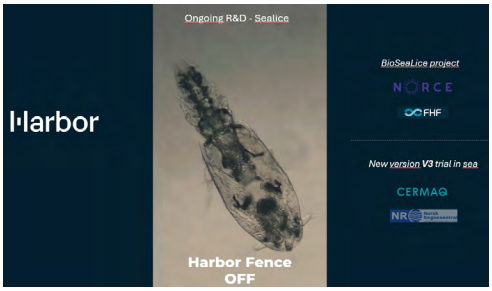
Sea lice *(Lepeophtheirus salmonis)* and *Caligus* elongatus remain major biological constraints for sustainable growth in Norwegian salmon aquaculture (Hemmingsen et al., 2020; Misund, 2022). Reduced efficacy of pharmaceuticals due to resistance has driven adoption of non-medicinal methods, many of which are stressful and associated with fish welfare and mortality concerns (Sommerset et al., 2024). Free-living salmon lice larvae primarily inhabit the upper 10 m to maximize host encounters, as salmon regularly surface to refill their swim bladders (Coates et al., 2020). Barrier technologies such as shielding skirts (Grøntvedt et al., 2018) and snorkel sea cages (Wright et al., 2017) exploit this behavior and provide welfare benefits compared to mechanical delousing (Overton et al., 2019; Barret et al., 2020). However, these barriers reduce water exchange, may deform in strong currents, and remain only partially effective (Barret et al., 2020).
Electric fence technology represents a non-invasive preventive approach, generating pulsed electromagnetic (EM) fields that damage free-swimming lice larvae with low resistance potential. Laboratory infection trials have shown a 60–80% reduction in lice settlement on salmon exposed to EM fields. To further explore underlying mechanisms, Harbor AS is currently conducting a large R&D project together with NORCE, Cermaq, and the Norwegian Computing Center. In this project, early-stage lice are experimentally exposed to different EM pulse patterns under controlled laboratory conditions, and outcomes on larval behavior, feeding activity, and survival are systematically assessed.
Parallel large-scale field trials since 2021 have confirmed stable cage-level operation and provided insight into protective performance in commercial aquaculture settings. These combined efforts suggest that EM-based electric fence technology has strong potential as a preventive tool, offering effective reduction of lice infestation pressure while avoiding the welfare costs of conventional delousing strategies.

Appendix









NON-PHARMACOLOGICAL TREATMENTS

KRILL MEAL REDUCES SEA LICE INFESTATION AND ENHANCES SKIN MUCOSAL HEALTH IN ATLANTIC SALMON

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Sea lice remain a major challenge for the salmon industry, requiring frequent treatments and handling due to limited management options and strict regulations. These practices can compromise fish health and welfare, underscoring the urgent need for alternative, low-stress control strategies. Functional nutrition offers a promising avenue, with dietary interventions designed to enhance host defenses. This study evaluated the efficacy of krill meal (KM) as a functional feed ingredient to reduce sea lice infestation and improve skin mucosal health in Atlantic salmon smolts reared in seawater. KM is rich in polyunsaturated fatty acids (PUFAs), including EPA and DHA, and phospholipids such as phosphatidylcholine.

Atlantic salmon (~170g) were fed diets containing 0% (control), 8%, or 12% KM for eight weeks, reaching ~310g. Following this, fish were exposed to a two-week sea lice challenge while continuing their respective diets.

After the initial pre challenge feeding phase, fish on the 8% KM diet exhibited the thickest skin epithelium (72.3 µm) compared to the 12% KM (51.3 µm) and control (43.8 µm) groups. Mucosal parameters—including cell size, density, and defense activity—were significantly higher in the 8% KM group. Post-challenge, these fish also had the fewest lice (median: 6.5 per fish), suggesting that dietary inclusion of KM at 8% may enhance mucosal barrier function and contribute to lice deterrence.

These findings highlight KM's potential as part of a functional nutrition strategy for lice mitigation in salmon. Further research is needed to identify the active compounds and biological mechanisms responsible for the observed benefits.

IN VITRO AND IN VIVO SUSCEPTIBILITY OF *Caligus rogercresseyi* TO PLANT-DERIVED PHYTOGENIC PREMIXES FOR IN-FEED USE

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Sea-lice infestations caused by *Caligus rogercresseyi* remain a major constraint for Chilean aquaculture, with control still relying on therapeutic baths and a limited set of registered drugs available. Functional feed additives with antiparasitic activity are promising approach to reduce dependence on conventional chemotherapeutants. Our objective was to evaluate the in vitro and in vivo susceptibility of *C. rogercresseyi* to a panel of additives. Seven plant-derived phytogenic additive premixes from Amaryllidaceae, *Rutaceae* and *Lamiaceae*, plus a Negative Control were evaluated in an acute immersion bioassay at concentration of 0, 0.01, 0.1, 1, 10 and 100 ppm, each tested in triplicate. Non-viable parasites (moribund or dead) were recorded at 0, 1, 24 and 48 h post-immersion. At ≤1 ppm, no parasites were affected by any additive. At 10 ppm, only one premix yielded 93% and 73% non-viability at 0 and 1 h, respectively. At 100 ppm, all premixes caused non-viability at every time point. This in vitro screening identified promising plant-derived additive premixes for in-feed in vivo validation. Five mixes were trialed using juvenile Atlantic salmon, by feeding them two weeks before experimental infestation. Feed intake, palatability, and effectiveness on *C. rogercresseyi* development were assessed. The efficacy on copepodites survival and evolution to adult stages will be discussed, as well as the capacity of gravid females to generate descendance.

NON-PHARMACOLOGICAL TREATMENTS

RECOMBINANT LICE ANTIGEN VACCINES AND WHOLE LICE ANTIGENS PROVIDE PROTECTION AGAINST SEA LICE IN ATLANTIC SALMON

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The ectoparasitic copepod Lepeophtheirus salmonis continues to pose a major challenge to Atlantic salmon *(Salmo salar)* aquaculture, causing substantial economic losses and raising concerns about fish health and welfare. Conventional control measures, such as chemotherapeutants and mechanical delousing, are limited by resistance development, environmental impacts, and stress to fish. Vaccination offers a more sustainable alternative, and promising results have been reported using both recombinant antigen-based vaccines and preparations derived from whole sea lice antigens.

In the present study, Atlantic salmon were intraperitoneally immunized with a recombinant protein formulated with an adjuvant and subsequently challenged with *L. salmonis* copepodids under experimental conditions. Post-challenge analyses showed a significant reduction in lice burden among vaccinated groups compared to controls, with the strongest effects observed during the chalimus and pre-adult stages. Elevated antigen-specific antibody levels confirmed induction of a robust humoral immune response.

Complementary trials using whole sea lice antigen preparations have also demonstrated protective effects against infestation, with reduced parasite loads and evidence of both mucosal and systemic immune activation. These responses included induction of serum and mucus immunoglobulins (IgM and IgT), as well as transcriptomic and proteomic changes linked to pathways such as complement activation, cytokine signaling, and antimicrobial peptide production. Importantly, combined antigen delivery routes (intraperitoneal, mucosal, or fin administration) enhanced both local and systemic immune responses, further supporting the utility of whole-antigen formulations.

Taken together, these findings indicate that both recombinant antigen vaccines and whole lice antigen preparations can elicit protective immunity by interfering with parasite development and host attachment. This highlights the potential of antigen-based vaccines as an integral component of sea lice management strategies in salmon aquaculture. Future work should focus on optimizing antigen selection, delivery routes, and dosing regimens, as well as testing combined or sequential use of recombinant and whole-antigen approaches under field conditions.

NON-PHARMACOLOGICAL TREATMENTS

REPROGRAMMING SEA LICE MICROBIOTA THROUGH PRECISION PROBIOTICS: A NOVEL PREVENTIVE TOOL FOR SALMON AQUACULTURE

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Salmon aquaculture is continually threatened by pathogens that affect fish health, welfare, and productivity. In particular, pesticides or delousing drugs are commonly used for sea lice control. However, the extensive use of antiparasitic treatments has led to sea lice losing sensitivity to these methods. Therefore, research efforts are focused on developing new sustainable solutions, such as vaccines. In arthropods like sea lice, it is reported that the microbiota can support metabolic pathways; thus, disrupting the microbiota may impact their development and survival. In this context, anti-parasite microbiota vaccines have emerged as a promising alternative for controlling parasitic infestations. This study reports a vaccination strategy that uses sea lice microbiota to disrupt the symbiotic bacterial communities of *Caligus rogercresseyi*, thereby impairing parasite fitness and boosting the immune response in Atlantic salmon. We isolated non-pathogenic bacteria from sea lice microbiota to immunize Atlantic salmon and challenged them with *Caligus rogercresseyi.* We analyzed the success of sea lice infestation after different immunization strategies using both inactivated and live vaccines delivered intraperitoneally and orally. A reduction in sea lice load was observed in salmon immunized with the anti-sea lice microbiota vaccine. Additionally, changes in the bacterial communities of sea lice were detected through 16S rRNA sequencing analysis. The skin transcriptome profile of the salmon showed an upregulation of mucosal immune responses. This study presents an innovative strategy for controlling sea lice infestations that can be applied throughout the entire production cycle without compromising fish welfare.

Funding: INCAR Center ANID Grant #1523A0007

NON-PHARMACOLOGICAL TREATMENTS

TECHNOLOGICAL TRANSITION AQUACULTURE: EVALUATING WELLBOAT PERFORMANCE AGAINST TRADITIONAL TARPULIN METHODS FOR CONTROL OF *Caligus rogercresseyi*

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This study provides a comparative analysis of wellboat and tarpaulin bath systems for managing *Caligus rogercresseyi* infestations in Chilean salmon aquaculture. Our results indicate that wellboat systems deliver more consistent outcomes with significantly lower variability, particularly for hydrogen peroxide and freshwater treatments, although no statistically significant difference in overall efficacy was observed compared to tarpaulin systems.

Distinct operational trends emerged: wellboats were primarily utilized for hydrogen peroxide and freshwater treatments, while tarpaulin dominated for essential oils and chemotherapeutants like azamethiphos.

Industry trends show increasing adoption of wellboat technology, driven by superior technical performance, enhanced safety, and improved operational control. However, barriers to widespread use include limited vessel availability, high costs, and competition from cost-effective alternatives like essential oils or chemotherapeutants. System selection hinges on multiple factors such as on treatment type, site-specific logistics, health and production constraints and associated costs. Theses comparative findings provide valuable information for decision taking in the health management for the Chilean Salmon Industry.

NON-PHARMACOLOGICAL TREATMENTS

EFFICACY OF NATURAL FEED ADDITIVES FOR THE CONTROL OF *Caligus rogercresseyi* IN ATLANTIC SALMON *(Salmo Salar)*

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Caligidosis, caused by the ectoparasite *Caligus rogercresseyi,* is a major health challenge in salmon aquaculture.
This study assessed the antiparasitic efficacy of feed formulated with natural additives in Atlantic salmon *(Salmo salar).*
In vitro assays with different natural formulations were initially conducted to determine optimal strategies for subsequent in vivo evaluation. Fish were maintained at 12 °C in tanks (density: 11 kg/m³) and fed for 42 days with diets containing different additives or a control diet. Two experimental infestations were performed: (I) 20 adult parasites/fish before the feeding period, and (II) 35 copepodids/fish after 27 days of ingestion (DDI).
In Infestation I, efficacy against adults at 13 DDI was S4 (19%), S10 (21%), S13 (15.7%), S16 (29.9%), and SD16 (45%), increasing at 20 DDI to S4 (39%), S10 (42%), S13 (19%), S16 (46.8%), and SD16 (89.7%). In Infestation II, efficacy against chalimus I–II (34 DDI, 7 DPI (post infestation days)) was S4 (15.3%), S10 (9.8%), S13 (16.2%), S16 (34.3%), and SD16 (59%); and against chalimus III–IV (42 DDI, 14 DPI) was S4 (13%), S10 (15.7%), S13 (17.1%), S16 (47.9%), and SD16 (72%).
No significant differences in porductive performance were observed among treatments and controls. Feed containing additive S16 showed consistent efficacy (>45% in adults and juveniles), whereas SD16 achieved the highest reductions (90.7% in adults; ~65% in juveniles). These findings indicate that the addite, particularly SD16, offers a promising and sustainable preventive approach for controlling *C. rogercresseyi* in salmon farming, potentially reducing the chemotherapeutics and minimizing environmental impacts.

NON-PHARMACOLOGICAL TREATMENTS

DEVELOPMENT OF A STANDARDIZED POLYVALENT PHYTOGENIC PRODUCT WITH MULTI-STAGE EFFICACY AS A TREATMENT FOR *Caligus rogercresseyi*

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Caligus rogercresseyi is a growing problem that induce economic impact in the Chilean salmon farming. Currently, some of the pharmacological treatments have shown lost effectiveness. Due to this, non-pharmacological treatments have achieved strong notoriety, even changing consumption trends. In this context, an antiparasitic formulation has been developed based on three extracts of endemic and exotic plants, named XX, YY, and ZZ. Adults, egg strings and copepodites of *C. rogercresseyi* were exposed to the extracts through *in vitro bioassays.* The results showed that above 5000 ppm of compound XX, the affected individuals reached 100% at 0- and 24-hours post-exposure (hpe), without detecting recovery in them. Compound YY at 1000 ppm yielded values of 100% and 83,3% of affected at 0 and 24 hpe, showing recovery between observation times. For compound ZZ from 2,5 (%w/v) and above, 100% of individuals were affected at 0 and 24 hpe. Also, the ZZ extract on egg strings showed 100% inhibition of nauplii hatch and development at a concentration of 10% w/w of the extract. When XX and ZZ extracts were combined, 55,6% and 100% of the adult stages were affected at 0 and 24 hours, respectively, showing an increase in affectation of the parasites. In addition, some egg strings showed hatching inhibition and nauplius were immobilized after treatment. It is suggested that this phytogenic product has good antiparasitic activity on *C. rogercresseyi under in vitro* conditions. Further studies will be carried out regarding safety and efficacy under controlled *in vivo conditions.*

SALMON LICE DISLODGED IN DELOUSING OF FARMED SALMON CAN SURVIVE AND REATTACH TO NEW FISH

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Open sea cages used in Atlantic salmon aquaculture can lead to infestation by the salmon louse *(Lepeophtheirus salmonis).* Salmon lice are ectoparasites and mechanical delousing treatments dislodge the mobile stages. This can lead to the release of large amounts of lice into the surrounding water. The aim of the study was to determine whether the mobile stages of the parasite can survive and spread to new fish.
Dislodged mobile lice were monitored in the laboratory for survival at different temperatures and salinities and their ability to attach to new fish. Furthermore, the sinking rates of lice were investigated experimentally in tanks and open water. These results were utilized in a hydrodynamic dispersal model to quantify potential spread to other farm sites.
All mobile lice exhibited long survival off fish with most lice surviving more than one week. Warmer temperatures and lower salinity reduced overall survival. Adult females survived longer than preadults and adult males. Also, lice reattachment to new fish was only slightly reduced even by prolonged removal from the host fish. Whereas the long survival times and high rates of reattachment highlight the risk of reinfestation, the mobile lice exhibited limited ability to remain in the water column. The rate of sinking was approximately 1metre per minute with adult females sinking faster than the smaller stages. Accordingly, modelled dispersal indicates that the highest risk of reinfestation is within farms and to wild fish in the immediate vicinity of cages.

NON-PHARMACOLOGICAL TREATMENTS

DRIVERS OF SEA LICE CONSUMPTION IN LUMPFISH *(Cyclopterus lumpus)* : EFFECTS OF PREY AVAILABILITY, LUMPFISH SIZE, AND DEPLOYMENT STRATEGY

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Lumpfish are used as a non-pharmacological method to control salmon lice in Atlantic salmon aquaculture. However, their effect as cleaner fish varies substantially. This study examines how alternative prey availability, individual characteristics and deployment strategy influence sea lice consumption in lumpfish, with the aim of improving the predictability and outcome of this treatment approach.

We analyzed stomach contents data from over 27,000 lumpfish sampled across multiple sites and production years in the Faroe Islands. The dataset included lumpfish size, cage-level lice pressure at deployment and on cohabiting salmon, and the presence of alternative prey items in the stomachs.

We assessed how fish weight and the availability of different prey items influenced lice consumption, and whether cage-level lice pressure at deployment was linked to the probability of lice ingestion. A large proportion of stomachs contained no lice, suggesting that many individuals may not engage in lice feeding.

Preliminary results suggest that lumpfish deployed under low lice pressure were more likely to remain non-feeders, possibly due to missed opportunities to initiate lice-feeding behavior. Fish weight showed a non-linear relationship with consumption, with peak predicted ingestion occurring at small to intermediate sizes. The presence of jellyfish in the stomach had a strong negative effect, with more lumpfish classified as non-feeders when jellyfish were present. Other prey types also influenced feeding outcomes.

These findings highlight the importance of early deployment conditions and the influence of prey availability in lumpfish feeding behavior and can contribute to more effective and targeted use of this cleaner fish.

NON-PHARMACOLOGICAL TREATMENTS

ASSESSMENT OF DIFFERENT KIND OF SHIELDING SKIRT TO REDUCE *Caligus rogercresseyi* INFESTATION IN SALMON FARMING SITES IN SOUTHERN CHILE

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Sea lice infestation remains a major challenge for salmon aquaculture in Chile. Currently, barrier methods, such as skirts, have become increasingly important, for preventing parasite infestation and reducing the need for treatments. This study assessed the efficiency of skirts as a physical barrier against larval stages of *Caligus rogercresseyi* in laboratory and at farm settings. Initially, laboratory experiments were conducted to evaluate different skirts fabrics and determine their optimal design for retaining larvae and preventing trespass. Subsequently, skirts were placed at multiple farms in Los Lagos and Aysen regions. Larval density was monitored from inside and outside the skirted cages at each site using data from Mowi´s zooplankton surveillance program. The effectiveness of the skirts was evaluated by measuring the number of weeks from fish stocking to the first required bath treatment . The results showed a consistent difference in larval density inside versus outside the skirted cages, with densities approximately 40–60% lower inside the cages than outside. A comparative analysis revealed that the time to the first treatment was extended in cages equipped with skirts, relative to historical data from unprotected cages at the same farming sites. This finding, coupled with the observed reduction in internal larval density, confirms the efficacy of skirts and validates their role as a key component in an Integrated Pest Management (IPM) strategy against *C. rogercresseyi*. Further research is needed to optimize skirt implementation by evaluating long-term effects on site-level infestation dynamics and fish welfare, alongside other pharmaceutical and non-pharmaceutical treatments.

MODELLING AND EPIDEMIOLOGY

PATTERNS OF ANTIPARASITIC USE IN ATLANTIC SALMON FARMING IN LOS LAGOS REGION, CHILE (2015–2024)

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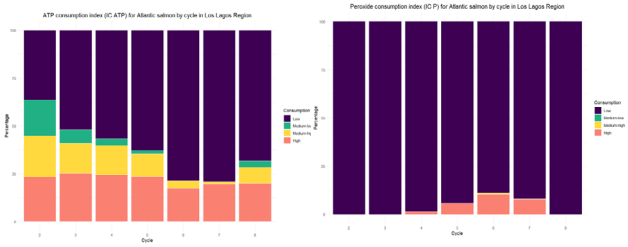
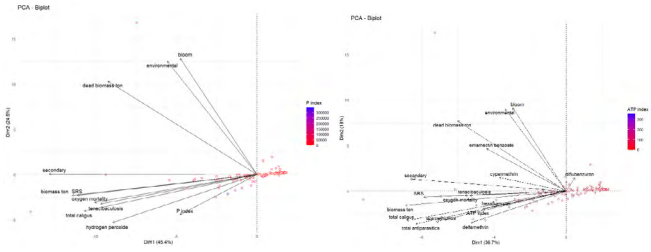
This study retrospectively evaluated antiparasitic usage patterns within Salmon Farming Concession Groupings (ACS) in Chile's Los Lagos region from 2015 to 2023. The objective was to identify the key productive, sanitary, and environmental variables associated with treatment application. We utilized a comprehensive dataset from Sernapesca's national surveillance program, containing weekly records from Atlantic salmon farms.
The analysis included farm location, fish density, biomass, environmental parameters (salinity and temperature), cause-specific mortality, kilograms of antiparasitics used, and stage-specific abundance of sea lice *(Caligus rogercresseyi).* An antiparasitic consumption index was calculated for each compound (azamethiphos, emamectin benzoate, cypermethrin, deltamethrin, diflubenzuron, hexaflumuron and for hydrogen peroxide), expressed as kilograms used divided by total produced plus dead biomass (in tonnes). Farm and neighborhoods antiparasitic consumption was classified into quartiles: low (≤ Q1), medium-low (Q1–Q2), medium-high (Q2–Q3), and high (≥ Q3). Patterns were described and visualized (Figures 1 - 2). A principal component analysis (PCA) was performed on antiparasitics consumption alongside production variables (Figures 3 - 4).
Most salmon farming fell in the low-consumption category, though a persistent subset remains in the high-consumption group over time.
In the PCA, azamethiphos, deltamethrin, and hexaflumuron use were associated with neighborhoods with higher biomass, while emamectin benzoate showed weaker association and diflubenzuron displayed no clear clustering with production patterns. For hydrogen peroxide a notable proportion of farms show high consumption, including recent production cycles. Antiparasitic consumption pattern in some neighbourhoods was related to some productive and health variables indicating heterogeneity in treatment strategies, differences in Caligus pressure across production areas, and the need for more targeted management approaches.



Figures N°1 and N°2: Antiparasitic and Hydrogen Peroxide Use in Production Centers by Consumption Indices.



Figures N°3 and N°4: PCA biplots of antiparasitic and hydrogen peroxide use by production factors and consumption indices.

MODELLING AND EPIDEMIOLOGY

HARNESSING CURRENTS TO QUANTIFY PARASITES: ddPCR ANALYSIS OF PASSIVELY COLLECTED PLANKTON PROVIDES NEW EMPIRICAL DATA FOR RISK ASSESSMENTS

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In Norway, hydrodynamic particle dispersion models (HPDM) are applied to salmon lice larvae to estimate densities of infective larvae along the Norwegian coast. These are used, among other things, to assess the risk of infestation for migrating wild salmonids. Because the models' results impact industry regulation, predictions in some regions are hotly contested and more empirical measurements would be a beneficial addition to risk assessments. Therefore, we designed a time-integrated passive sampler that filters large volumes of near-surface seawater and captures louse for quantification by ddPCR. We deployed 12 passive samplers in Hardangerfjord, where louse infestation experiments are conducted annually and Havforskningsinstituttet's HPDM is calibrated.

Comparing the number of lice larvae captured with modeled estimates and the number of lice infesting experimental salmon smolts, we found that spatial and temporal patterns of louse distribution were mostly consistent across methods. We observed that sites near a high density of farms had a significantly greater chance of having lice caught by passive collection than those without. In Etnefjord, a protected area closed to fish farming, experimental smolts became infested with louse during both two-week cage deployments but the passive sampler failed to capture parasite larvae, likely due to its collection depth, highlighting areas for improvement in the sampler's design. Overall, our tests show that passive collection methods function to generate substantiated larvae abundances and deploying this approach on a large scale could be used to fortify risk assessments and identify areas with heightened HPDM uncertainty.

MODELLING AND EPIDEMIOLOGY

CAN WE PREDICT *Caligus rogercresseyi* ABUNDANCES IN FARMING SITES BY MONITORING THE PLANKTON STAGES OF THE PARASITE?

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The planktonic stages of *Caligus rogercresseyi* may spend from 5 to 12 days in the upper water column searching for hosts, before initiating the parasitic stage. Better understanding of the distribution pattern of these stages around salmon farms would allow us to estimate infestation pressure which in turn will contribute to develop new strategies and solutions to reduce the sea lice impact on salmon farming in Chile. This study aims to determine the correlation between larval density and parasite abundance on the fish to enhance our understanding of caligus population dynamics. Larval density patterns were analyzed and characterized against environmental variables (salinity and temperature), caligus abundance on fish and farming practices. Data were collected from the zooplankton monitoring program conducted by the Mowi Chile Laboratory as part of health surveillance programs. Farming sites were classified as challenging (historically high load of sea lice) and standard (historically media or low load of sea lice) The analysis included data from 2019 to 2025 in Los Lagos and Aysén region. Linear mixed-effects models and Principal Components Analysis were used to establish relationships between larval density against sea lice abundance on salmon. The results indicate that including larval density as a variable helps to improve the model's predictability of caligus abundance. Furthermore, it contributes to refining caligus simulation models, providing deeper insights into estimating infestation rates—a parameter that still remains poorly understood. This is the first study conducted in Chile that establishes relationships between larval density and parasite abundance in farming sites.

MODELLING AND EPIDEMIOLOGY

DETECTING LONG-TERM ABUNDANCE CHANGES AND EPIDEMIC TRANSITIONS OF SEA LICE IN SALMON FARMING AREAS USING COMPLEXITY MEASURES AND EARLY WARNING INDICATORS

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Outbreaks of sea lice *(Caligus rogercresseyi)* in highly infested salmon farming areas of southern Chile were analyzed within the framework of dynamical system theory considering the salmon farming activity and oceanographic variability between 2012 and 2022 as major stressors of Patagonian aquatic ecosystems. Transitions points, which demark changes in the state of an epidemic, were detected in total adult sea lice abundance time series at a weekly scale. Several generic early warning indicators were applied to sea lice abundance series from salmon neighborhood areas 10A and 10B, and the autoregressive at lag-1 coefficient successfully detected increases in sea lice abundance several months in advance of two major transition points marked by large scale atmospheric/oceanographic disturbances caused by El Niño 2016 and the abnormal low precipitation level detected during spring-summer of 2019-2020. When complexity measures- Hurst coefficient *(H)* and weighted permutation entropy *(WPE)*- were used as indicators to track the evolution of the epidemiological status of farming areas across several years, an opposite pattern of fluctuation was detected. As the long-term memory quantified by *H* in sea lice abundance series decreases, WPE increases, demarking a decrease in the predictability of sea lice dynamics. This pattern was interpreted as the continuous development of antiparasite resistance in the study area, and by the effect of high host biomass levels on sea lice abundance. These results suggest that complexity measures and generic EWIs can be useful tools for farm managers to track sea lice epidemic transitions and for the early detection of major outbreaks.

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MANAGEMENT AND REGULATIONS

ASSESSING GOVERNANCE INFLUENCE ON SUSTAINABLE SEA LICE MANAGEMENT: AN AQUACULTURE GOVERNANCE INDICATORS (AGIS) APPROACH ACROSS KEY SALMON-PRODUCING NATIONS

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Sea lice threaten global salmon farming. Their effective management and the adoption of new solutions depend on each nation's governance frameworks—rules, practices, and institutions guiding aquaculture. This study examines how governance in Chile, Canada, and Norway impacts the speed of sustainable sea lice management.

A comparative analysis of salmon aquaculture governance profiles in Chile, Canada, and Norway was conducted, based on the Aquaculture Governance Indicators (AGIs) assessment reports from 2019-2020. Four key governance dimensions were examined: legislation, voluntary codes and standards, collaborative arrangements, and actor capabilities.

The unique governance profiles of each country differentially influence their capacity to respond to and implement solutions for sea lice. Norway, with its mature industry and innovation drive, appears better poised to accelerate solutions despite some gaps in standard coordination. Canada, with high adoption of certifications, faces challenges in governmental promotion and cumulative impact assessment. Chile, with a strengthened post-ISA regulatory framework and growing inter-industry collaboration, shows potential, yet limited civil society inclusion and a lack of trust among actors may slow problem resolution at the local level. More inclusive, coordinated, and proactive governance, with an explicit focus on cumulative impacts, is crucial for accelerating sustainable sea lice management in global salmon aquaculture.

MOBILE LICE DETACH DURING CROWDING BUT CAN BE RETAINED BY FINE-MESHED CROWDING NET

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Salmon are crowded during lice treatments or harvesting operations whereby mobile lice may detach, spread and pose a risk of reinfestation for farmed and wild fish. Such lice detachment and future retainment are not well understood. The aim was therefore to quantify lice detachment, determine the mesh size needed to capture all lice stages, and assess the feasibility of using a fine-meshed net to collect the detached lice.

Crowding trials were conducted in cages, using standard or fine-meshed nets. Crowding duration (10-120 min), fish size and number and louse density varied. Detached lice were captured using plankton nets. A benchtop model was made simulating the retention of detached lice using various mesh types and sizes (0.8–2.0 mm) as well as precise measurements of each louse stage size metrics.

Mobile lice detached at 2 to 38%, with higher rates during longer crowding duration (up to 2 h) and with larger fish. Smaller pre-adults detached more frequently than adults. A nominal mesh size of 0.8 mm effectively captured all lice stages, 1.6 mm retained most pre-adults and adult males, while 2.0 mm retained adult females. Mesh opening size (diagonally) corresponded to cephalothorax width of the louse stage captured. In cages, the fine-meshed crowding net successfully retained 75% of adult females and improved fish welfare. Lice loss can occur during handling of salmon and collecting lice during crowding operations could be an effective management strategy to limit their release into the environment and thereby lower the reinfestation risks.

MANAGEMENT AND REGULATIONS

IMPACT OF ANTIPARASITIC TREATMENTS ON THE EFFICACY OF BEST MANAGEMENT PRACTICES AND SANITARY TOOLS IN CHILEAN SALMON AQUACULTURE: EVIDENCE FROM THE PINCOY INITIATIVE

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The sustainability of the salmon farming depends on the implementation of best management practices (BMPs). These practices are essential for production efficiency, fish welfare, and reduced environmental impact. However, the type and intensity of treatments for control of lice, one of the main hurdles in salmon farming, impose continuous challenges to the effectiveness of strategies to ensure Aquaculture sustainability. A comprehensive understanding of how these treatments influence the outcomes of BMPs is therefore needed.
The Pincoy Initiative is a collaborative program encouraging strategies based on scientific evidence and data collection. The initiative maintains a comprehensive database integrating fish health indicators, farming practices, and production records. For the present study, data analysis of a range of treatments was conducted for the development of statistical models and evaluation using farming records over six years.
The analysis demonstrated that the type and frequency of treatments can significantly impact the efficacy of BMPs as preventive strategies, fish genetic background, functional feeds and the target for antibiotic reduction. These results show, with empirical evidence, the relationship among antiparasitic treatments, the mortality by Salmon Rickettsial Syndrome (SRS), antibiotic consumption, and the overall effectiveness of adopted aquaculture practices.
Our findings highlight the need for developing integrated and sustainable strategies for parasite management that minimize their deleterious impact on the efficacy of salmon aquaculture Best Management Practices (BMPs). The presented evidence emphasizes the association between therapeutic interventions and overall production performance, highlighting the importance of holistic approaches for ensuring the long-term sustainability and resilience of the salmon farming industry.

MANAGEMENT AND REGULATIONS

BALANCING REGULATION AND SUSTAINABILITY: MANAGING SALMON LICE IN IRELAND'S ORGANIC AQUACULTURE SECTOR

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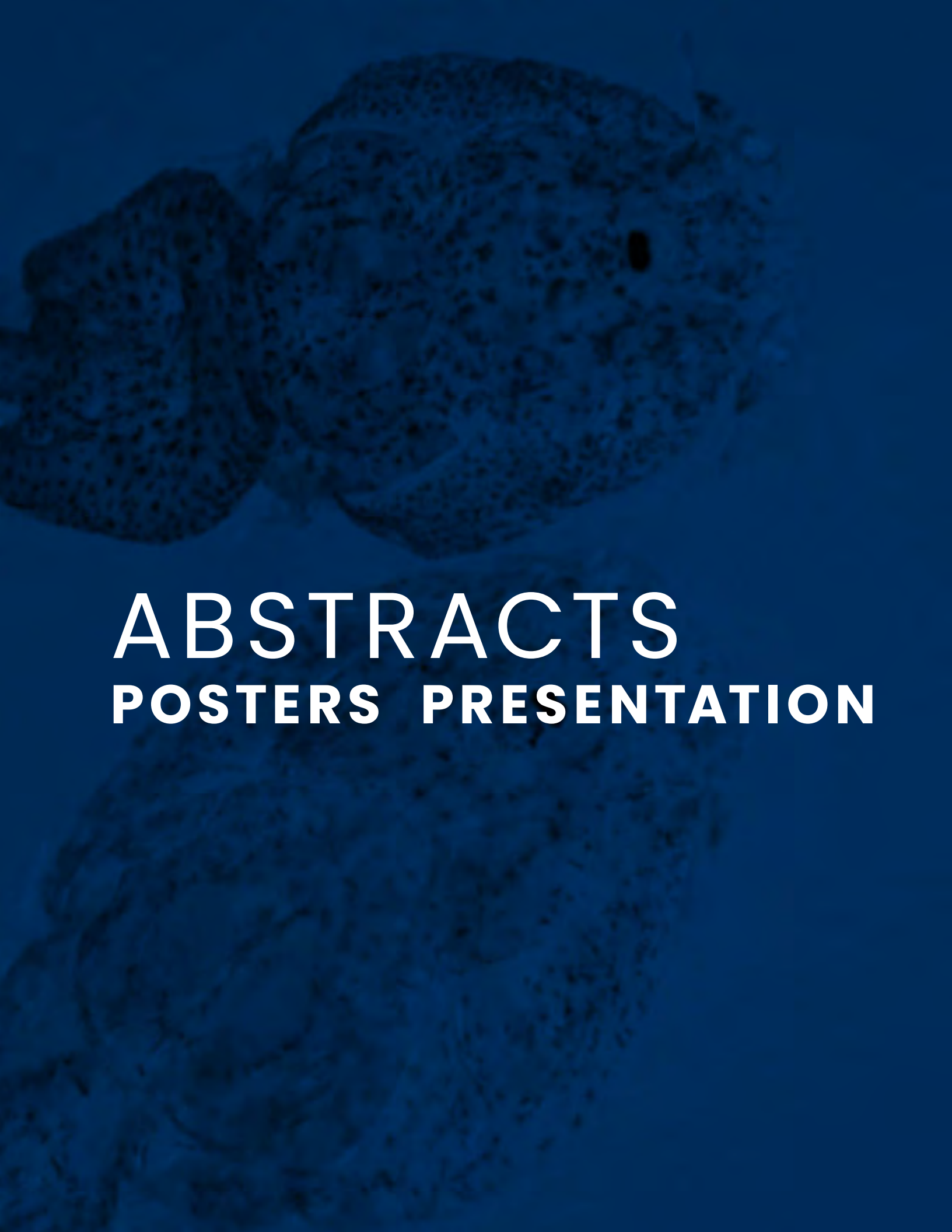
Ireland produces approximately 12,000 tonnes of Atlantic salmon annually, valued at €120 million. While modest by global standards, the sector is unique in that all production is certified organic, commanding premium prices and supporting rural coastal economies. Central to maintaining both fish health and certification standards is the management of *Lepeophtheirus salmonis* (salmon louse), a persistent parasitic threat to farmed Atlantic salmon. Since 1991, Ireland has implemented an independent sea lice monitoring programme (for both *L. salmonis* and *Caligus elongatus*) led by the Marine Institute, ensuring scientific integrity and transparency. This system has evolved through the introduction of a formal monitoring protocol (2000) and a national strategy (2007), with compliance embedded as a core condition of aquaculture licensing.

Drawing on over 30 years of monitoring data, this presentation explores long-term trends in salmon lice abundance and the influence of temporal, geographic, and environmental variables. It evaluates the effectiveness of key management strategies, including stakeholder-led 'management cells', treatment trigger thresholds, and the increasing use of non-medicinal control methods. The analysis also considers the unique challenges faced by Ireland's small-scale, organic-certified industry in maintaining low lice levels amid shifting environmental conditions, evolving EU regulations, and emerging international frameworks such as traffic light systems and risk-based management approaches.



ABSTRACTS
POSTERS PRESENTATION

FISH WELFARE

CRITICAL EVALUATION OF NOCICEPTIVE ASSESSMENT TOOLS USED TO ESTIMATE ANIMAL WELFARE IN ATLANTIC SALMON *(salmo salar)* INFESTED WITH *Caligus rogercresseyi*

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This study aimed to evaluate the relationship between the degree of animal welfare compromise—assessed through behavioral indicators of pain—and the severity of macroscopic and histopathological lesions in Atlantic salmon *(Salmo salar)* suffering from moderate to severe infestations of *Caligus rogercresseyi.*

A total of 60 clinically affected fish were selected from marine aquaculture sites in southern Chile. Welfare impairment was classified using a behavioral scoring system based on nociception-associated responses documented in existing literature. Macroscopic lesions were recorded, and skin and muscle samples were collected from various anatomical regions (head, abdomen, and flank), fixed in 10% buffered formalin, and processed for histopathological examination. Tissue sections were stained with hematoxylin and eosin and evaluated under light microscopy.

Fish identified as experiencing sustained pain exhibited multifocal cutaneous lesions, which included petechiae, ecchymosis, epidermal erosion, hemorrhages, and ulcerations. The histopathological analysis revealed epidermal disruption, chronic dermal inflammation, hypodermal involvement, diffuse hemorrhage in the underlying musculature, and necrotic foci. Fish displaying mild to moderate pain presented similar types of lesion but with differences in their distribution, extent, and severity. The findings suggest a lack of consistent correlation between behavioral pain scores and the severity of tissue damage. This discrepancy highlights the limited sensitivity of current behavioral assessment tools in accurately detecting pain intensity in *C. rogercresseyi*-infested salmon.

FISH WELFARE

TARGETING SEA LICE WITH AI AND REVERSE VACCINOLOGY: ORAL VACCINE ANTIGEN DISCOVERY FOR ATLANTIC SALMON

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The salmon louse *(Lepeophtheirus salmonis)* causes over 1 billion USD in annual global losses and limits growth in the UK's £1 billion salmon industry. There are major challenges with the current treatment regimens, and there is a need to develop more sustainable, vaccine-based control strategies to improve fish health and welfare.

The project aims to develop an *in silico* antigen screening platform for mucosal salmon louse vaccine candidates, using an artificial intelligence-aided reverse vaccinology (RV) approach.

Methodology: Our innovative approach will use reverse vaccinolo mgy to identify key biological targets utilising a bioinformatic predictive pipeline, using an artificial intelligence prediction platform to assist in the discovery of epitopes that can be recognised by fish immunoglobulins. Predicted epitopes will be integrated into a mucosal antigen-presenting scaffold carrying multiple antigen epitopes (EpitoGenX), allowing the rapid screening of vaccine candidates. Vaccine delivery and efficacy will be assessed on its ability to stimulate mucosal immunity and protection after a *L. salmonis* challenge.

A newly annotated *L. salmonis* genome assembly was generated, yielding 27,290 transcripts and 20,650 predicted proteins. SignalP 6.0 analysis identified 4,117 proteins with signal peptides, including 66 lipoproteins. Of those, 2,908 are predicted to be secreted into the extracellular space. Epitope prediction was performed using EpitoPredikt™. Ultimately, we aim to establish a vaccination strategy using the most efficacious antigens selected from pooled epitope groups, targeting a reduction of sea lice burden and/or fecundity in vaccinated fish.

WILD FISH INTERACTION

VALIDATING TOLERANCE LIMITS TO LICE IN WILD SALMON

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The spread of salmon lice *(Lepeophtheirus salmonis)* from farmed to wild salmon has been identified as one of the greatest threats to Norwegian wild populations of Atlantic salmon. Salmon farms are often located near the migration routes of wild post-smolts on their way to the sea. The early marine migration is a vulnerable life stage with high natural mortality rates, and additional mortality due to raised parasite load may therefore have large consequences for the overall marine survival of wild salmon populations. In Norway, estimates of lice-induced mortality in wild salmon post-smolts are used to regulate the salmon farming industry, and it is therefore important to have precise estimates of how lice infestation affects marine mortality in wild salmon. However, tolerance limits to lice have mainly been estimated in small-scale lab and field experiments using farmed or cultivated salmon, and we need more knowledge on how much lice wild salmon can tolerate before it has lethal or sublethal effects in the wild. In this study, the aim is to validate lice tolerance limits estimated in the lab and in situ experiments by investigating how variation in lice infestation pressure manifests in observed marine return rates in wild populations of Atlantic salmon. We will model observed return rates as a function of estimated lice infestation using data from 13 Norwegian rivers where the timing of out-migration and return of individual fish have been registered using PIT-tagging (10 rivers) or in-river traps (3 rivers).

WILD FISH INTERACTION

EVALUATING THE EFFECT OF SEA LICE FROM AQUACULTURE ON WILD SEA TROUT, THE ANADROMOUS BROWN TROUT

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Norwegian sea trout, the anadromous fraction of brown trout *(Salmo trutta)* populations, have severely declined during the last decades. Of 1251 Norwegian sea trout populations, almost 40% are in a poor or very poor state, or lost entirely. Among the anthropogenic factors affecting the state of sea trout populations, salmon lice from Atlantic salmon aquaculture have been found to have the largest negative impact. The Norwegian "traffic light system" regulates the production limits of the salmon aquaculture industry. However, the agreed system has not yet integrated the effect on other species than Atlantic salmon, such as sea trout and anadromous Arctic charr, due to poor effect indicators for these species.
Sea trout spend their marine feeding period in the same coastal waters as salmon aquaculture and are therefore very susceptible to lice infestations associated with aquaculture. Sea trout may respond to high lice infestations by prematurely returning to freshwater, thus the direct effects of lice infestations, besides increased marine mortality, will be reduced marine growth. At the population level, sea trout spawning biomass will be reduced.
We will here present a sea trout effect indicator that measures the negative impact from lice on the sea trout's reproductive success (fitness) by estimating lice-induced population change. This effect indicator, based on well documented knowledge of sea trout life history, the concept of mean individual fitness, and all available data will be an important tool to guide the sustainable management of Atlantic salmon aquaculture.

WILD FISH INTERACTION

INVESTIGATION OF SESSILE SEA LICE ON ATLANTIC SALMON POST-SMOLTS REVEALS SUBSTANTIAL PREDOMINANCE OF *Lepeophtheirus salmonis* COMPARED TO *Caligus elongatus*

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Atlantic salmon *Salmo salar* are exposed to parasitic sea lice as they enter the marine environment, particularly in areas with high densities of open net-pen salmon farming, where infestations can reach harmful levels. Given the correlation between infestations on farmed and wild fish, the highly specialized *Lepeophtheirus salmonis*, which often cause disease outbreaks in salmon farms, is considered the primary causal agent. However, this has not been validated, as sessile life stages of *L. salmonis* cannot easily be distinguished from other caligid sea lice species. Through diagnostic PCR analysis of sessile sea lice (n = 1 247) collected from wild Atlantic salmon post-smolt in Central and Western Norway, we document large disparity in the occurrence of *L. salmonis* (98 %) compared to *Caligus elongatus* (2 %); the other sea lice species found on salmonids in Norway. The occurrence of *L. salmonis* was substantially greater among sessile sea lice in all fjords (n = 6) and samplings (n = 9), and the contribution of C. elongatus only exceeded 3 % in the two samplings with the lowest prevalence and mean intensity of sea lice. Combined with the finding that sessile sea lice constituted 97 % of the total lice burden, this demonstrates that *L. salmonis* is the dominant caligid sea louse on wild Atlantic salmon post-smolts migrating through fjords in Central and Western Norway.

SEA LICE MONITORING ON PINK SALMON IN NORWAY

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In recent years, pink salmon *(Oncorhynchus gorbuscha)* have been returning in increasing numbers to rivers in Northern Norway, particularly in odd-numbered years. The ecological impacts of this invasion remain uncertain, including potential transmission of pathogens and parasites to native salmonids. In Norway, the salmon louse *(Lepeophtheirus salmonis)* is a major threat to wild salmonids, while *Caligus* elongatus is less common. Pink salmon are natural hosts for *L. salmonis,* and in Western Canada, mature pink salmon often carry heavy infestations. Returning pink salmon linger in bays and estuaries before entering rivers, potentially serving as hosts and vectors of sea lice to native species. Pink salmon were officially included in Norway's salmon lice monitoring program (NALO) in 2023, and the first official records of lice on this species are presented here. A total of 216 pink salmon were caught at sea using fyke nets at three sites in northern Norway in 2023. Both *L. salmonis* and C. elongatus were observed, with attached stages comprising ~6% of lice. *L. salmonis* prevalence ranged from 4–70% (mean intensity: 1–3.8 lice per fish, max: 17), while C. elongatus prevalence ranged from 1–96% (mean intensity: 1–9 lice per fish, max: 49). Notably, sea trout caught concurrently showed increased prevalence and intensity of both lice species compared to previous years. These findings suggest pink salmon could become an important source of sea lice for wild salmonids in Norway, underscoring the need for continued monitoring. Additional data from 2025, currently under collection, will also be presented.

WILD FISH INTERACTION

THE NORWEGIAN SALMON LICE SURVEILLANCE PROGRAM (NALO): METHODS AND FINDINGS FROM THE LAST DECADE OF MONITORING WILD SALMONIDS

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The Norwegian salmon lice surveillance program for wild salmonids (NALO) annually monitors the occurrence and intensity of salmon lice *(Lepeophtheirus salmonis)* on wild hosts. Established in response to concerns over the ecological impact of lice from aquaculture on wild populations of Atlantic salmon *(Salmo salar)*, sea trout (Salmo trutta), and Arctic char (Salvelinus alpinus), the program is currently conducted on behalf of the Norwegian Food Safety Authority and the Ministry of Industry and Fisheries.

NALO combines field monitoring and modeling to forecast lice pressure and identify high-risk areas and periods. Each year, teams systematically sample juvenile and adult salmonids in fjords and coastal areas using standardized methods, including pelagic trawling for migrating Atlantic salmon post-smolts, gill and trap nets for sea trout and Arctic char in littoral zones, and sentinel cages stocked with hatchery-reared smolts to assess infestation levels across spatial and temporal scales. Sampling is carried out at numerous coastal and riverine stations distributed along the Norwegian coastline. At each station, individual fish are examined, and lice counts are recorded.

Over the past decade, more than 16.000 salmon and 32.000 trout have been sampled at more than 40 different stations along the Norwegian coast, providing a robust dataset on temporal and spatial trends in lice infestation on wild salmonids. This long-term monitoring program has become a cornerstone of salmonid conservation and management in Norway, informing risk assessments and regulatory measures to mitigate aquaculture impacts on wild populations. Here, we present an overview of the program's methodology, spatial coverage, and sampling results from the last ten years.

SEA LICE BIOLOGY AND ECOLOGY

THE CONTRIBUTION OF HOST TRANSFER TO THE INFECTION DYNAMICS OF *Caligus elongatus AND Lepeophtheirus salmonis* IN A SALMON FARMING NETWORK

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Salmon lice *(Lepeophtheirus salmonis)* typically infect through the planktonic copepodite stage. However, high level of handling of farmed fish, where a portion of the mobile lice fall off, may induce host transfer if mobile lice survive and successfully find a new host. The other sea lice species affecting farmed salmon in the north Atlantic is the generalist *Caligus elongatus.* This species has different behavioural traits than salmon lice, and the relative contribution of host transfer to the overall infection pressure on farmed fish is not well understood.

In the Faroe Islands, third-party sea lice monitoring ensures consistent data collection across farms. These data are stored in a database, including farm specific information. Host transfer was identified when mobile or adult lice were found on newly deployed fish before development from a copepodite was possible, according to temperature-based development models for the two species.

Transfer of preadult and adult salmon lice between cages was highly unusual, with little impact on the overall epidemiology in farmed fish. In contrast, transmission of adult *C. elongatus* was common, accounting for ~44% of the total infections. *C. elongatus* abundances on farmed salmon were seasonal, and new infections were dominated by adult host transfer in July, when abundances started to increase. These contrasting transmission patterns highlight the need for species-specific management strategies and further research into the ecology and transmission dynamics of C. elongatus, which remain less well understood despite its significant role in infection pressure.

SEA LICE BIOLOGY AND ECOLOGY

SALMON LOUSE (*Lepeophtheirus salmonis*) FROM SUBMERGED SALMON FARMS: LARVAE DEVELOPMENT AND RESPONSE TO PRESSURE BETWEEN FAMILY GROUPS

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Infestations by the salmon louse *Lepeophtheirus salmonis* pose a serious threat to the salmon aquaculture industry. In recent years, cages submerged below 20 m depth have emerged as a seemingly effective measure to reduce infestation levels. Still, some infestation do occur, and history has demonstrated the ability of the lice to adapt to the measures against it. The infestation mechanisms at depth and the potential for the lice to adjust its behaviour to locate hosts in submerged cages more effectively is not known.
In this study, egg strings produced at depth were collected from submerged farms and hatched in the laboratory, and the development of the larvae was followed. Furthermore, for copepodites from 24 family groups, swimming behaviour was observed in an aquarium in a pressure tank at 0, 2.5 and 5 bar additional pressure. Each sibling group was observed separately to investigate if the response to increasing pressure differed between them.
Eggs produced at depth hatched and developed as normal, producing 128-233 active copepodites per egg string on average. Copepodites from different family groups mostly displayed clear negative barotaxis, moving upwards as pressure increased. However, the magnitude of the response was varying, and preliminary analyses indicate that some family groups did not change their position significantly between pressures, indicating that some may not be inclined to swim up and away from the deep cages.

EFFECTS OF IN VITRO pH VARIATION ON BIOLOGICAL-BEHAVIORAL ASPECTS OF SEA LICE *(Caligus rogercresseyi)*

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Chile is a major global aquaculture producer, with production focused mainly on salmonids, generating significant income through exports. However, intensive farming leads to infectious, viral, bacterial, and parasitic diseases. *Caligus rogercresseyi* is a parasite with a major economic impact on Chilean salmon farming, and controlling it is key to the industry's sustainability. The development of this parasite is related to a number of environmental factors, such as temperature, oxygen, and salinity, among others, although the effects of other variables such as pH, a relevant variable in the context of climate change, are unknown. The objective of this study was to determine the impact of environmental pH levels below 8.0 on *C. rogercresseyi* larvae under controlled conditions. This was done by exposing nauplius I, II, copepodite, and egg strings to a decreasing pH gradient, thereby determining the effects on parasite survival, hatching rate, and behavior in the face of ocean acidification. Exposure of *C. rogercresseyi* to pH <5.0 generates negative effects in the early stages of the parasite, reducing the hatching rate of females, the survival of nauplius and altering the behavior of copepodites. These results suggest that small pH variations (around pH 8.0) do not induce negative effects on the parasite larvae, which are tolerant to very acidic pH. To understand the effects of climate change on parasite biology, it is necessary to conduct studies with other variables associated with this climate event.

SEA LICE BIOLOGY AND ECOLOGY

RE-EVALUATING THE DEVELOPMENT RATE OF THE DIFFERENT STAGES OF *Caligus rogercresseyi* UNDER CONTROLLED CONDITIONS

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The sea louse *Caligus rogercresseyi,* recorded since 1997 infesting Atlantic salmon *(Salmo salar)* and rainbow trout *(Oncorhynchus mykiss)* reared in cages in seawater, is the major challenge to the Chilean salmon industry. The life cycle consists of three planktonic developmental stages, two nauplius and the infective copepodid, and four sessile stages (chalimus) on the host, before becoming adult. Although it has been widely documented that the development of the different stages of sea lice are dependent directly on water temperature, the objective of the study was to elucidate other factors besides temperature that could influence the development rate of *Caligus rogercresseyi.* In this way, data of the life cycle generated under controlled conditions in the periods 2007-2008 and 2016-2018, were analyzed. Results showed that in the free life stage, temperature controlled the development rate of each stage through both periods with no annual differences. The copepodid stage was reached in 5 days at 12°C (60 degree-days), while after the copepodid infestation on the fish, a difference around 70 degree-days less was recorded to reach the adult stage in the period 2016-2018 respect to 2007-2008. These differences could be explained by other additional factors.

SEA LICE BIOLOGY AND ECOLOGY

TRANSCRIPTOMIC AND MORPHOLOGICAL CHANGES TRIGGERED BY SALINITY AND TEMPERATURE IN *Caligus rogercresseyi* COPEPODITES

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Caligus rogercresseyi copepodite phase is responsible for host recognition and initiates the parasitic stages. The processes inherent to this phase can be affected by environmental variations. This study aimed to explore the environmental influence on the copepodite transcriptome and morphology during Atlantic salmon infestation under different salinity and temperature conditions. Two hundred salmon were infested with 35 copepodites per fish under four combined salinity and temperature conditions (32 and 26 PSU; 8 and 16 °C). After 48 hours of infestation, the attached copepodites were counted and collected for photographing under magnification for geometric morphometric and transcriptome analysis. Also, gene regulation by alternative splicing was analyzed. Fish in the 26PSU/16°C group showed the highest *Caligus* attachment rate, with 38.92%. Temperature-related morphological changes were observed. Copepodites cultured at 8°C displayed a streamlined body in the thorax region and elongation of the antennules toward the anterior region. Moreover, transcriptome analysis revealed modulation dependent on S/T conditions. Notably, there was a reduction in gene expression of cuticle proteins and trypsin in the 8°C-cultured groups. In the 32PSU/8°C and 26PSU/8°C groups, a lower enrichment of metabolic processes was observed. Furthermore, an increase in alternative splicing processes was evident in genes associated with growth and development in the 26PSU/16°C group. This study demonstrates the influence of environmental conditions on the molecular mechanisms occurring during the *C. rogercresseyi* copepodite stage.

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GENETICS AND GENOME

AQUACULTURE BIOTECHNOLOGY AND GENOMICS LABORATORY, 12 YEARS OF RESEARCH ON *Caligus rogercresseyi*

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The Aquaculture Biotechnology and Genomics Laboratory (LBGA) at the University of Concepción celebrates 12 years associated with the ectoparasite *Caligus rogercresseyi.* This period of intellectual and technical effort began in 2013 with *Caligus*-SEQ, the starting point for the implementation of future genomic tools in the study of the parasite. In 2014, the laboratory delved into the life stages of *Caligus*, generating relevant transcriptomic information. Between 2015 and 2017, the LBGA was designated by SERNAPESCA as a reference laboratory for *Caligus* research. With the aim of articulating national science around the parasite, the laboratory dedicated itself to characterizing and maintaining Caligus strains. Between 2018 and 2020, the laboratory explored the scope of *Caligus* non-coding RNAs. We report the expansion of the Caligidosis range to Magallanes and contribute to the study of drug resistance, emphasizing the importance of using genomic tools for vaccine development. We participated in the standardization of the minimum functional concentration of hydrogen peroxide for the removal of *Caligus.* Between 2021 and 2024, the LBGA published the complete genome of *Caligus rogercresseyi,* a baseline template for future research. Thanks to all the information gathered over the years, the laboratory published the IPath® vaccine, based on a chimeric protein that confers immunity to Caligus in Salmo salar.

Currently, the LBGA continues to research and work on tools to counteract the effects of Caligidosis in our country.

GENETICS AND GENOME

UNVEILING THE ROLE OF RNA EDITING AND lncRNA SPLICING AFTER INFESTATION WITH SEA LICE (*Caligus rogercresseyi*) IN ATLANTIC SALMON

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RNA editing is a process that happens either during or after the RNA is made, allowing some cells to change specific parts of the RNA molecule. Earlier research, in model organisms, has shown that RNA editing plays a key role in several diseases. However, its role in Caligidosis in salmonids is completely unknown. In this study, we looked at DNA/RNA sequencing data and found that 33. 5% of Atlantic salmon RNA muscle editing sites showed changes in their editing levels during parasite infestation. Also, RNA editing was more common at places where the RNA is cut and joined together, called splicing sites. We found that editing sites on long non-coding RNAs (lncRNAs) were more often at splicing sites compared to mRNA editing sites. This was shown by the odds ratio of 2. 35 for lncRNAs and 0. 45 for mRNAs. In addition, the rate of RNA editing on lncRNAs was closely related to how much of the lncRNA is spliced, as shown by the high correlation coefficient of 0. 77. This suggests that RNA editing might help control how Atlantic salmon lncRNAs are spliced during parasite infestation. Overall, this study offers new information about the role of RNA editing on the regulation of lncRNAs in salmonids under Caligidosis.

PHARMACOLOGICAL TREATMENTS

EFFECTS OF CYPERMETHRIN AND DELTAMETHRIN ON HATCHING AND SURVIVAL OF SEA LICE LARVAE OF *Caligus rogercresseyi*

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The welfare of salmon in aquaculture is crucial for good production, being Caligus rogercresseyi the main parasite that disrupts this welfare, causing lesions and immunosuppressing the host. Pharmacological is one of the most emblematic problems in recent times due to the resistance that this parasite has produced. Among these drugs is the pyrethroid family (deltamethrin and cypermethrin). Some studies have shown that adult parasites show some loss of sensitivity to this family. However, there are insufficient studies on the effects on juvenile and larval stages. The present study aimed to elucidate the effects of pyrethroids on hatching and survival of larval stages of C. rogercresseyi. We sought to determine toxicity in nauplius I, evaluate survival in nauplius II and copepodite, identify the most affected stage and compare survival between the different stages. For this purpose, individuals of *Caligus rogercresseyi* were collected from salmon farms of Los Lagos region, Chile, selecting females with mature ovigerous sacs and classifying larval stages (nauplius I, II and copepodite). Individuals were distributed in culture plates and applying deltamethrin and cypermethrin solutions in concentration gradients. Effects were assessed by classifying individuals as live, moribund or dead, and statistically analyzed to determine the impact per larval stage. It was observed that cypermethrin and deltamethrin did not prevent hatching of the egg strings but did affect larval survival of the parasite at nauplius I stage. It was found that copepodites were more sensitive to deltamethrin, while cypermethrin had a similar effect on both stages (nauplius II and copepodite).

NON-PHARMACOLOGICAL TREATMENTS

DERIVATION OF A THERAPEUTIC DOSE FOR A NOVEL PARASITICIDE'S USE IN PIVOTAL FIELD TRIALS

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Ectoparasite infestations, especially by *Lepeophtheirus salmonis*, remain a persistent challenge in salmonid aquaculture. This report presents the structured dose titration program used to derive an effective and safe dose of an in-feed treatment for a novel isoxazoline parasiticide (S-Roxapin), recently granted US patent protection. The approach followed a stepwise empirical design to identify the lowest non and efficacious dose. An initial laboratory tank study explored a range of sub-optimal doses. Subsequent refinement demonstrated near-total parasite clearance at 0.025, 0.05, and 0.125 mg/kg/day, while confirming reduced efficacy (< 90%) at 0.010 mg/kg/day, thus establishing the therapeutic threshold. A third study, to evaluate duration of efficacy, revealed >99% reduction in lice burden up to 8 days post-treatment when administered at 0.06 mg/kg/day for 7 consecutive days. This regimen was effective against all parasitic life stages and confirmed as safe with a high safety margin. This dose determination strategy culminated in the identification of a therapeutic window significantly lower than that of earlier compounds in the same class. The findings were unexpected based on known structure-activity relationships and are now protected by US Patent No. 12,280,040. The therapeutic dose will underpin pivotal field trials under commercial conditions.

NON-PHARMACOLOGICAL TREATMENTS

COMPARATIVE SENSITIVITY OF *Caligus rogercresseyi* TO LYPTUS PLUS®, ROSSEUS PLUS® AND HYDROGEN PEROXIDE

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Pharmacovigilance through bioassays of *Caligus rogercresseyi* sensitivity to chemicals and other control measures is essential to detect changes before observing a reduction in clinical efficacy. This study compares sensitivity of *C. rogercresseyi* to Lyptus Plus®, Rosseus Plus®, and hydrogen peroxide (HP), having different potency, using bioassays according to SERNAPESCA Technical Standard No6. Adults were collected from an Atlantic salmon farm. Bioassays were conducted using seven concentrations and three replicates (five males and five females). Concentration affecting 50% of exposed individuals (EC50) and that affecting 100% of individuals were estimated at 1- and 24-h post-exposure (1h and 24h pe, respectively) along with relative potency.
EC for Lyptus Plus® was 104.7ppb and 201.5ppb (1 and 24h pe, respectively), affecting 100% of parasites at 200ppb (1h pe) and greater 24h pe, and presenting a temporary sensitivity loss (92% of increase between both exposure times). EC for Rosseus Plus® was 40.6ppm and 36.3ppm (1 and 24h pe, respectively), affecting 100% of parasites at 15ppm at both evaluations, and showing a temporary cumulative effect (11% of decrease between both exposure times). EC for HP was 752.6ppm (1h pe) and 1465ppm (24h pe), requiring more than 3000ppm to affect 100% of individuals and showing a marked temporary loss of efficacy (95% increase between both exposure times) and evidence of recovery. The study revealed potency differences spanning orders of magnitude with distinct temporal patterns and provides stratified therapeutic options for integrated parasite management. It contributes to national pharmacovigilance systems, since these products have not yet been included.
Acknowledgements to Tirsa Valenzuela, Nitza Vera, and Oscar de Lázaro from the Laboratory of Ecological Interactions, Institute of Aquaculture and Environment, Universidad Austral de Chile (UACh) for the collaboration during the implementation of the bioassays.

EVALUATION OF *P. pastoris* EXPRESSED IPATH INCORPORATION IN ATLANTIC SALMON FEED AS A SEA LICE CONTROL METHOD

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Due to the widespread use of antiparasitic treatments, sea lice have developed resistance to these control methods. As a result, research efforts are continuously directed toward developing vaccines as a sustainable solution. Previously, the INCAR research group demonstrated that the Ipath® recombinant vaccine effectively controls sea lice when administered intraperitoneally. However, the high cost of recombinant protein purification limits its widespread application. In this study, the impact of adding P. pastoris yeast expressing the Ipath® protein into salmon feed was examined, along with its effectiveness as an immune stimulant in Atlantic salmon for sea lice control. Therefore, Atlantic salmon were fed a formulated pellet for one month and then challenged with sea *Caligus rogercresseyi.* Differences in the number of sea lice per fish were observed in salmon immunized with P. pastoris+Ipath®. Additionally, changes in immune gene expression were recorded between salmon immunized with P. pastoris+Ipath® and the control group. Notably, a high level of IgT gene expression was observed in the immunized salmon. Our study presents a promising strategy for sea lice control using modified yeast integrated into salmon feed, which could help reduce stress during sea lice treatments.

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NON-PHARMACOLOGICAL TREATMENTS

EFFICACY OF DIFFERENT TREATMENTS AGAINST CALIGUS: A CHILEAN FIELD EXPERIENCE

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This work presents the analysis of a field case study performed during four months in 2024 comparing the efficacy of six treatment against *Caligus rogercresseyi* infestation levels in a seawater cages located in the X Region, Chile. Underwater camera footage was used to monitor Caligus levels, specifically counting female Caligus and total adult Caligus daily. The selection of the type of product used and application of it were performed by the producer´s health team and producer´s site personnel respectively.
Camera-based counts before and after treatment indicated variability in treatment effectiveness, with some treatments demonstrating higher efficiency than others. Additionally, the efficacy of some products varied between applications.
Our results suggest that the performance of Caligus control treatments is not consistent across different products, and even repeated applications of the same product can yield variable results. Further research is needed to understand the underlying causes of such variability. Factors such as incorrect dosage, bath duration, or potential resistance development in lice populations may contribute to reduced treatment effectiveness and inconsistent outcomes.

NON-PHARMACOLOGICAL TREATMENTS

BOTANICAL-BASED STRATEGIES FOR SEA LICE (*Lepeophtheirus salmonis*) CONTROL: IN VITRO AND IN VIVO ASSESSMENT IN ATLANTIC SALMON

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Norway's salmon farming industry, a major contributor to global aquaculture, faces persistent challenges from *Lepeophtheirus salmonis*, a parasitic causing significant economic losses and health impacts in Atlantic salmon *(Salmo salar).* Conventional control measures are increasingly limited by parasite resistance and their environmental impact.
The objective was to evaluate the antiparasitic efficacy of a botanical supplement (Rosseus Plus®), in Atlantic salmon infested with *Lepeophtheirus salmonis,* through therapeutic baths.
An in vitro trial was conducted at 12 °C to test three concentrations (5, 7,5, and 10 ppm) against mobile and preadult stages of both sexes. Parasite mortality was assessed at 0, 90, 120 minutes, and 24 hours post-exposure. For the in vivo trial, the same three concentrations were evaluated through baths on a sample of Atlantic salmon.
The same formula was used to calculate efficacy for both trials.
At 5 ppm, efficacy values were 55%, 50%, 50%, and 45%. At 7.5 ppm: 95%, 84%, 84%, and 79.5%, while at 10 ppm: 100%, 100%, 95%, and 95%. For the timepoints of 0, 90, 120 minutes, and 24 hours respectively.
In vivo evaluation confirmed that 7.5 and 10 ppm doses maintained over 90% efficacy and were well tolerated by fish.
These findings indicate that botanical supplement, provides high, sustained antiparasitic activity at safe concentration. Its botanical origin and lack of observed adverse effects make it a promising tool for integrated parasite management. Incorporating Rosseus Plus® into treatment protocols could reduce reliance on synthetic chemotherapeutants, support fish welfare, and mitigate environmental impacts in salmon aquaculture.

NON-PHARMACOLOGICAL TREATMENTS

NON-PHARMACOLOGICAL APPROACHES AGAINST *Caligus rogercresseyi* AND THEIR IMPACT ON HEALTH AND ANIMAL WELFARE IN ATLANTIC SALMON

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Global aquatic animal production has increased. In Chile, Atlantic salmon *(Salmo salar)* dominates the export-oriented aquaculture industry but faces challenges, especially caligidosis caused by the ectoparasite *Caligus rogercresseyi.* This parasite disrupts immune, neuroendocrine, and metabolic functions, reducing fish welfare and causing economic losses. Concerns over drug resistance, environmental impact, and animal welfare have driven interest in sustainable, non-pharmacological alternatives.
This study assessed the antiparasitic efficacy and physiological benefits of Rosseus Plus®, a botanical supplement, administered via therapeutic baths and water exposure.
In vitro analyses were carried out at 12 different concentrations, and in vivo trials were conducted at Atlantic salmon farming sites, obtaining samples at different timepoints (0, 24, 48 y 120 hours).
In vitro assays showed 93–100% effectiveness against adult *C. rogercresseyi,* while field trials using wellboats achieved parasite reductions of 98% in adults and 85% in juveniles. Waterborne delivery enhanced immune responses, lowered stress markers, and improved the intake of the feeding and the growth.
Molecular, histological, and physiological analyses revealed significant reduction in acute and chronic stress, mitigation of oxidative stress, lowering inflammation in gill and skin. Treated fish maintained stable cortisol levels, preserved lysozyme activity, uncompromised immune response, balanced osmoregulation and consistent serotonin concentrations, indicating improved neuroendocrine balance and welfare. The inclusion of antioxidant, serotonergic biomarkers provides a robust, science-based framework to monitor fish resilience and welfare, offering precise evaluation of health and treatment effects.
The study supports to Rosseus Plus® as a safe and effective solution for integrated parasite management in Atlantic salmon aquaculture.

NON-PHARMACOLOGICAL TREATMENTS

EVALUATION OF FIVE BOTANICAL FORMULATIONS WITH ANTIPARASITIC EFFECT AGAINST *Caligus rogercresseyi* VIA FEED

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The sea lice *Caligus rogercresseyi* is one of the most significant ectoparasites affecting Chilean salmon aquaculture, causing severe economic and welfare impacts.
This study evaluated the antiparasitic efficacy of five hybrid formulations (S15, S17, S18, S20, and S21) incorporated as dietary additives for *Salmo salar* under controlled laboratory conditions.
Atlantic salmon were maintained at 12 °C in tanks at a density of 12 kg/m³ and fed experimental diets for 42 days. Two parasite challenges were conducted: Infestation I, with 20 adult lice per fish before diet administration, and Infestation II, at 27 days of diet ingestion (DDI), with 35 copepodites per fish. Parasite detachment was monitored daily, and relative control effectiveness was calculated for adult and chalimus stages at defined sampling points. At 10 days, treatments S17, S18, S20, and S21 showed 19–21% adult detachment. In adult stages, S18 and S21 reached 76% and 72% control at 20 DDI:39 DPI, respectively. Against chalimus I–II (27 DDI:7 DPI (post infestation days)), effectiveness ranged from 72.4% (S15) to 86% (S18). Against chalimus III–IV (34 DDI:14 DPI), S18 and S21 achieved the highest control rates (97.8% and 97.3%, respectively).
No significant differences in growth or feed conversion ratios were observed between treated and control groups. The results indicate that the compounds, particularly S18, can achieve over 98% reduction across parasite stages without compromising fish performance.
These findings support the use of dietary hybrids additives as a preventive and sustainable strategy for integrated sea lice management in salmon aquaculture.

NON-PHARMACOLOGICAL TREATMENTS

TRANSCRIPTOME SURVEY OF PRECISION PROBIOTICS AGAINST *Caligus rogercresseyi*

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In all organisms, health balance is related to the interaction between the immune system and its microbiota. Sea lice microbiota play a symbiotic role in the organism, facilitating metabolic processes throughout the life cycle. When this balance is disrupted, it can lead to a weakening of the body's immune system. The use of precision probiotics against sea lice can cause dysbiosis, weakening the host's microbiome and increasing the severity of infestation. The objective of this study was to evaluate the transcriptomic response of genes involved in the success and reproduction of *C. rogercresseyi* infestation in Atlantic salmon in response to the use of precision probiotics. In this study, salmon fed with the precision probiotic every 21 days were infected in a tank with 35 copepodids per fish. Twenty-five days later, adult sea lice were counted and collected for qPCR analysis. A significant reduction in sea lice was observed in salmon fed with precision probiotics, along with phenotypic changes. Expression levels of genes associated with reproduction in sea lice exposed to salmon fed with the probiotic also changed. Additionally, an overexpression of secretome-related and ROS-related genes was observed in male sea lice obtained from salmon fed with the probiotic. This study demonstrates that using precision probiotics against sea lice induces molecular changes that diminish the success of sea lice infestations.

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MODELING DYNAMICS OF ADULT FEMALE LICE AT SALMON FARMING SITES IN EASTERN CANADA: A STOCHASTIC, STATE-BASED APPROACH

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A stochastic, state-based, time-dependent epidemiological model was developed to assess the dynamics of adult female sea lice *(Lepeophtheirus salmonis)* infestations in Atlantic salmon farms in New Brunswick, Canada. The model incorporated farming practices (stocking week, production cycle, and treatment timing), environmental covariates (sea surface temperature and seasonality), and spatial connectivity based on seaway distance among 57 farming sites. Weekly transitions among infestation states (free, low, high, and recovered) were modeled using Markov-chain methods, with treatment effects integrated as state-dependent modifiers. Exploratory analyses highlighted the relationship between outbreak-to-treatment timing and recovery, showing that immediate treatments were associated with higher recovery rates, whereas delayed treatments reduced effectiveness. Model results revealed that spring and summer stocking increased the probability of high infestation levels, while reduced winter treatments contributed to elevated sea lice concentrations. Furthermore, treatment outcomes seems to be often undermined by continued waterborne transmission from neighboring farms, underscoring the importance of coordinated management. Predictive accuracy averaged 59%, reaching up to 74% in the initial 10 weeks post-stocking. Limitations included reliance on seaway distance as a proxy for hydrodynamic connectivity and the absence of data on salinity, fish growth, and farm density. This modeling framework advances understanding of infestation dynamics and supports development of improved strategies for sea lice management in Eastern Canadian aquaculture.

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