IDENTIFYING ENVIRONMENTAL AND PRODUCTION RELATED RISK FACTORS ASSOCIATED WITH ULCER OUTBREAKS IN SALMON FARMING

RISKIDENT: 901837

Final report – 26 February 2026

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1 Summary

1.1 Project organization – FHF901837

Project group

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1.2 Main findings

This report identifies critical risk factors associated with winter ulcer disease outbreaks in Norwegian salmon aquaculture, combining large-scale commercial data analysis, controlled laboratory experiments, and longitudinal field monitoring to provide actionable insights for disease mitigation.

1. Temperature and salinity are the primary environmental determinants of *Moritella viscosa and Tenacibaculum sp.* abundance.

From the commercial experiment, *M. viscosa* was only detected at temperatures < 10°C and salinities > 30 ppt salinity. *Tenacibaculum spp*. showed broader tolerance, but severe ulcers occurred exclusively at temperatures <10°C and salinities >30 ppt. These thresholds provide clear indicators for high-risk periods.

Chlorophyll, turbidity, and phycoerythrin sensors showed no correlation with pathogen abundance or ulcer severity, indicating these are not useful indicators for winter ulcer disease.

2. Environmental conditions significantly impact wound healing.

Experimentally, low dissolved oxygen and high current speeds significantly delayed wound healing. Reduced salinity accelerated healing and led to reduced wound size by 7 days after injury. Sub-optimal environmental conditions can interact synergistically.

3. Repeated handling (4+ events) poses high ulcer and mortality risk regardless of method or temperature.

Analysis of 452 commercial handling events revealed that wound frequency remained stable after first and second handling events but increased markedly after the fourth event. This cumulative stress effect demonstrates the importance of minimizing handling frequency through use of louse preventative strategies.

4. Thermal and mechanical delousing methods increase mortality risk but minimally impact wound scores.

While chemical methods (freshwater, medicinal baths) resulted in slightly elevated wound frequencies after repeated handling, physical removal methods (thermal, mechanical) showed higher mortality rates post-treatment regardless of past experience. This suggests that physical methods lead to mortality in already compromised individuals, while chemical methods allow survival but with an increased risk of ulcer development.

5. Environmentally responsive lice prevention can also reduce winter ulcer disease.

A commercial-scale trial demonstrated that environmentally-responsive use of skirt barriers, oxygenation and behavior modification using lights and feeding delayed the need for first delousing by 2 months and reduced delousing frequency by 50%. Fish in cages using the dynamic strategy maintained consistently lower wound scores compared to controls, even before the first delousing. Similarly, ulcer causing agent abundance (*M. viscosa & Tenacibaculum spp*.) was lower in both water samples and skin mucous swabs in dynamic cages compared to controls.

1.3 Summary

The RiskIdent project represents a multi-faceted investigation into winter ulcer disease in Norwegian Atlantic salmon aquaculture, examining the complex interactions between environmental conditions, farm management practices, and host susceptibility. This study employed three complementary approaches: analysis of 452 commercial handling events across farms in northern Norway, controlled laboratory experiments examining wound healing under varied environmental conditions, and longitudinal monitoring of pathogen abundance and ulcer severity and prevalence at a commercial farm over 16 months. The research addresses the persistent challenge that ulcerative disease remains among the top five health concerns despite widespread vaccination against *Moritella viscosa*, indicating that effective prevention requires understanding the broader context of when and why salmon become susceptible to infection.

Commercial data analysis revealed that handling frequency significantly affects ulcer risk. While first and second handling events showed no increase in wound prevalence, wound frequency increased after the fourth event, demonstrating cumulative stress effects. The relationship between delousing method and outcomes proved nuanced: physical removal methods (thermal and mechanical) were associated with elevated mortality but were preferentially used when fish were in better condition, while chemical methods (freshwater baths and medicinal treatments) showed slight increases in wound frequency following treatment but lower mortality. This suggests physical methods may select for mortality in vulnerable individuals, while chemical methods allow survival but with increased susceptibility to winter ulcer disease. Results were consistent even when considering triploid salmon, generally considered to be more susceptible to wound development.

The longitudinal study established temperature and salinity as the primary correlates with pathogen presence and virulence. *M. viscosa* was detected exclusively when water temperatures fell below 10°C and disappeared completely at salinities less than 30 ppt, appearing in autumn and persisting through winter. *Tenacibaculum* spp. showed broader tolerance (6-19°C throughout most of the year), but severe ulcer scores occurred exclusively at temperatures below 10°C and salinities above 30 ppt. An exponential relationship emerged between *Tenacibaculum sp.* load and ulcer severity, with total ulcer scores increasing rapidly when qPCR cycle threshold values fell below 25. M. viscosa load correlated directly with ulcer severity, with lower Ct values associated with increased ulcer severity. Chlorophyll concentration, turbidity, and phycoerythrin showed no correlation with pathogen abundance (water or skin mucous) or ulcer prevalence, indicating these parameters have no predictive value for winter ulcer risk.

A dynamic lice prevention strategy was evaluated using test cages equipped with 10 m skirt barriers, automated aeration/oxygenation systems, and submersible lights and feeding equipment, with deployment driven by real-time environmental data. The strategy deployed skirts and aeration when salinity was uniform to shield fish from surface waters with high copepodid densities, while raising equipment during brackish layer presence to guide fish into low-salinity surface waters. When properly implemented, test cages required half as many delousing treatments as controls, maintained consistently lower wound scores, and had reduced abundance of *M. viscosa* and *Tenacibaculum spp*. in both skin mucous and water samples. Critically however, increased severity of Amoebic gill disease in incompletely prepared test cages early in the trial highlight the importance of deploying the dynamic strategy in full, as a package, to minimize the risk of poor gill health outcomes. Effectiveness in this experiment was limited by implementation challenges including initial lack of submerged feeding equipment and discontinuation during a mass *Apolemia* sp. jellyfish aggregation.

Controlled experiments demonstrated that environmental factors can significantly impact wound healing in Atlantic salmon. The highest current speed and lowest oxygen saturation (60%) led to the largest wound areas, which were 37 and 57% greater than in the control on day 7, respectively. Within the photoperiod groups, long-day had the largest wound sizes on all days, and the greatest relative difference to the control when the wound was significantly 70% larger on day 28. Wound area tended to be negatively associated with salinity from day 7 onwards, and on days 7 and 14 this was significant with the 22 ppt group having 20 and 22% smaller wound areas than the controls, respectively. These findings demonstrate how environmental stressors compromise host resilience even in the absence of pathogen exposure.

The integration of findings enabled development of evidence-based guidelines for risk-controlled production planning. Specific quantifiable thresholds define high-risk periods: water temperatures below 10°C combined with salinities above 30 ppt and dissolved oxygen concentrations less than 8 mg/L create conditions where pathogen abundance is elevated and host healing capacity is compromised. Real-time environment monitoring at 5m inside cages is critical to accurately assessing risk.

The strongest risk predictor with regards to delousing that we identified was repeated handling. Regardless of method and temperature, the first and second delousings are relatively low risk while four or more present high risk of both ulcer development and mortality. Thus, although no louse prevention strategies are 100 % effective, use of any strategy which can reduce delousing events to 3 or less will considerably reduce the risk of winter ulcer disease. The dynamic strategy described herein both delayed and reduced the need for delousing, while maintenance of optimal oxygen conditions and increased residence in the brackish water layer reduced ulcer prevalence.

Strategic use of chemical delousing methods (medicinal or freshwater) when high-risk cold water delousing is unavoidable can reduce the risk of poor outcomes (mortality or winter ulcer disease). Similarly, supplemental oxygenation in the 14 days following delousing can support wound healing, minimizing the window of susceptibility to infection. Feeding and lights can also be used to encourage fish into the surface brackish layer (28ppt or less at 5 m), when present, where *Tenacibaculum sp*. and *M. viscosa* do not thrive.

Apolemia sp. jellyfish are a major risk factor for ulcer development, even in low densities. Standardized, daily, on-site monitoring from August through February will facilitate rapid response. A 10 m skirt barrier with aeration, at a site with no brackish layer, reduced entry of *Apolemia sp.* fragments to the cage by 63%. This exact setup will not work on all farms in all conditions (Ex. *Apolemia* shift deeper when a brackish layer is present), but demonstrates that a barrier strategy adapted to local conditions can provide real protection.

These results provide immediately implementable knowledge for reducing ulcer disease through risk-controlled production, enabled by environmental and operational optimization.

1.4 Norsk sammendrag

RiskIdent-prosjektet representerer en flersidig undersøkelse av vintersår i norsk lakseoppdrett, der komplekse samspill mellom miljøforhold, driftspraksis og vertens mottakelighet blir analysert. Studien benyttet tre komplementære tilnærminger: analyse av 452 kommersielle håndteringshendelser ved oppdrettsanlegg i Nord-Norge, kontrollerte laboratorieforsøk som undersøkte sårheling under varierende miljøforhold, samt longitudinell overvåking av patogenforekomst og sårgrad og -prevalens ved et kommersielt anlegg over 16 måneder. Forskningen tar for seg den vedvarende utfordringen med at vintersår fortsatt er blant de fem største helseproblemene i lakseoppdrett til tross for utbredt vaksinering mot *Moritella viscosa*, noe som indikerer at effektiv forebygging krever en bredere forståelser når og hvorfor laks blir mottakelig for infeksjon.

Analysen av kommersielle data viste at håndteringsfrekvens har betydelig innvirkning på risikoen for å utvikle sår. Mens første og andre håndtering ikke viste økning i sårprevalens, økte sårfrekvensen etter fjerde hendelse, noe som demonstrerer kumulative stresseffekter. Sammenhengen mellom avlusningsmetode og utfall viste seg å være nyansert: mekaniske metoder (termisk og mekanisk) var assosiert med økt dødelighet, men ble fortrinnsvis brukt når fisken var i bedre kondisjon, mens medikamentelle metoder (ferskvannsbad og medikamentelle behandlinger) viste en svak økning i sårfrekvens etter behandling, men lavere dødelighet. Dette tyder på at mekaniske metoder kan selektere for dødelighet hos sårbare individer, mens medikamentelle metoder tillater overlevelse, men med økt mottakelighet for vintersår. Resultatene var konsistente også når triploid laks. generelt ansett som mer mottakelig for sårutvikling, ble inkludert.

Den longitudinelle studien identifiserte temperatur og saltholdighet til å være de primære faktorene som korrelert med tilstedeværelse av patogen og virulens. *M. viscosa* ble utelukkende påvist når vanntemperaturen falt under 10 °C og forsvant fullstendig ved salinitet under 30 ppt, med hovedsakelig forekomst om høsten og gjennom vinteren. *Tenacibaculum sp.* viste bredere toleranse for vanntemperatur (funnet ved 6–19 °C gjennom store deler av året), men alvorlige og høye skårer for sår forekom kun ved temperaturer under 10 °C og salinitet over 30 ppt. Det ble observert en eksponentiell sammenheng mellom mengde *Tenacibaculum sp.* og sårgrad, der total skår økte raskt når qPCR Ct-verdier falt under 25.

Mengde *M. viscosa* korrelerte direkte med sårgrad, der lavere Ct-verdier var forbundet med økt sårgrad. Klorofyllkonsentrasjon, turbiditet og fykoerytrin viste ingen sammenheng med mengde patogen (i vann eller hudslim) eller sårprevalens, noe som indikerer at disse parameterne ikke har prediktiv verdi for risiko for vintersår.

En dynamisk preventiv strategi for forebygging av lus ble evaluert ved bruk av testmerder utstyrt med 10 m dype skjørt, automatiserte luftings-/oksygeneringssystemer samt nedsenkbart lys- og fôringsutstyr. Implementeringen av det ulike utstyret ble styrt med hjelp av sanntidsmålinger av miljødata. Strategien innebar bruk av skjørt og lufting når saltholdigheten var jevn gjennom hele vannmassen for å skjerme fisken fra overflatevann med høy tetthet av kopepoditter, samt heving av utstyr ved tilstedeværelse av brakkvannslag for å lede fisken inn i overflatevann med lav saltholdighet. Når strategien ble korrekt implementert, krevde testmerdene halvparten så mange avlusninger som kontrollmerdene, opprettholdt gjennomgående lavere sår skår og hadde redusert forekomst av M. viscosa og *Tenacibaculum sp*. både i hudslim og i vannprøver. Det viste seg imidlertid at når den dynamiske strategien ikke ble implementert korrekt i en tidlig fase av forsøket forkom det økt alvorlighetsgrad av amøbegjellesykdom. Dette viste tydelig viktigheten av å implementere den dynamiske strategien i sin helhet, som en samlet pakke, for å minimere risiko for dårlig gjellehelse. Effektiviteten i dette forsøket ble begrenset av tidlige utfordringer med korrekt implementering av den dynamiske strategien, inkludert mangel på nedsenkbart fôringsutstyr, samt avbrudd i bruk av dynamisk strategiunder en masseforekomst av *Apolemia sp.*-maneter mot slutten av forsøket.

Kontrollerte labforsøk demonstrerte at miljøfaktorer kan ha betydelig innvirkning på sårheling hos atlantisk laks. Høy strømhastighet og lav oksygenmetning (60 %) ga størst sårareal, henholdsvis 37 og 57 % større enn hos kontrollfisken etter 7 dager. Innen lysgruppene hadde gruppen med 24 t kontinuerlig lys størst sårareal på alle prøvetakingstidspunkt, og med størst relativforskjell fra kontrollgruppen da såret var 70 % større på dag 28. Sårareal viste seg å ha en negativ trend sammen med saltholdighet fra dag 7 og utover,, og på dag 7 og 14 var dette signifikant, der gruppen som ble holdt i 22 ppt vann hadde henholdsvis 20 og 22 % mindre sårareal enn kontrollgruppene. Disse funnene viser hvordan miljøstressorer svekker vertens robusthet selv i fravær av patogener.

Kombinering av alle disse funnene muliggjorde for en utvikling av kunnskapsbaserte retningslinjer for risikokontrollert produksjonsplanlegging. Høyrisikoperioder for sårutvikling kan derfor bli definert som: vanntemperatur under 10 °C kombinert med salinitet over 30 ppt og oppløst oksygen under 8 mg/L som skaper forhold der patogenmengden er forhøyet og vertens helingskapasitet er svekket. Sanntids overvåking av miljøforhold på 5 m dyp inne i merdene vil derfor være avgjørende for korrekt risikovurdering.

Den høyeste risikofaktoren knyttet til avlusning var gjentatt håndtering. Uavhengig av metode og temperatur hadde første og andre avlusning relativ lav risiko, mens etter fire eller flere ble det betydelig høyere risiko for både sårutvikling og dødelighet. Selv om ingen forebyggende strategier mot lus er 100 % effektive, vil bruk av enhver strategi som kan redusere antall avlusninger til tre eller færre, betydelig redusere risikoen for vintersår. Den dynamiske strategien som er beskrevet her, både forsinket og reduserte behovet for avlusning, samtidig som opprettholdelse av gunstige oksygenforhold og økt opphold i brakkvannslaget reduserte sårprevalens.

Strategisk bruk av medikamentelle avlusningsmetoder (medikamentelle eller ferskvannsbehandlinger) når avlusning i kaldt vann med høy risiko er uunngåelig, kan redusere risikoen for dårlige utfall (dødelighet eller vintersår). Tilsvarende kan ekstra oksygenering i 14 dagene etter avlusning støtte sårheling og dermed minimere perioden med mottakelighet for infeksjon. Fôring og lys kan også brukes for å lede fisken inn i brakkvannslag (28 ppt eller lavere på 5 m) når dette er til stede, hvor *Tenacibaculum sp*. og *M. viscosa* ikke trives.

*Apolemia s*p. maneter er en betydelig risikofaktor for sårutvikling, selv ved lave tettheter. Standardisert, daglig overvåking av disse fra august til februar vil muliggjøre rask respons. Et 10 m dypt skjørt med lufting, på en lokalitet uten brakkvannslag, reduserte inntrenging av *Apolemia sp*.-fragmenter i merden med 63 %. Denne løsningen vil ikke fungere på alle lokaliteter eller under alle forhold (for eksempel trekker *Apolemia* seg dypere når brakkvannslag er til stede), men demonstrerer at en barrierestrategi tilpasset lokale forhold kan gi reell beskyttelse mot manetene.

Disse resultatene gir nyttig kunnskap som kan implementeres umiddelbart i næringen for å redusere vintersår gjennom risiko-kontrollert produksjon, ved å optimalisere miljøovervåking og driftspraksis.

2 Introduction

2.1 Background

Ulcerative disease in salmon is not caused by a single pathogen, but rather is the result of opportunistic bacterial infections when hosts have limited ability to fend off infection. As a result, focusing preventive efforts on pathogens without considering the wider context of host susceptibility can only have limited impact. This point is exemplified by the case of classical 'winter-ulcers' which remain among the top 5 health concerns of the Norwegian salmon industry despite most fish being vaccinated against *Moritella viscosa*, the aetiological agent [1]. In this project we propose a 'back to basics' approach to, (i) identify and clearly define environmental and production related risk factors which increase salmon susceptibility to ulcerative disease, (ii) develop early warning environmental indicators of infection risk by mapping the abundance and distribution of pathogens throughout production on a commercial farm, and (iii) collate the knowledge gained in this project with previous work to create best practice guidelines for risk-controlled production planning. In this way the project will provide immediately implementable recommendations to minimize risk, as well as the foundational knowledge required to develop preventive interventions.

Given the impact of ulcers on downgrading and the growing concern around antibiotic resistance, prevention is the only path forward with regards to ulcerative disease. However, given the complexity of the condition, prevention must go beyond vaccines and requires an in-depth understanding of how environmental variation affects both host susceptibility and pathogen infectivity. Two primary pathogens are associated with the recent ulcerative disease outbreaks in Norway, *Moritella viscosa* is the causative agent of 'classical winter-ulcers' while *Tenacibaculum finnmarkense* is the primary pathogen associated with 'atypical winter-ulcers' [2]. Both are gram-negative bacteria which thrive in high-salinity, and low-temperature conditions [3,4]. Although infection routes are not entirely clarified for either species, both appear to readily colonize open wounds or areas where the epidermal-mucus barrier is degraded [4,5]. Unconfirmed reports from industry also suggest a correlation between poor gill health during summer/autumn and increased severity of ulcer outbreaks through winter, a finding supported by challenge trials which detected the presence of *M. viscosa* on gills within 2h of exposure, well before the bacteria were present in skin, muscle and intestine samples [5,6]. Similarly, transmission mechanisms also remain unclear. Both *T. finnmarkense* and *M. viscosa* can be detected directly in seawater samples, and no intermediate hosts have been identified. Taken together with the observation that outbreaks frequently only affect a subset of cages within a site, some researchers suggest that colonization directly from seawater is more common than horizontal transmission [4]. In contrast, others suggest that seawater is an unlikely transmission route for *Tenacibaculum* and that spread through fish mucus, biofilms or parasites is more likely [2].

In Norway onset of ulcer outbreaks typically occurs in the autumn/winter period, most commonly in fish which are either transferred to sea or undergo non-medicinal delousing at temperatures ≤ 10 °C [1]. One possibility which could explain this timing is pathogen abundance – both *T. finnmarkense* and *M. viscosa* are phsychrophilic [7]. However, since adjacent cages and sites are frequently differentially impacted by ulcers, it is clear that pathogen abundance is not the only variable of importance. Understanding host susceptibility is also key to preventing ulcer outbreaks.

A multitude of factors can impact salmon susceptibility to infection, but in the case of winter-ulcer disease there are some obvious candidates for consideration. During

smoltification salmon experience systemic immune suppression [8], resulting in increased susceptibility to infection [9,10]. Since the majority of ulcer outbreaks occur in fish weighing ≤ 1 kg [4], in the period when the skin barrier is adapting to seawater [11], the type and age of smolt as well as the timing of sea-transfer may be critical.
There is considerable variety throughout the industry in the methods used to prepare parr for sea-transfer including photoperiodic cues, specialized feed and combinations of both, as well as variation in the age at transfer, and it is unclear exactly how this variation affects susceptibility to infection [12].

Other production related decisions which appear to be important are the timing and type of delousing treatment. The skin-mucus microbiome is sensitive, and can be significantly impacted by procedures such as basic as netting [13]. While chemical delousing is gentler with better welfare outcomes for salmon, resistance and sustainability concerns have led to an industry-wide shift toward non-medicinal delousing [14]. Comprised of thermal, mechanical and combined strategies, non-medicinal delousing is associated with elevated mortality, increased percentage of damaged gill tissue, increased prevalence of putative gill pathogens [15], and increased likelihood of ulcer outbreaks, particularly if performed in the autumn/winter[1]. The relative risk posed by the different strategies, and the interaction between ambient environmental conditions and treatment method, however, are unknown.

Environmental variability can also directly impact salmon susceptibility. Because salmon are poikilothermic their wound healing rate and immune capacity are both directly linked to sea temperature [16] . Temperature has therefor been identified as a risk factor for ulcer outbreaks, although no specific risk-related thresholds have been determined. What is clear is that the lower the temperature, the slower injuries heal [17], and the greater the likelihood of bacterial colonization. Similarly, dissolved oxygen is highly variable in sea cages with the lowest concentrations typically occurring during the autumn [18], and is also an important modulator of immune response and tissue repair. Exposure to 60% oxygen saturation resulted in earlier onset, increased disease severity and ultimately increased mortality compared to fish held at 80% saturation in a challenge trial with *M. viscosa* [19].

Less obvious but also potentially interesting variables to consider are daylength and fish swimming speed. Light is one of the primary drivers of behavior in sea-caged salmon, with fish swimming more slowly and diffusely dispersed in the dark [20]. The autumn through early spring period when ulcer outbreaks are most common coincides with low light intensities and long periods of darkness, particularly in northern Norway. In such conditions, particularly combined with low temperatures[21], cardiac output and blood flow are minimal[22] and the oxygen supply available to support tissue repair may be insufficient [17,23]. Untested reports from industry suggest that ulcer outbreaks start improving in late spring when daylength and light intensity are increasing, even though temperatures remain low. Examining the relationships between daylength, tailbeat frequency and wound healing could provide valuable insights and potential avenues for interventions to support wound healing in high-risk situations.

2.2 Objectives

This application addressed the FHF call, “Forebygging mot sårutfordringer i laksenæringen (22/00104)”. Specifically, sub-goal: *Utvikle hensiktsmessige produksjonsforbedrende tiltak som vil øke biosikkerhet og forebygging mot sårutfordringer.*

By examining both the intrinsic and extrinsic factors necessary for ulcerative disease to develop, we aimed to identify critical connections between the host, agent and environment which through risk-controlled production planning or interventions can be used to mitigate the extent of ulcer outbreaks.

To that end we have defined the following specific objectives:

1. Identify and clearly define the environmental and production related risk factors which increase salmon susceptibility (WP1 & WP3).
2. Identify the environmental conditions in which the risk of infection is high by mapping the abundance and distribution of pathogens throughout commercial production (WP2).
3. Create best practice guidelines for risk-controlled production planning (WP4).

3 WP1 – Identify production related risks associated with ulcer development in commercial salmon production

3.1 Impact goals

Mitigating risk requires identifying and defining which situations are dangerous and which are not. While it is clear that non-medicinal delousing during the autumn/winter can lead to an increased incidence of ulcers, no information is available regarding the relative risk posed by different delousing techniques, nor the impacts of other basic management practices such as the ploidy, type of smolt (size, smoltification method) or timing of sea transfer.

The objective of WP1 was to examine how production and management practices contribute to the risk of ulcer outbreaks. Specifically, (1) how management decisions and repeated handling impact the likelihood of ulcer outbreaks, and (2) determination of the relative risk posed by different delousing techniques in varied conditions.

3.2 Methods

The dataset used originated from the TripVel project, reports of which can be found at Stein et al., (2023, 2024). The project covered diploid and triploid productions in northern Norway since 2014, but the data from fish transferred to sea prior to 2020 was not collected in a way that could be compared to data post 2020. As such, only the higher quality data which was collected post-2020 was usable in these analyses.

Data from commercial sources

The data follows from the production of diploid and triploid salmon transferred to sea between 2020 and 2022 at commercial farms in Troms and Finnmark. The proprietary data was generated through a collaboration between different aquaculture firms and includes information on the transfer time, size and number of fish, daily cage level mortality, including the number of fish euthanized, the assessment of external injuries of a subsample of 20 fish/cage done as part of the weekly lice counts by the farmers, and key data for delousing treatments. All types of delousing treatments and fish transfers are classed as handling events for the purposes of this report. For some analyses, mechanical and thermal delousing were grouped as physical removal methods, while freshwater, hydrogen peroxide and medicinal treatments were grouped as chemical removal methods. Inaccuracies in fish counts can result from either the error margin of the counting process or any unobserved loss of any fish that is not found in the collector.

Wound data

Norwegian salmon farmers are obliged by regulation (FOR-2012-12-05-1140) to sample at least 10 to 20 fish (depending on season) and count the number of adult female, mobile and sessile salmon lice (*Lepeophtheirus salmonis*) on each of them. As part of the TripVel dataset, farmers were also trained and encouraged to collect data on the physical condition of the fish using the LASVEL scoring system during lice counts.

As far as possible, welfare data were recorded weekly for each cage at each farm. In short, the farmers inspected each fish, and scored them from 0 to 3, where 0 implies no injury/defect, 1 denotes minor or very mild, 2 is moderate, and 3 represents severe injury/defect. To facilitate the recordings, the companies commissioned the development of a software program (Røkter 2.0) that saves and enables downloading of these data. The scoring system was developed in collaboration with the LAKSVEL-project and includes images of each indicator and scoring level (Nilsson et al., 2022).

Environmental data

Although the commercial companies provided some temperature data recorded on site, the data was often incomplete. Therefore, we combined the commercial data on fish condition with environmental data (temperature, salinity, and current speed data at 5m intervals from 1 to 40m) from the NorFjord160 hydrographic model.

Fish groups

In total, fish were transferred into 120 sea-cages between 2020 and 2022. However, as fish grew they were occasionally split between cages that can legally hold up to 200 000 fish at 25kg/m3. Few farms collected welfare data within the first month of sea transfer, so this could not be explored. Some fish also swapped localities as they were moved between farms. At harvest, based on the criteria that we needed both mortality and wound data, the data came from 134 sea-cages.

Calculations

To examine the impact of handling events on wounds and mortality two indicators were calculated, loss delta (%) and wounds delta (%). For wounds delta (%) the percentage of fish with a score of 2 or higher at each sampling for the category “sår” (moderate or severe wounds/ulcers) was determined. We then calculated the difference in mean percentage of moderate/severe wounds/ulcers in the 3 weeks before from the 3 weeks after each event. For loss delta (%) we calculated the difference in mean mortalities for a period of 3 weeks before and 3 weeks after each event. In some cases, there were less than 3 weeks between events, meaning some overlap for the before or after scores. We tested windows of 1-3 weeks for both indicators with little change in results, and ultimately chose to use 3 week windows as that is sufficient time to ascertain whether an injury is healing or has turned into an ulcer.

3.3 Results

In total the TripVel dataset encompassed 452 individual handling events divided amongst 19 farms where it was possible to calculate the change in mortality and wound frequencies before and after an event. Of these cases, 123 were from diploid fish while 329 were from triploid fish. The temperature at 5m depth at each event ranged from 2.4 °C to 12.2°C. Each sea cage experienced between 1 to 5 handling events during the sea phase.

Table 1.

	Chemical			**Physical**	
	H_2O_2	**Freshwater**	**Medicinal**	**Mechanical**	**Thermal**
# events	7	36	87	111	121
Method selection	Little available data	Preferred when wounds are present prior to treatment		Used more often when fish are healthy prior to treatment	
Impact on wound scores		Slightly elevated after treatment		No change	
Impact on mortality		No change		Elevated after treatment	

3.3.1 Ploidy

Overall, the frequency of wounds did not change as a result of handling events. The same is true for both diploid and triploid fish (Figure 1a). In contrast, mortality was elevated after handling for both diploid and triploid fish (Figure 1b). Additionally, there were no relationships between change in mortality and change in frequency of wounds for either diploid or triploid fish (Figure 1c).

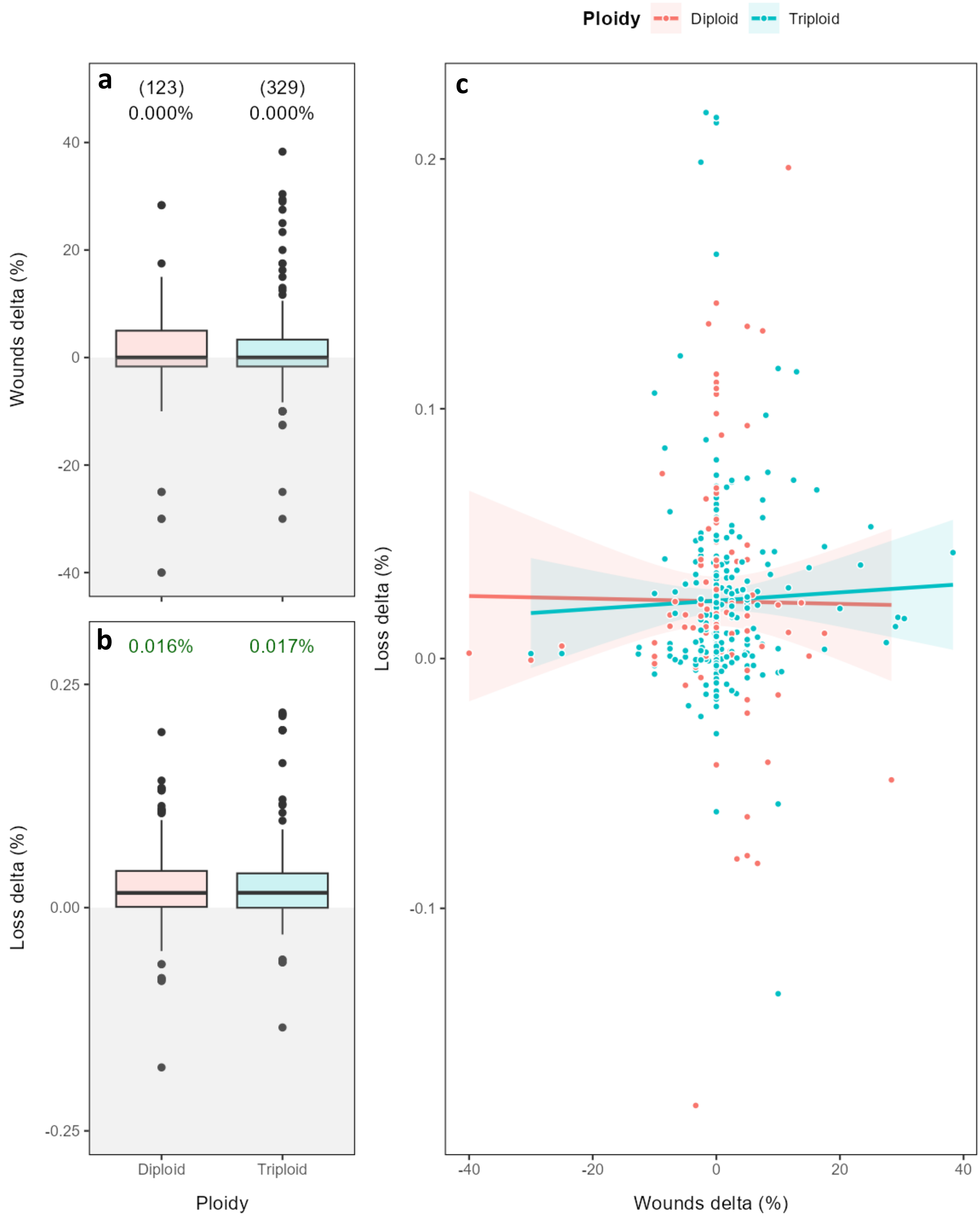

Figure 1. Impact of handling on wounds and mortality. Plot (a) shows the change in frequency of moderate to severe wounds after handling, plot (b) shows the change in mortality after handling, and plot (c) shows the relationship between mortality and wound frequency. Diploid fish are shown in pink, while triploid fish are shown in blue.

3.3.2 Event number

Every sea cage in the study experienced between one to five handling events. Only a few cages (n = 19) experienced five handling events. The frequency of wounds, on average, did not change following handling events one and two. However, with repeated handling the frequency of wounds after handling increased, especially after the fourth event (Figure 2a). Mortality, on the other hand, was increased after all handling events regardless of prior experience (Figure 2b).

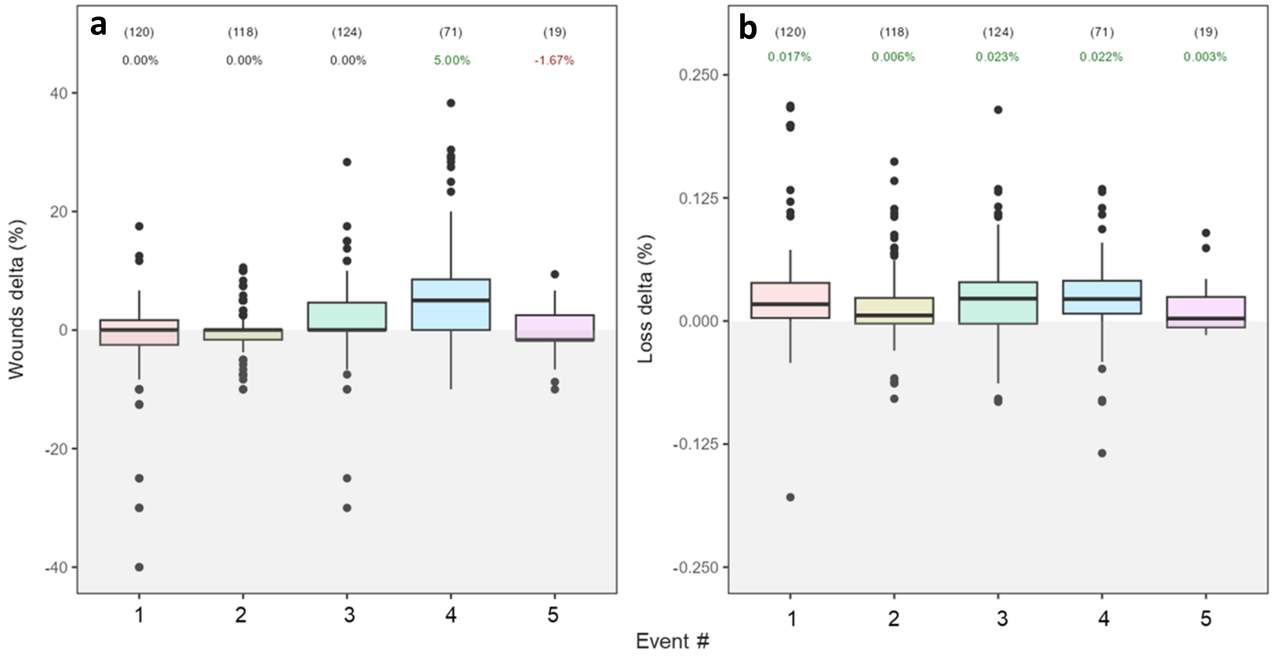

Figure 2. Impact of repeated handling on wounds and mortality. Plot (a) shows the change in frequency of moderate to severe wounds after one to five handling events, while plot (b) shows the change in mortality after handling.

3.3.3 Delousing method

The most common handling event was thermal delousing (121), followed by mechanical delousing (111), medicinal delousing (87), fish transfer (84), freshwater bath (36), and hydrogen peroxide (7). Initially there is a preference for physical louse removal methods, with thermal delousing alone accounting for nearly 75% of first handling events. While the preference for physical removal remains through handling events two and three, the dynamic splits to a more event distribution between thermal and mechanical treatments. By the fourth handling event the preference shifts to chemical delousing methods, with more than half of all treatments being medicinal. There were no examples of mechanical or thermal delousing being used during the fifth handling event (Figure 3).

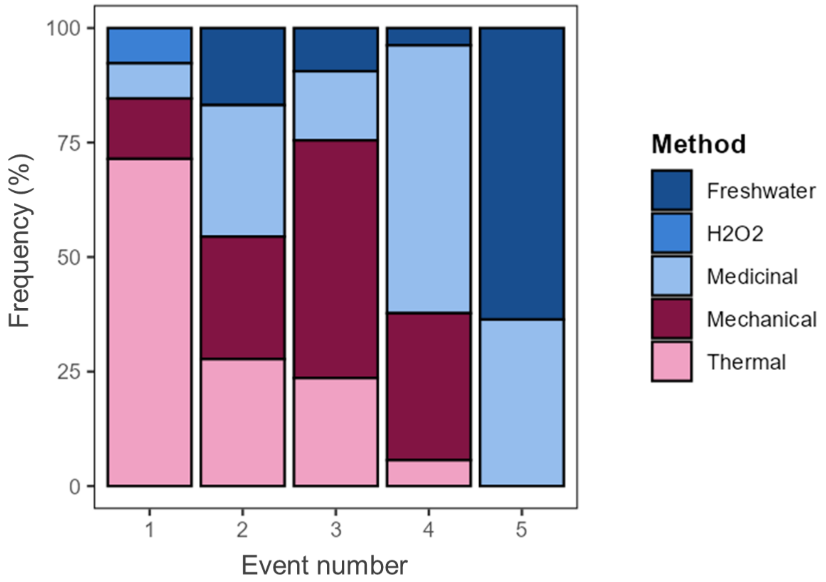

Figure 3. Frequency of use of different delousing techniques at handling events one through five. Chemical removal methods are shown in shades of blue, while physical removal methods are shown in shades of red.

Similarly, thermal and mechanical delousing are only used when the frequency of wounds is low, while freshwater and medicinal treatments are used when wounds are more prevalent (Figure 4).

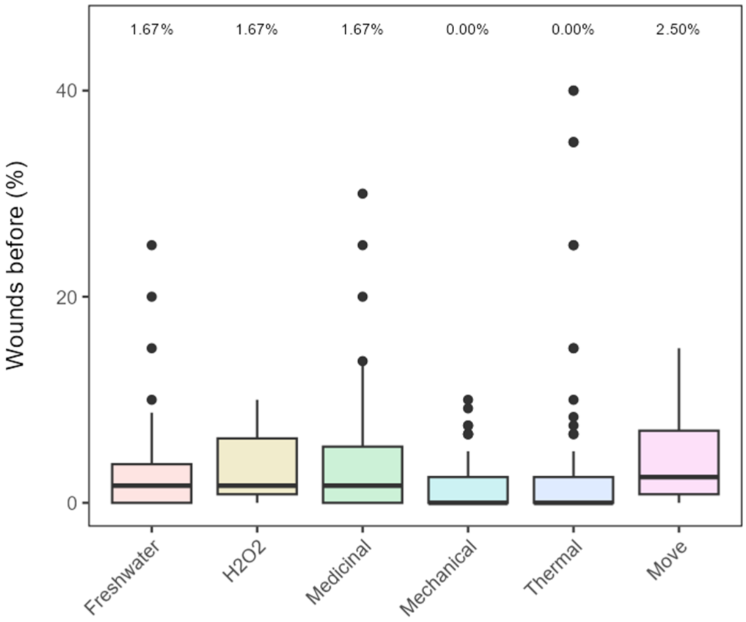

Figure 4. Wound prevalence in cages during the week prior to handling.

The frequency of wounds was not affected by fish transfer, thermal delousing, mechanical delousing, or hydrogen peroxide treatment. There was, however, a slight increase in the frequency of wounds following freshwater and medicinal baths (Figure 5a). The inverse pattern was true of mortality, with losses elevated after thermal and mechanical delousing, but no other treatments (Figure 5b).

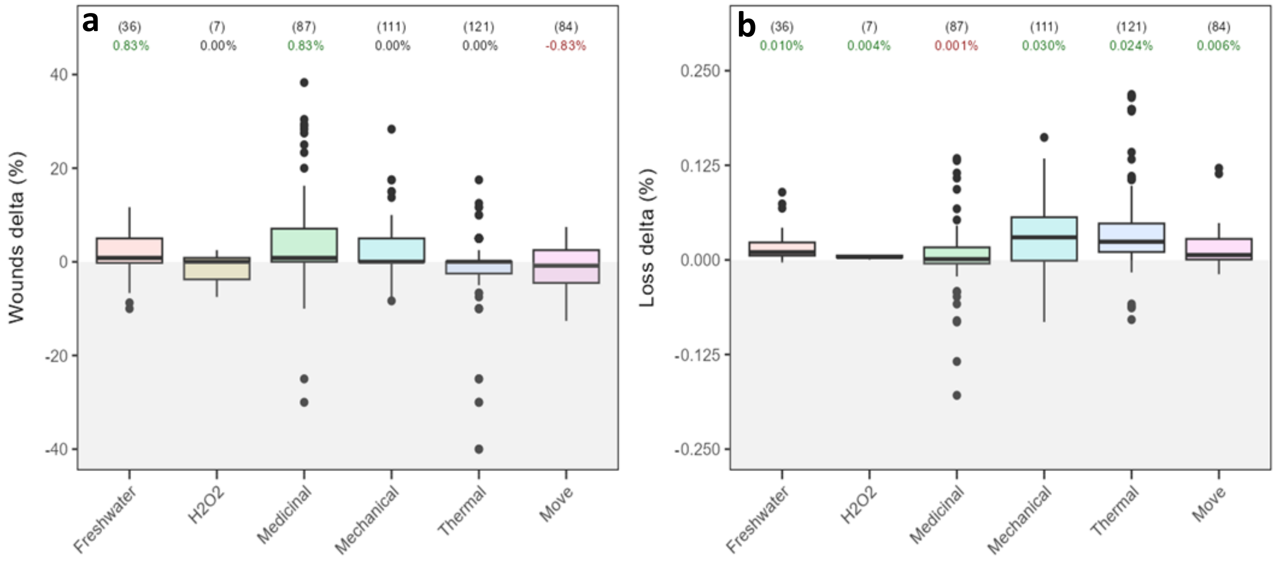

Figure 5. Impact of handling method on wounds and mortality. Plot (a) shows the change in frequency of moderate to severe wounds after handling, while plot (b) shows the change in mortality after handling.

To consider the interaction between type of handling event and prior exposure it was necessary to use grouped handling categories due to limited sample size. The frequency of wounds was not affected by any methods following handling events one through three. Following the fourth handling event the frequency of wounds was elevated after both physical and chemical delousing treatments, but not following simple transfer (Figure 6a). Mortality on the other hand was elected after physical louse removal regardless whether it was the fishes first or fourth handling event. Mortality was less affected by chemical louse removal, which did not differ in impact from fish transfer alone (Figure 6b).

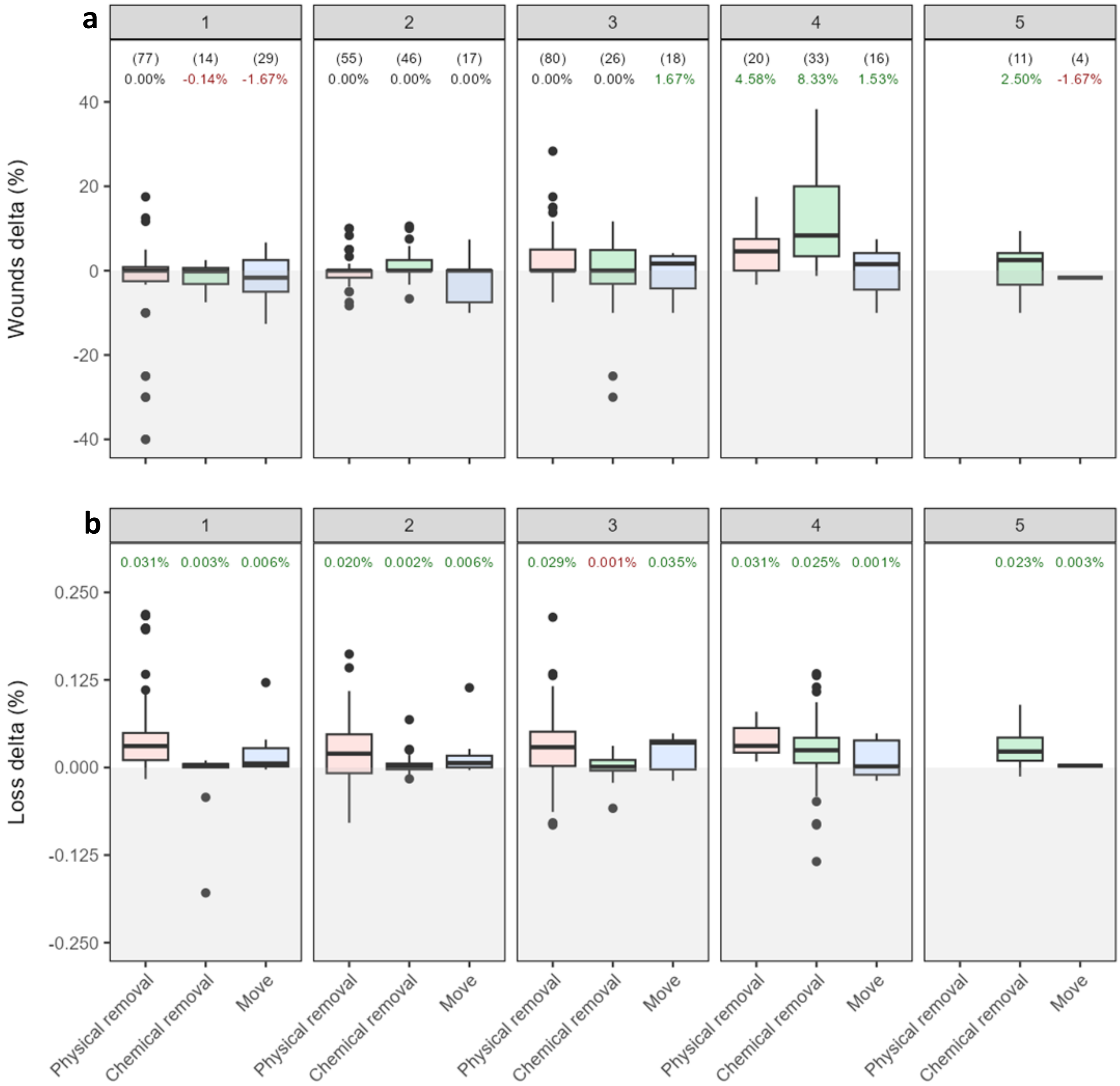

Figure 6. Interaction of handling method and repeated exposure. Plot (a) shows the change in frequency of moderate to severe wounds after handling, while plot (b) shows the change in mortality after handling. Each column indicates handling event 1-5.

3.3.4 Temperature

There were 239 handling events which occurred when sea temperatures ranged from 8 – 12 ˚C, 197 events at 4 – 8 ˚C, and 16 events which occurred at 0 – 4 ˚C. No mechanical delousing treatments were performed below 4 ˚C, while freshwater bathing was not used in the warmest temperature range (Figure 7c). The frequency of wounds did not change after handling in either the 4 – 8 ˚C or 8 – 12 ˚C ranges. The frequency of wounds was actually reduced after handling at 0 – 4 ˚C, but as this is based on only 16 events no conclusions should be drawn based on these data (Figure 7a). Mortalities were slightly elevated after handling in all three temperature groupings (Figure 7b).

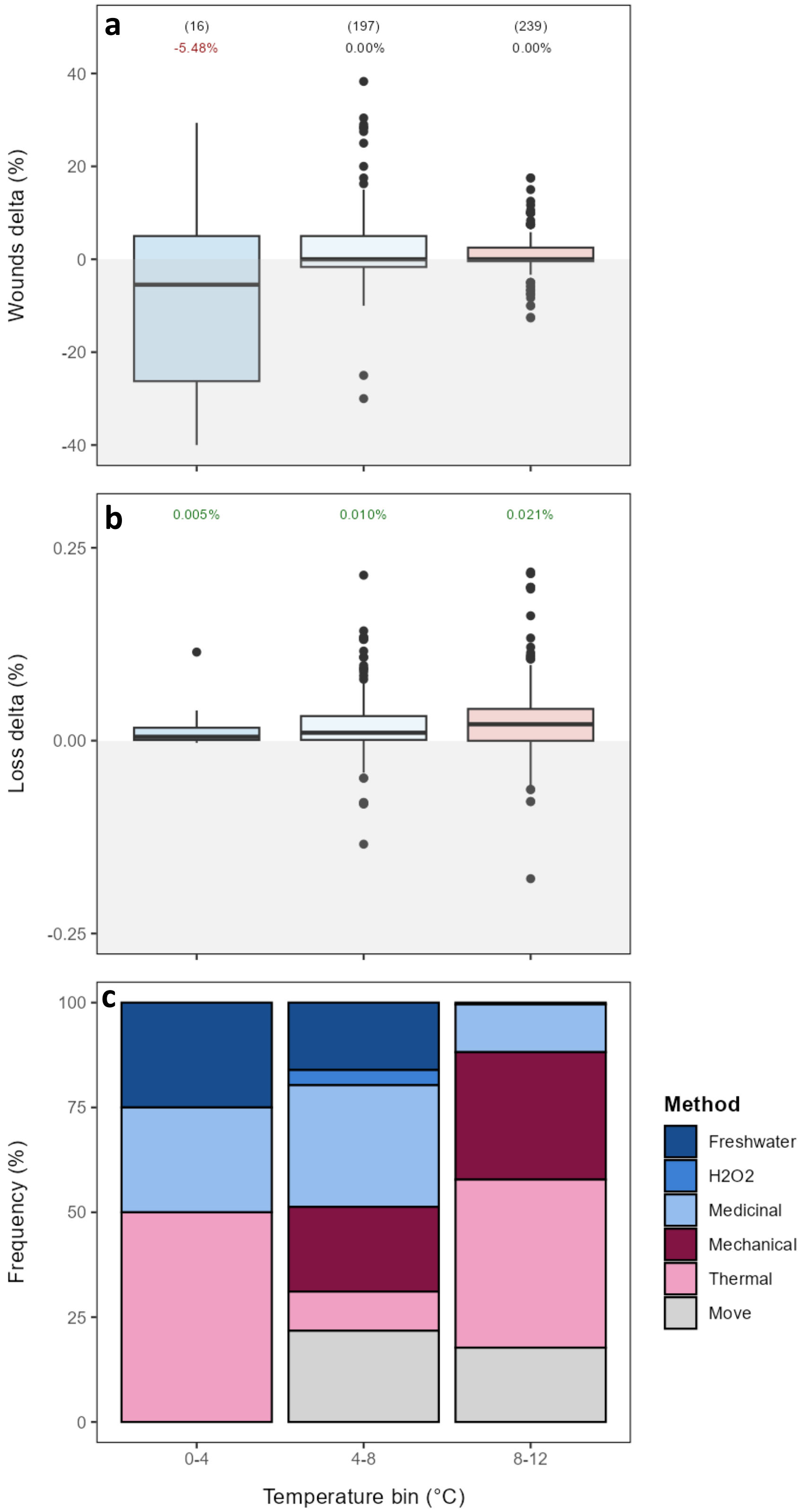

Figure 7. Impact of temperature on treatment choice and outcome. Plot (a) shows the change in frequency of moderate to severe wounds after handling, while plot (b) shows the change in mortality after handling, and plot (c) shows the frequency of each type of handling event in each temperature range.

4 WP2 – Map the infection pressure of ulcer causing pathogens on a commercial farm in relation to environmental conditions and louse management

4.1 Impact goals

The two widely accepted risk factors for ulcerative disease in farmed salmon are, (i) cold temperatures, and (ii) delousing in the autumn/winter[1]. Given the psychrophyllic nature of *M. viscosa* and *T. finnmarkense*, one of the major influences driving the timing of ulcer outbreaks in Norway could be increased pathogen abundance in the autumn/winter. At present however, no systematic studies have examined variation in ulcer-causing pathogen abundance and distribution in relation to seasonal variation and environmental conditions.

Second, while it is clear that delousing predisposes salmon to ulcerative disease, farmers often have no choice but to treat in the autumn/winter periods given the limited options available and strict regulations. One possibility which could alleviate some of the burden is lice preventive tools such as barriers and/or behavior modification. However, although the lice preventive efficacy of some strategies is well documented, it is unclear how these tools may impact ulcerative disease. Reports from industry suggest that the static use of skirts or snorkel barriers can exacerbate gill problems. Promisingly however, in a trial which tested the efficacy of a dynamic lice prevention strategy using skirt barriers, aeration, and adjustable depth lights and feeding, in addition to reducing the need for delousing treatments by 25%, wound scores were consistently lower in the test cages compared to controls, particularly during the second autumn and winter at sea. Understanding the impacts of lice prevention strategies on ulcerative disease outbreaks, and ensuring that farmers are not fixing one problem while worsening another, is key to developing holistic solutions which have real-world applicability.

The objectives of this WP were therefore, (i) identify periods when pathogen abundance is high, (ii) determine the predictive ability of potential proxy environmental indicators, and (iii) examine how environmentally responsive lice prevention affects prevalence of pathogens.

4.2 Methods

4.2.1 Experimental set-up

The study was conducted at a commercial salmon farm (Fosså) in Ombofjorden, southwestern Norway (59.27° N, 6.14° E). The farm had six circular sea cages (C = 200 m, 36 m deep) arranged in two parallel rows from the feed barge, perpendicular to the shore. The cages were stocked with Atlantic salmon (Salmo salar, autumn transferred smolts from Fjæra, MOWI strain), with four cages stocked 12.10.2022 and the last two cages stocked two weeks later (28.10.2022). At transfer, the number of salmon per cage ranged between 199 850 – 199 998 fish with an average weight of 130 ± 4.6 g. The study lasted for 16 months, spanning the entire production cycle.

The trial consisted of two treatment groups each replicated in three cages, (a) standard cages which followed normal production procedures, and (b) test cages which utilized a dynamic parasite prevention strategy. The test cages were equipped with adjustable depth lights (150 W/1200 W Aurora SubLED Combi light, AKVA group) and feeding (SubFeeder, AKVA group), a 10 m deep skirt barrier (Skirt type) and two bubble aeration devices (Midt-Norsk ringen, NorseAqua, Norway) positioned below the skirt to create vertical water exchange within the barriers. The facility followed standard husbandry protocol during the trial, including daily feeding and dead fish registration. Salmon were fed size appropriate pellets according to standard growth tables.

Throughout the experimental period the farm was managed according to standard rearing and feeding procedures in salmon aquaculture. When lice infestations at the farm exceeded the maximum allowed limit of 0.5 adult female lice per fish or 0.2 adult females during migration season (weeks 16–21) (Lovdata, 2012), delousing treatments were administered on a cage-basis according to standard commercial practice. All preventive tools, including skirts, had to be removed for delousing. Medicated feed (emmamectin benzoate) was distributed in all cages from 16.03–24.03.2023.

4.2.2 Dynamic strategy

The principle for the dynamic strategy was to optimize and add automation to the environmentally-driven deployment strategy described in Oldham et al. (2023). In short, the strategy is intended to shield the fish from surface waters with highest densities of copepodids when salinity is uniform via skirt barrier. To create vertical water exchange, aeration is always provided when skirt barriers are down. When a brackish layer is present (< 28 ppt @ 5 m) skirts are pulled up, aeration turned off, and lights moved to the surface to attract the fish into the brackish layer to facilitate self-treatment of gills. Feeding depth was chosen as the position with temperature closest to 14 ˚C. If no thermocline was present, feeding was at the same depth as the lights. To ensure sufficient oxygen availability, supplemental oxygenation was also available for all dynamic strategy cages.

Table 1. Summary of the planned dynamic strategy. x denotes intervention is off, ✓denotes intervention is deployed.

		Optimal thermal zone**: Shallow**	**Optimal** thermal zone**: Deep**	**No optimal** thermal zone
Brackish layer: Present	**Skirt**	x	x	x
	Aeration	x	x	x
	Feeding	Shallow	Deep	Shallow
	Lights	Shallow	Shallow	Shallow
Brackish layer: Absent	**Skirt**	✓	✓	✓
	Aeration	✓	✓	✓
	Feeding	Shallow	Deep	Deep
	Lights	Deep	Deep	Deep

In previous trials of the dynamic strategy it was clear that an important factor limiting efficacy was response time, and difficulty changing the state of the preventive tools quickly enough.

To reduce the response time in this trial winches were attached to two points on each skirt barrier as well as all lights. Additionally, control of oxygenation was automated (InnovaSea, aquaControl system, USA) to maintain dissolved oxygen concentrations above 8 mg L^{-1} at 5 m.

4.2.3 Environmental monitoring

The site was equipped with a real-time environmental monitoring array comprised of sensors which could be remotely viewed online (InnovaSea, Realfish Pro Platform, USA). A set of sensors measuring temperature, salinity, dissolved oxygen, turbidity, chlorophyll a, phycoerythrin and dissolved organic matter were placed in each cage at depths of 5 m (mid-skirt) and in one cage per treatment at 15 m (below skirt). An additional set of sensors measuring the same conditions were placed at 5 and 15 m at a reference site at least 100 m from the nearest cage. A multi-sensor CTD (SD204, SAIV AS) was also used to collect a daily vertical profile of temperature, salinity and dissolved oxygen from the surface to 40 m from a reference point at the barge.

4.2.4 Sampling protocol

Throughout production salmon were regularly assessed for louse infestation, gill health and welfare, with sampling every 2–4 weeks throughout the trial period. Fish were captured using a jump net set out on the morning of sampling. During each sampling 20 fish per cage were subjected to a lethal dose of anaesthetics in individual buckets (Benzoak vet., Benzocaine, 500 mg L^{-1} or Finquel MS-222, 500 mg L^{-1}).

For the first 5 fish in each cage skin mucous samples were collected by rubbing a sterile swab along the lateral line from the caudal fin to opercula on the left side of the fish. Swabs were placed directly in tubes with eNAT fixative. Skin swabs were sent to a commercial company for qPCR analysis of *Tenacibaculum spp*. and *Moritella viscosa*.

After skin swab collection, the number of lice were counted and classified according to life stages: copepodid, chalimus I, chalimus II, preadult I, preadult II (male and female) and adult (male, and female with and without egg-strings). Counts were performed on fish in water filled trays using headlamps to ensure good visibility. Counts of mobile stages that had detached from the host and found in the buckets were also included in the total lice count per cage.

Fish welfare was scored using adapted morphological indicators from the FISHWELL scoring system, with 0 = ideal, 1 = light, 2 = moderate and 3 = extreme (Nilsson et al., 2022). Specifically, the fin condition, snout injury and wound scores were each separated into two categories, either fresh injuries (injury), or a wound with visible infection (ulcer). Gill health was further evaluated by visually scoring each arch using the standard 0 to 5 scale for amoebic gill disease (AGD) (Taylor et al., 2016).

For the 10 first fish in each cage gill swabs were also collected by gently swabbing each gill arch on the left side of the fish after the visual scoring. The swab was then placed in eNAT fixative and sent to a commercial company for qPCR analyses of *Neoparameoba perurans*, *Candidatus* Branchiomonas cysticola, *Paranucleospora theridion, Tenacibaculum spp.* and Salmon gill poxvirus (SGPV). Additionally, samples were collected from the 2nd right arch of the first 5 fish for histological analysis.

Also, at each sampling, water samples (2 L) were collected from 2 depths (5 m and 15 m) in each cage using a Niskin sampler. Each water sample was then filtered through a 47 mm 0.45 um filter via a sterilized vacuum filtration system (PALL, 47mm lab manifold). Filters were then placed in tubes with eNAT and sent to a commercial company for qPCR analysis of *P. perurans*. Additionally, after each sampling, 1L of freshwater was filtered as a negative control.

4.2.5 Apolemia sp. monitoring

A mass aggregation of *Apolemia sp*., a colonial Siphonophore with high stinging capacity, occurred along the Norwegian coast from August 2023 through February 2024 (Figure 9). Lack of knowledge at the time meant that nobody knew how best to protect fish from *Apolemia sp*, and the site manager at the study facility was concerned that *Apolemia sp.* would become trapped within the skirts and cause even more damage. As a result, the dynamic strategy was discontinued from August 2023. However, to study how skirts and aeration impacted *Apolemia sp*. intrusion to cages, a study was set up with one empty cage with a 10 m skirt barrier and aeration, and one standard empty cage as a control. Remotely operated underwater vehicles (rovs) were used to perform daily vertical profiles from the surface to the net bottom in each of the empty cages at four cardinal directions in line with (NW, SE) and perpendicular to (NE, SW) the primary current direction. In each profile the depth of all *Apolemia sp*. fragments was recorded as well as size classification (small < 20 cm, medium 21 – 100 cm, large > 100 cm) and position (inside the cage, stuck in the net, outside the cage) (Figure 8).

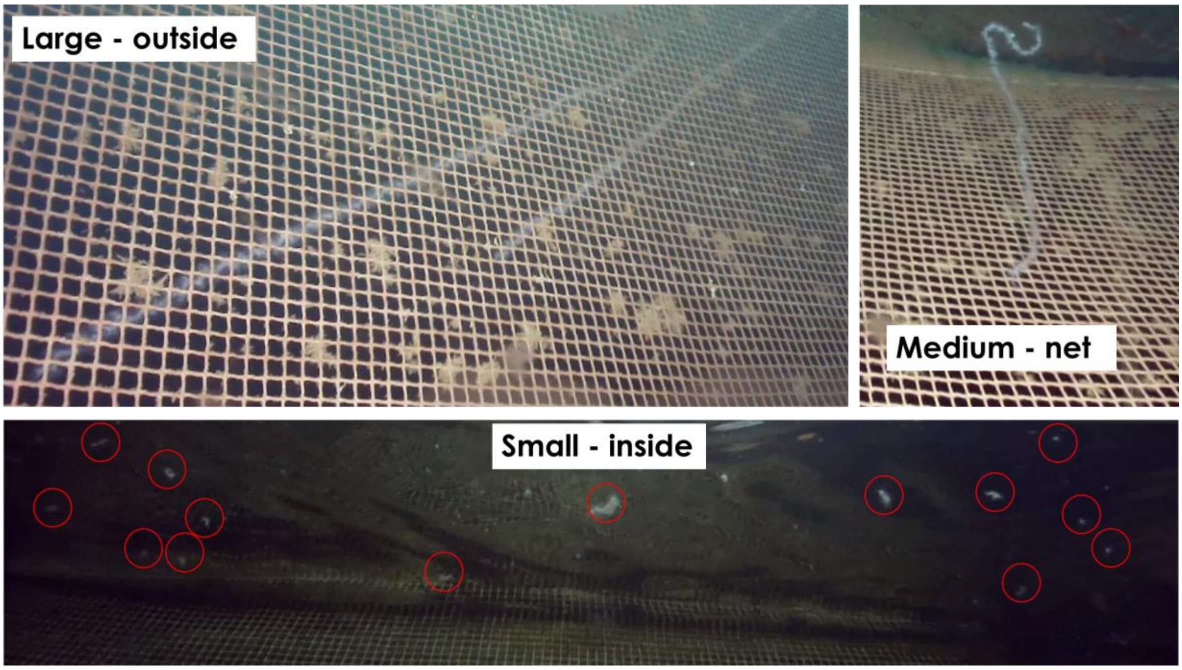

Figure 8. Illustrative examples of the size classifications (small, medium, large) and position locations (inside, net, outside) of Apolemia sp. fragments observed via ROV.

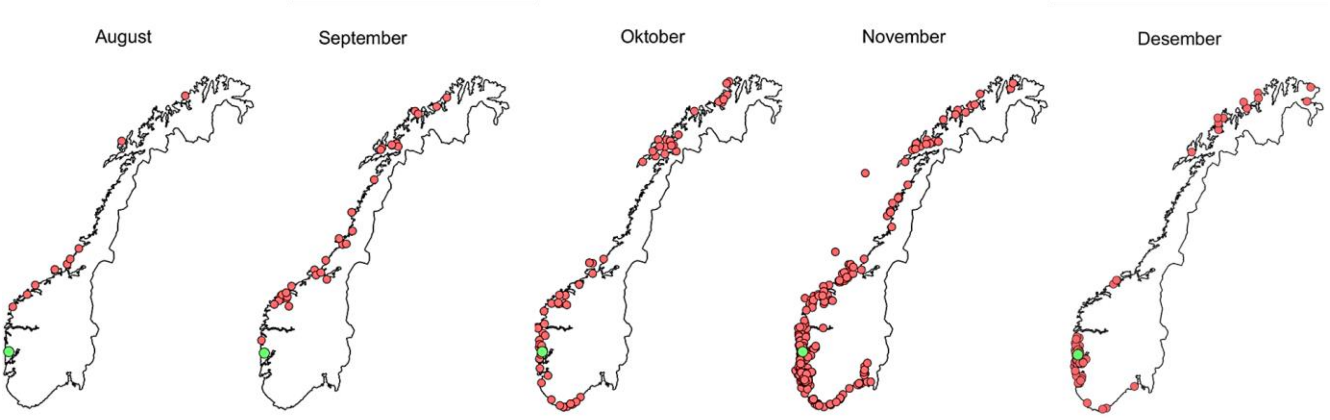

Figure 9. Reported sightings of Apolemia sp. along the Norwegian coast in 2023 from the citizen science portal, dugnadforhavet.no.

4.2.6 Descriptive analysis

Sampling events were split into three observation windows to account for changes deployment of the dynamic strategy (Figure 10). Observation window 1; November 2022-February 2023 was the initial introduction period allowing fish sufficient time to acclimate following transfer to sea. Observation window 2; February 2023- July 2023 the dynamic strategy was deployed in the test cages. Observation window 3; July 2023- February 2024 fish groups were mixed and use of all preventive tools was discontinued as per the decision of the commercial partner in response to a nationwide mass occurrence of *Apolemia sp*.

Dynamic tool use was tracked via a daily activity log then transcribed to create a dataset of daily activity for all test cages. The use of each tool (lights, feeding, skirt usage and aeration) was recorded using binary coding, as well as the presence or absence of a brackish layer and the location of optimal temperature (shallow or deep). The ideal use for each tool was calculated based on the corresponding environmental condition (brackish for aeration, lights and skirt, temperature for feeding) and the difference from the actual tool implementation was determined to visualise the frequency of correct strategy implementation.

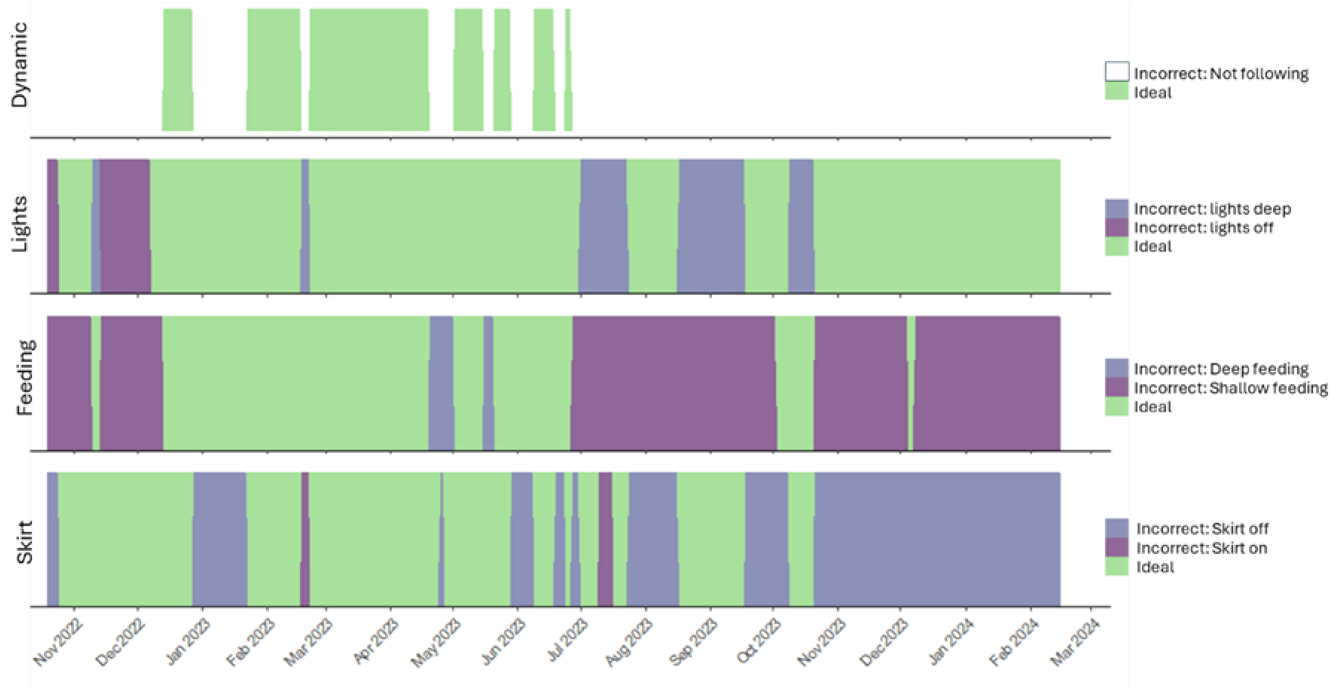

Figure 10. Implementation of dynamic strategy interventions. The upper panel shows the overall dynamic strategy, below which are light position, feeding depth and skirt status in relation to the planned dynamic strategy deployment criteria.

4.3 Results

4.3.1 Dynamic strategy implementation

Despite the addition of the winches and automation for oxygenation control, the dynamic strategy was still implemented as intended for less than half of the production cycle (Figure 10). During observation window 1 (Nov 22'– Jan 23') the initial lack of submerged feeding equipment made it impossible to properly implement the dynamic strategy. Instead, skirts were deployed with surface feeding attracting the fish into the skirt volume when they should have been fed below it, where natural water exchange occurs. Subsequent elevated AGD scores relative to control cages necessitated skirt removal when no brackish layer was present.

During observation window 2 (Feb 23' – July 23') all required equipment was available and the dynamic strategy was followed as closely as possible. Observation window 3 (Aug 23' – Feb 24') began when all preventive equipment was removed for delousing. Following delousing, a mass aggregation of *Apolemia sp*. occurred along the entire Norwegian coast, and the facility management decided to discontinue the use of all preventive tools.

4.3.2 Environmental conditions

At the reference location, recorded sea temperatures ranged from 5 – 19 ˚C, salinity ranged from 17 – 34 ppt, and dissolved oxygen saturation remained above 90% (Figure 11). Although surface waters were over-saturated throughout most of the trial, lowest saturations were consistently recorded at the 15 m position, and highest at 1 m.

Initially temperatures were similar at the 1, 5, and 15 m positions, until late January – March 2023 when 15 m was the warmest position. After a brief mixing period in April a thermocline again developed, this time with warmest temperatures nearer the surface. Surface waters then remained the warmest until October 2023 when there was another mixing event. From October 2023 through February 2024 temperatures remained similar at all 3 depths (Figure 11).

When a halocline was present, salinity was always highest at the 15 m position. Throughout the trial salinity was quite variable at the 1 m and 5 m positions. There were two brief periods with a halocline at the start of the trial (Nov 22’, Feb 23’), followed by an extended period with a strong brackish layer from May through October 2023 (Figure 11).

Figure 11. Dissolved oxygen saturation (%), salinity (ppt), and temperature (˚C) recorded at a reference location throughout the trial. The purple lines indicate levels at 1m water depth, teal lines 5 m, and yellow lines 15 m.

Chlorophyll red, blue, and phycoerythrin followed similar patterns where they all peaked in March 2023, to varying extents in July 2023, and again in September 2023 (Figure 12). Compared to chlorophyll-red the phycoerythrin sensor was very similar only less sensitive, while chlorophyll-blue followed a similar but exaggerated pattern compared to Chlorophyll-red, with peaks at the same times, but more extreme. Measured concentrations at 5 m and 15 m mostly followed the same trends, with some notable exceptions. Both the Chlorophyll-red and phycoerythrin sensors detected spikes at 15 m which did not occur at 5 m, for example in May and October 2023

Turbidity did not, for the most part, follow the same trends as the other sensors (Figure 12). During the apparent phytoplankton bloom indicated by the other sensors in March – April 2023, turbidity was consistently low.

Figure 12. Turbidity (A), Phycoerythrin (B), Chlorophyll-blue (C), and Chlorophyll-red (D) recorded at a reference location throughout the trial. Teal lines indicate sensors at 5 m water depth, and yellow lines 15 m.

4.3.3 Louse protective efficacy

Louse infestation pressure was generally low during observation windows 1 and 2, but even so the dynamic strategy delayed the need for first delousing by 2 months. Ultimately, during the portion of the trial when the dynamic strategy was in use, control cages were deloused twice while test cages were deloused once (Figure 13).

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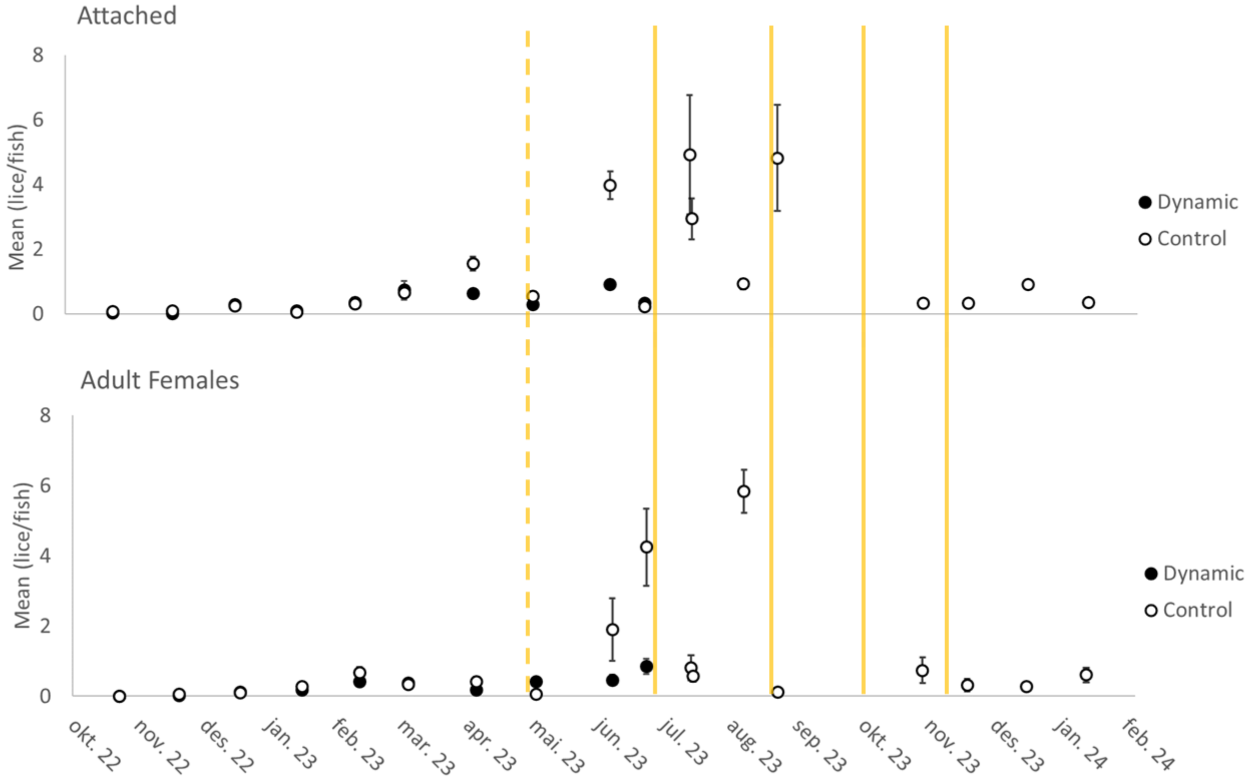

Figure 13. Attached and adult female lice per fish in cages using the dynamic strategy (solid point) versus control cages (open circles). Data shown are mean ± SEM. Orange vertical lines indicate when delousing treatments were performed, with dashed lines indicating only control cages were treated, while solid lines indicate all cages (although there were no test cages after July 2023).

4.3.4 Growth

All fish grew well, with no differences in weight or condition factor between treatment groups throughout the trial (Figure 14).

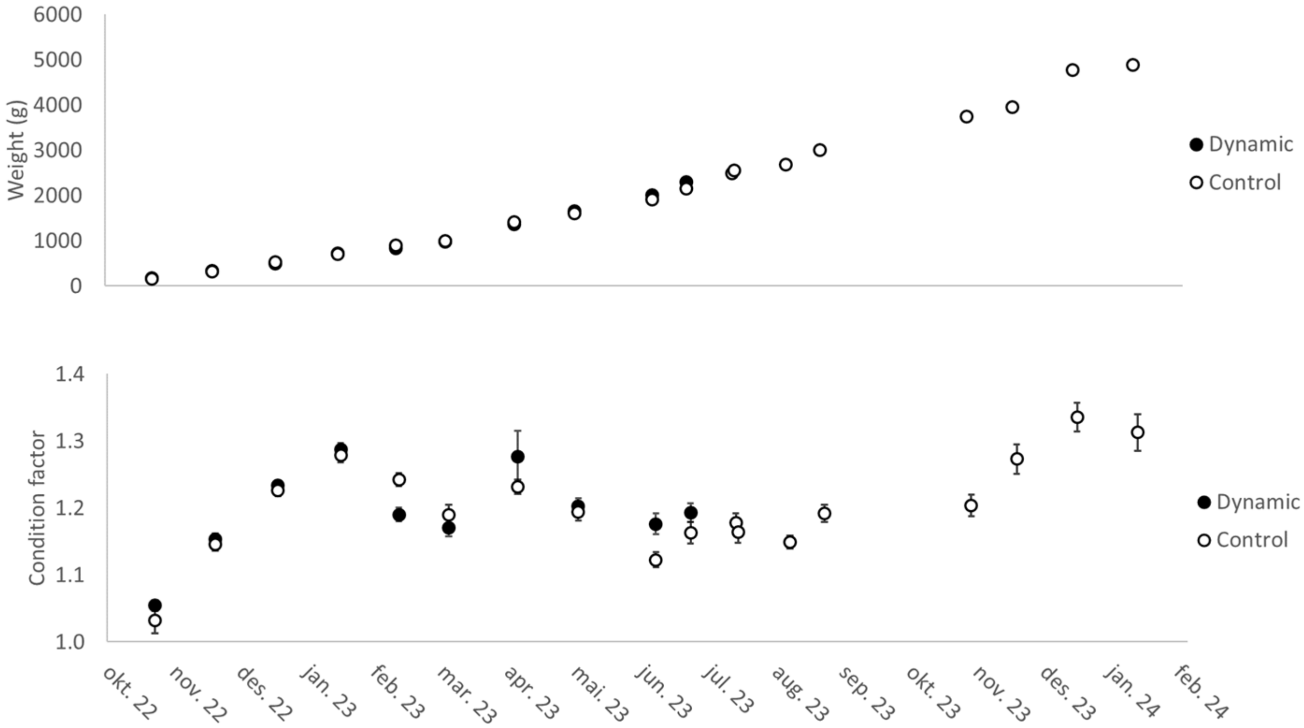

Figure 14. Weight and condition factor in cages using the dynamic strategy (solid points) versus control cages (open circles). Data shown are mean ± SEM.

4.3.5 Wounds & ulceration

Injuries followed the same trends in both test and control cages, with no differences in severity or prevalence throughout the study. In contrast, while ulcer scores were low throughout observation windows 1 and 2, during window 2 there were 3 sampling events where ulcer scores were significantly higher in the control cages compared to cages using the dynamic strategy. Two of the three periods where ulcer scores were higher in control cages were prior to any delousing treatments (Figure 15).

During observation window 3 injury scores began to increase after control fish underwent their third delousing. Injury scores peaked in November, following the 5th and final delousing. In contrast, ulcer scores remained low in test cages through early November 2023, but increased sharply by late November and remained high until harvest (Figure 15).

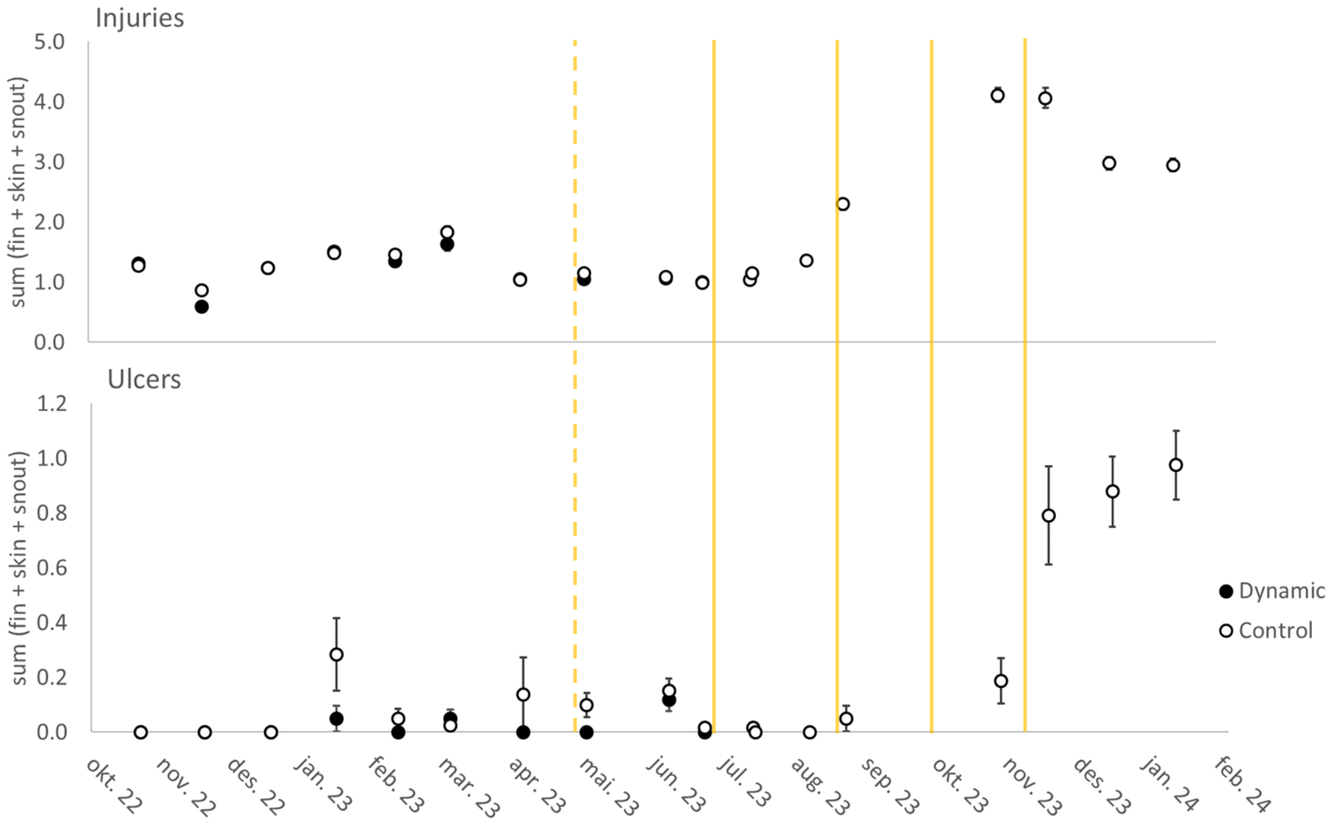

Figure 15. Injuries and ulcers in cages using the dynamic strategy (solid points) versus control cages (open circles). Data shown are mean ± SEM of the total score for injuries and ulcers, each scored from 0 to 9 on an ordinal severity scale. Orange vertical lines indicate when delousing treatments were performed, with dashed lines indicating only control cages were treated, while solid lines indicate all cages (although there were no test cages after July 2023).

4.3.6 Gill health

Throughout the trial both amoebic gill disease (AGD) and complex gill disease (CGD) posed challenges. Gross AGD scores began increasing immediately after sea transfer in all cages, peaking in January 2023 in both control and test cages. Gross AGD scores peaked higher in the test cages (mean = 1.5) compared to controls (mean = 0.9), at which point the skirts were removed. Gill scores in all cages declined into February, when skirts were re-deployed in test cages and the dynamic strategy was followed until July 2023. Gross AGD scores were at their lowest in both control and test cages in May 2023 (Figure 16).

Gross CGD scores followed a different pattern, with bimodal peaks in February 2023 and July 2023. Similar to AGD scores, the initial peak during observation window 1 when skirts were on but the fully dynamic strategy was not deployed, was higher in test cages than controls. Throughout observation window 2, when the dynamic strategy was in use, gross CGD scores decline in all cages to a minimum in May 2023 (Figure 16).

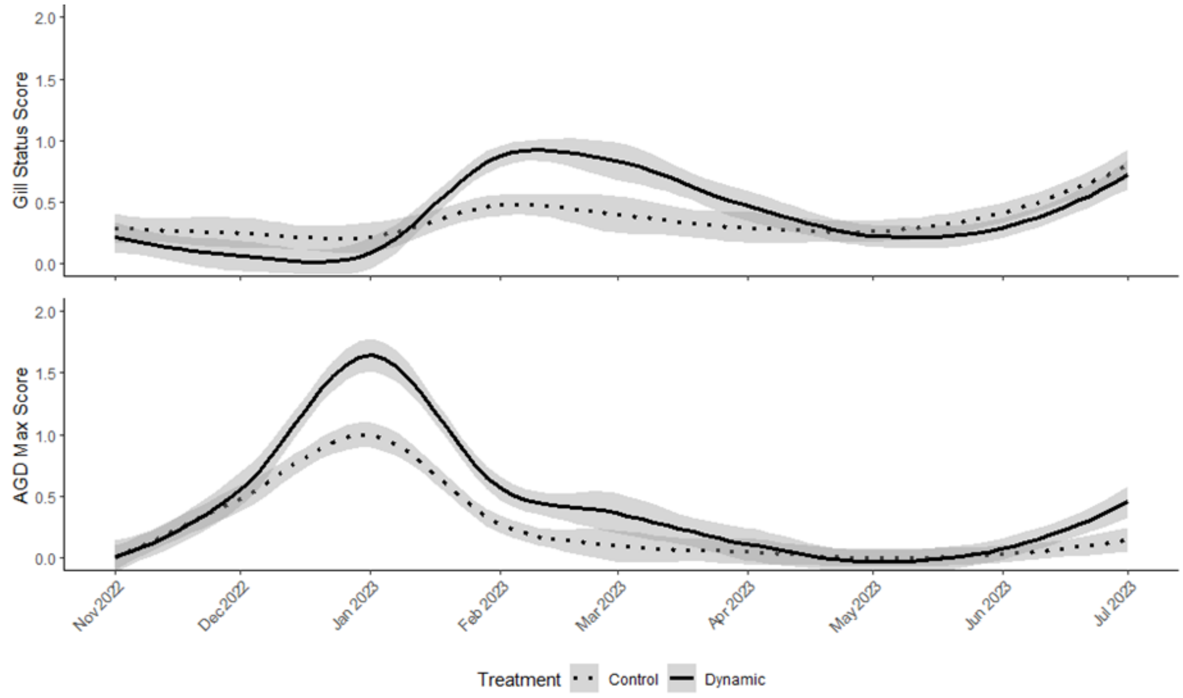

Figure 16. Patterns in gross scores for Complex Gill Disease (Gill status score) and Amoebic Gill Disease (AGD max score) throughout observation windows 1 and 2 of the trial. Control cages are indicated by dashed lines while test cages are indicated by solid lines. Data shown are mean ± 95 % CI.

Abundance patterns of potential gill disease causing agents detected on gill swabs varied considerably, although there were no significant differences between test and control groups (Figure 17). Salmon gill pox virus peaked in December 2022 and then remained low throughout the rest of the trial. *N. perurans* peaked slightly later, just after pox virus, in January 2023, gradually declining until May, and then started increasing again over summer. P. theridion was present from the start of the trial, and did not change. B. cysticola followed a completely different pattern, gradually increasing from January 2023 through the rest of the trial period. Tenacibaculum sp. was low or absent throughout most of the trial, until gradually increasing from May 2023.

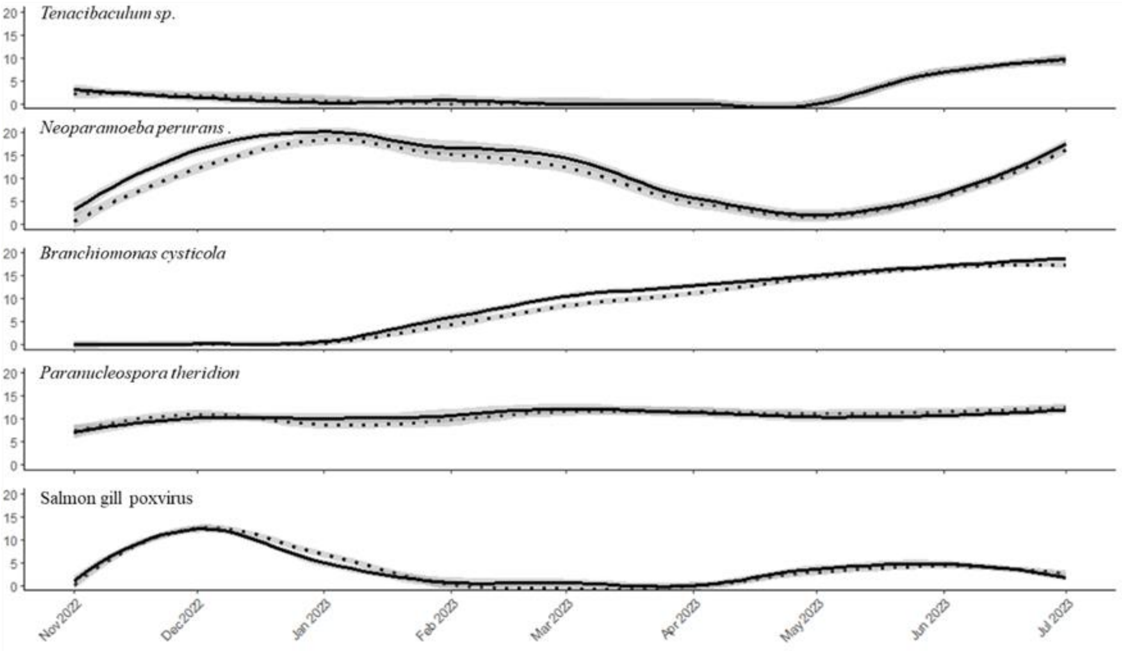

Figure 17. Mean qPCR load index ± 95% confidence intervals for five gill-associated agents in Atlantic salmon: (a) Tenacibaculum sp., (b) Neoparamoeba perurans, (c) Branchiomonas cysticola, (d) Paranucleospora theridion and (e) Salmon gill poxvirus (SGPV). Results are shown for control (dotted lines) and test cages (solid lines).

4.3.7 Pathological agents - water

The load index for all potential pathological agents was consistently lower from water samples compared to mucus samples from the fish (Figure 18). The only case in which load index differed between test and control cage samples was for *N. perurans* in January 2023, where the amoebae were more abundant in the test cages. In water samples, *N. perurans* peaked at the same time as gill mucus samples, January and August 2023. *Branchiomonas* began increasing later and also peaked in August 2023. No water samples tested positive for *Moritella viscosa*, and although *P. theridion* was present on fish gills throughout the trial, there were few positive detections from water samples. Pox virus was similarly only present in very small quantities in water samples. *Tenacibaculum sp*. was also only present in small quantities in water samples, with peaks matching but weaker than from gill mucus samples.

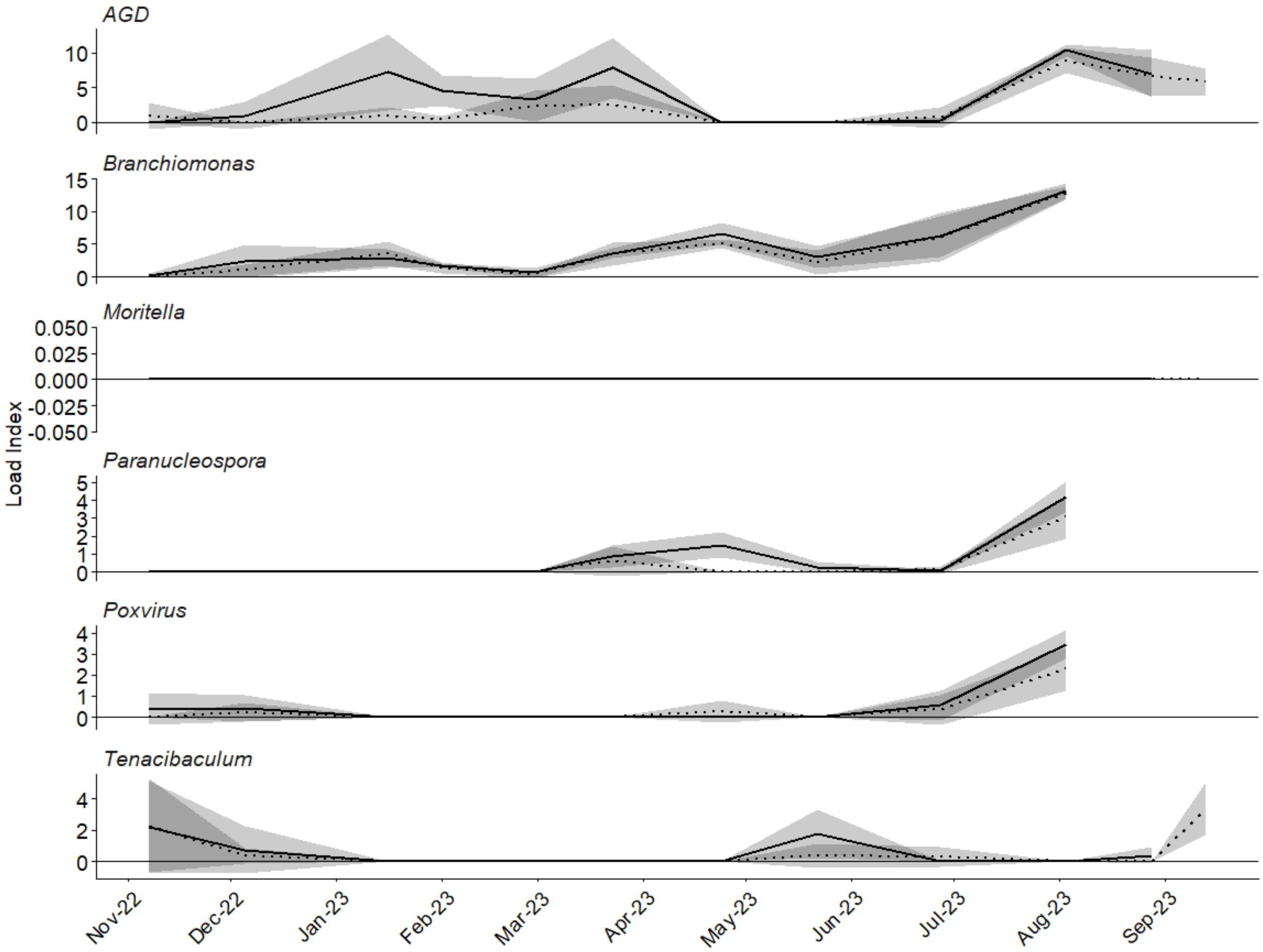

Figure 18. Mean qPCR load index ± 95% confidence intervals for potentially pathological agents from filtered water samples: Neoparamoeba perurans, Branchiomonas cysticola, Moritella viscosa, Paranucleospora theridion, Salmon gill pox virus, and Tenacibaculum sp. Results are shown for control (dotted lines) and test cages (solid lines).

4.3.8 Pathological agents - skin

For the first few months of the experiment both *M. viscosa* and *Tenacibaculum spp*. were largely absent from the skin mucous samples in both test and control cages. There was a spike in both *M. viscosa* and *Tenacibaculum spp*. in mid-April, only in control cages. Dynamic cages were unaffected. In July, following delousing, *Tenacibaculum spp*. load spiked in both groups. The dynamic strategy was discontinued beyond this point, but in the remaining cages both *M. viscosa* and *Tenacibaculum spp*. increased throughout the second autumn at sea, concurrent with the *Apolemia sp*. bloom and increasing ulcer prevalence and severity.

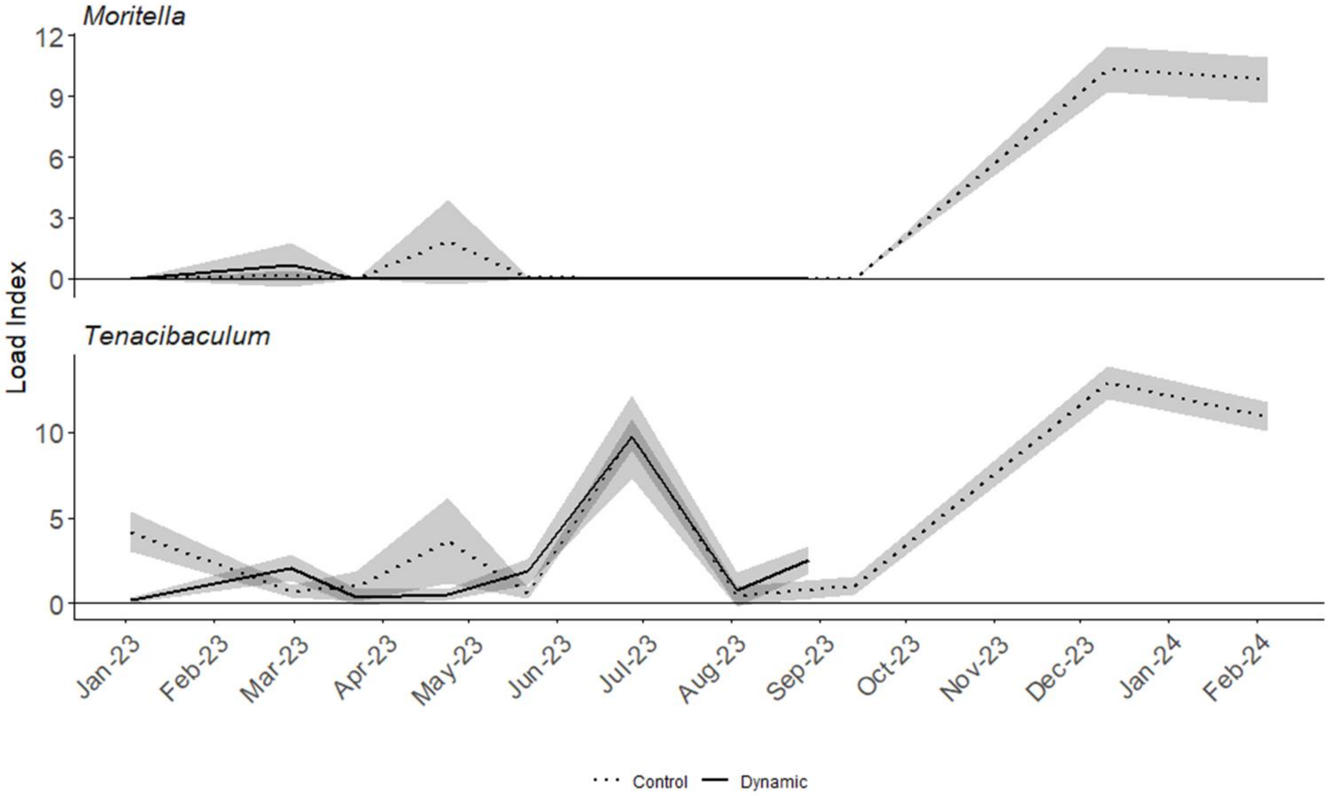

Figure 19. Mean qPCR load index ± 95% confidence intervals for Moritella viscosa and Tenacibaculum sp. in skin mucous samples. Results are shown for control (dotted lines) and test cages (solid lines).

Strong correlations were detected between both *M. viscosa* and *Tenacibaculum sp.* and wound abundance and severity. There was an exponential relationship between *Tenacibaculum sp.* load index and total ulcer score, with a rapid increase in severity with Ct values less than 25 (load index > 12). Total ulcer scores were lower in the test cages than controls, and load index never exceeded 12 (Figure 20).

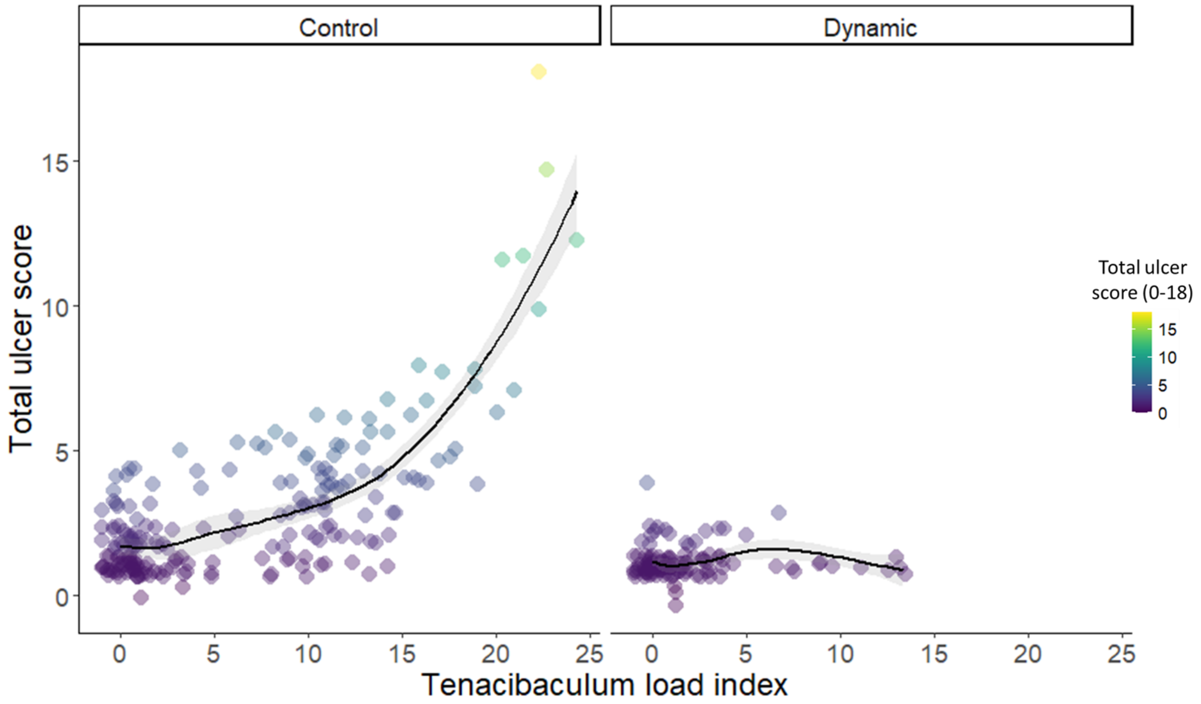

Figure 20. Total ulcer score in relation to study group (Control or Dynamic) and Tenacibaculum sp. load index in skin mucous samples.

In contrast to *Tenacibaculum sp.*, there was linear relationship between *M. viscosa* load and total ulcer score, where greater *M. viscosa* prevalence resulted in higher total ulcer scores (Figure 21). *M. viscosa* load index was considerably lower in test cages compared to controls, with only a single sample reaching a load index of 10 (Ct = 27), while all others were below 5.

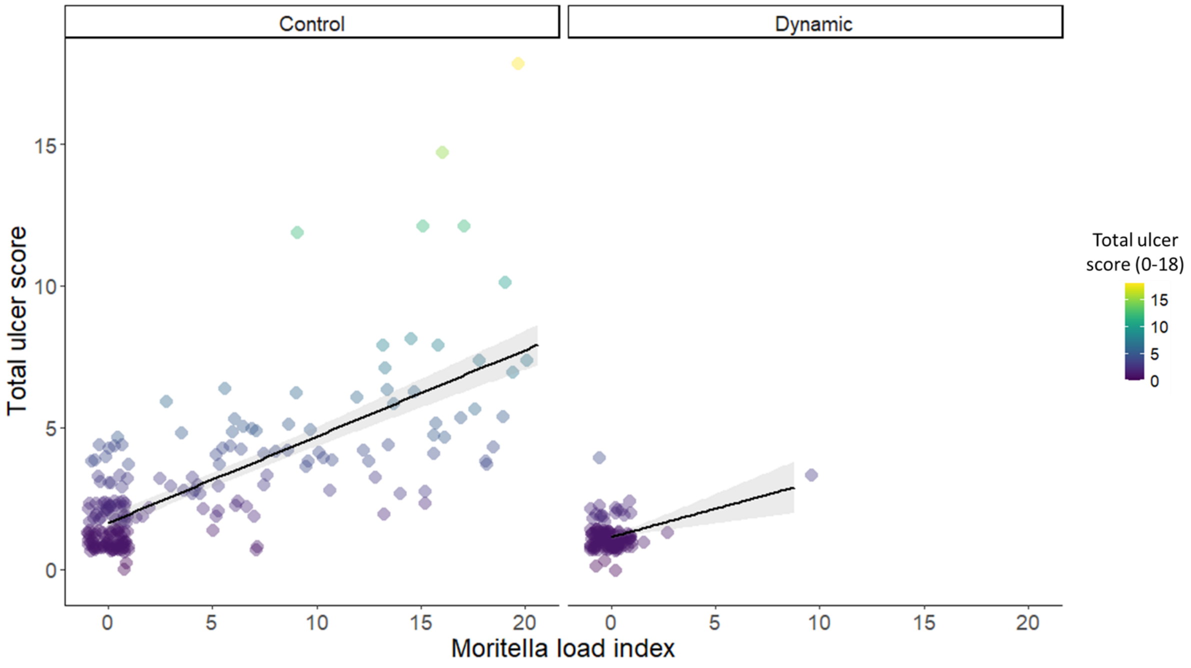

Figure 21. Total ulcer score in relation to study group (Control or Dynamic) and M. viscosa load index in skin mucous samples.

4.3.9 Environment x pathological agents

Despite reasonable variability and seasonal coverage in all parameters, there were no correlations between chlorophyll concentration, turbidity, or phycoerythrin and ulcer severity or abundance. Further, there were also no correlations between the ulcer causing agents, *Tenacibaculum sp.* or *M. viscosa*, and chlorophyll concentration, turbidity, or phycoerythrin (Figure 22).

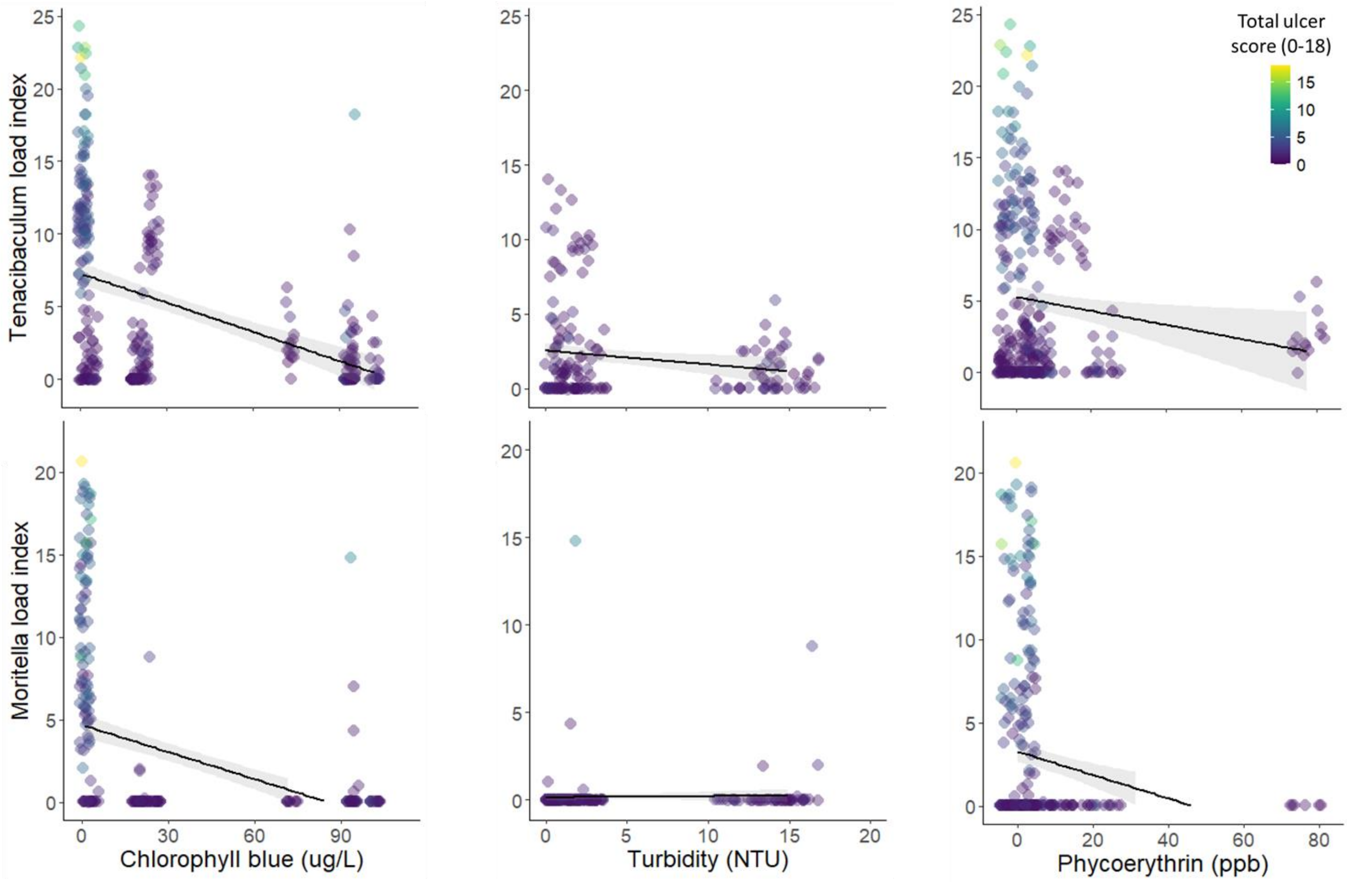

Figure 22. Tenacibaculum spp. (upper panels) and M. viscosa (lower panels) load index from skin mucous swabs in relation to Chlorophyll blue (ug/L), Turbidity (NTU), and Phycoerythrin (ppb). Each point represents an individual fish, coloured according to total ulcer score (0 = purple (minimum), 18 = yellow (maximum).

The only environmental variables which did correlate with total ulcer score and pathogen loads were salinity and temperature. For *Tenacibaculum sp*. the load index decreased with decreasing salinity, with high total ulcer scores (>10) only occurring at salinities higher than 30 ppt. *M. viscosa* was even more sensitive to reduced salinity, with no positive detections from skin swabs at salinities of 30 ppt or less (Figure 23).

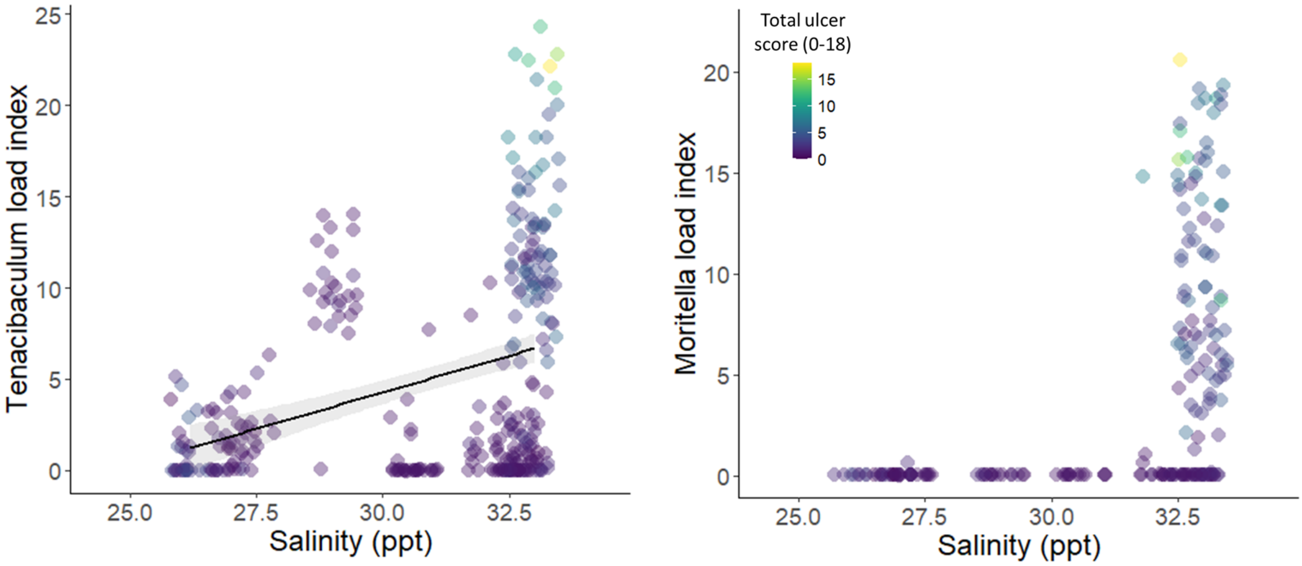

Figure 23. Tenacibaculum spp. and M. viscosa load index from skin mucous swabs in relation to salinity at the time of sampling. Each point represents an individual fish, coloured according to total ulcer score (0 = purple (minimum), 18 = yellow (maximum).

Tenacibaculum sp. was present and abundant at a wide range of temperatures, from 6 – 19 ˚C. However, total ulcer scores only exceeded 5 at temperatures < 10 ˚C. One possible explanation for this is that we employed a generic qPCR test for *Tenacibaculum sp*., and it could be that the more virulent species only thrive at lower temperatures. As with salinity, *M. viscosa* followed the same pattern as *Tenacibaculum sp*., but more extreme. There were no positive detections of *M. viscosa* above 10 ˚C (Figure 24).

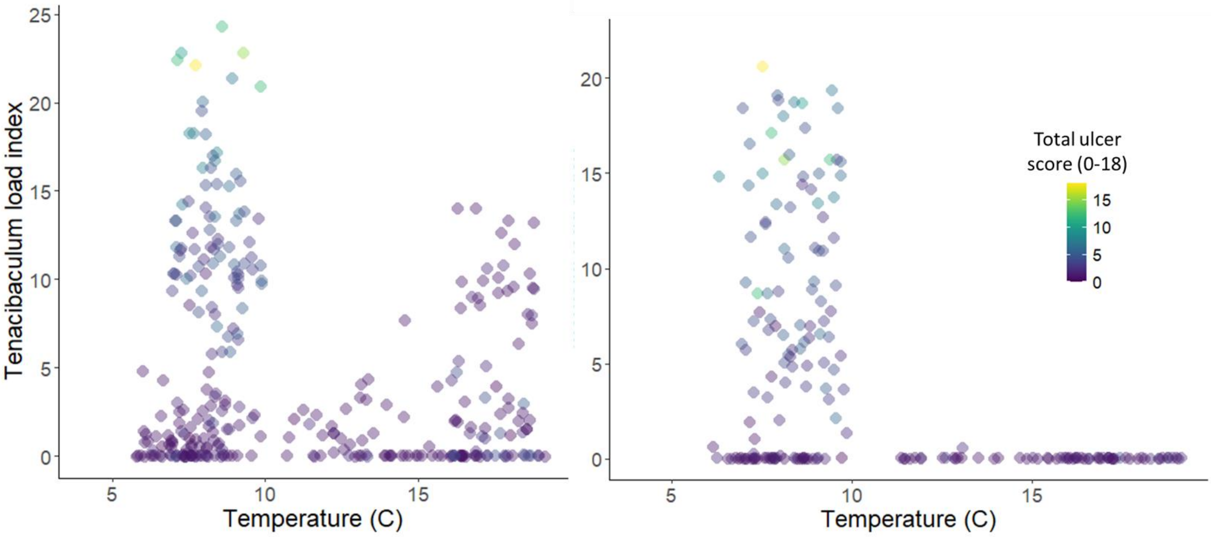

Figure 24. Tenacibaculum spp. and M. viscosa load index from skin mucous swabs in relation to sea temperature at the time of sampling. Each point represents an individual fish, coloured according to total ulcer score (0 = purple (minimum), 18 = yellow (maximum).

4.3.10 Apolemia sp. observations

When nearby farms began reporting acute mortality events related to mass aggregations of *Apolemia sp*. in October 2023 a decision was made to adjust our trial to better understand *Apolemia sp*. distribution and abundance. *Apolemia sp.* observations were performed daily from 1 December 2023 through 15 January 2024 for a total of 360 profiles over 45 days. The number of fragments observed each day was hugely variable, ranging from 0 to 80, and changed quickly (Figure 25).

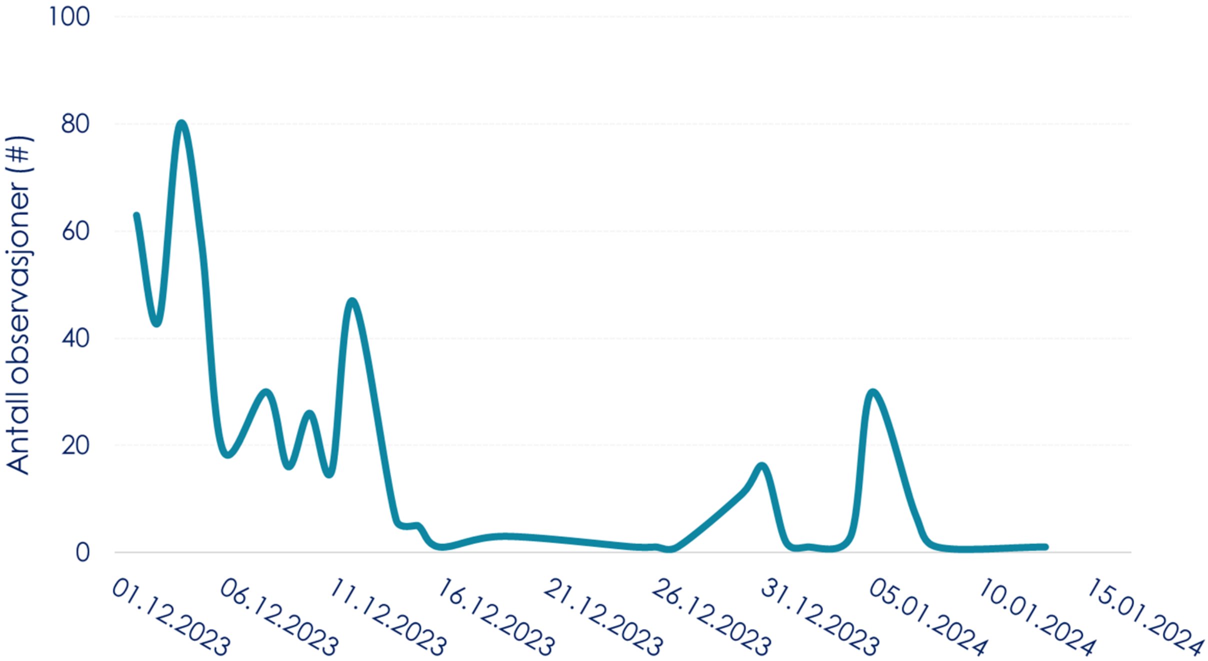

Figure 25. Daily total number of fragments observed in control cages from 1 Dec 2023 through 15 Jan 2024.

The majority of fragments were observed in line with the primary current direction (NW = 45 %, SE = 23 %), while 32 % of fragments were found in the profiles perpendicular to prevailing currents. Fragments were observed throughout the cage, from right at the surface all the way down to 35 m. In the cage without a skirt, 76 % of fragments were above 15 m water depth (Figure 26).

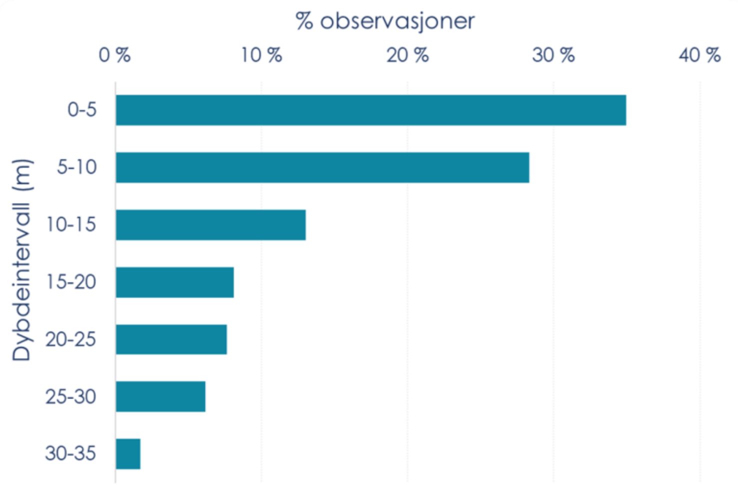

Figure 26. Depth distribution of all Apolemia sp. fragments observed in the control cage.

The use of a 10 m skirt barrier with aeration reduced the amount of Apolemia sp. fragments observed in the cage by more than half (63 %) relative to controls (Figure 27). The largest differences were in the 0-5 m and 5 -10 m depth ranges, where the skirt provided direction protection.

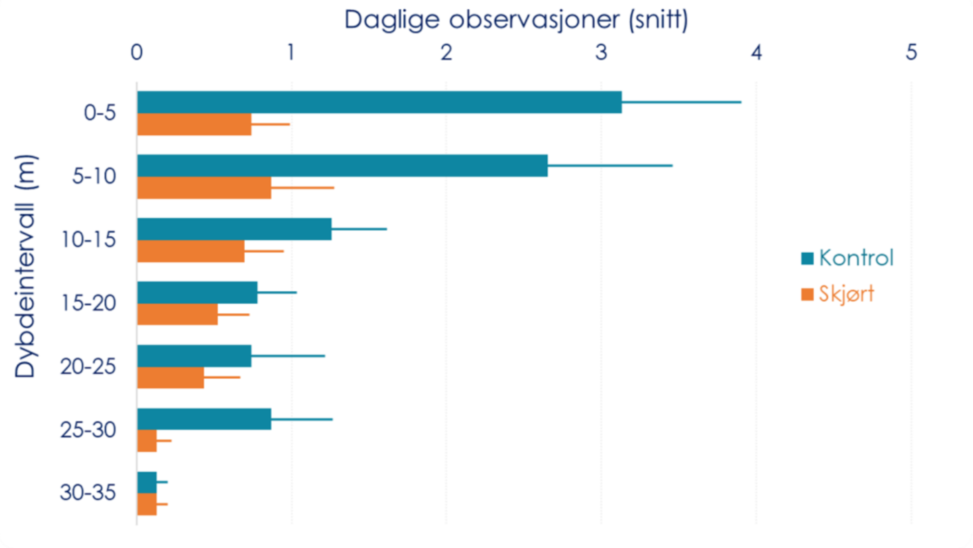

Figure 27. Depth distribution of the mean daily number of fragments observed in control cages (teal) and cages equipped with 10 m skirt barrier and aeration (orange). Data shown are mean ± SEM.

5 WP3 – Examine the influence of key environmental factors on tissue repair and wound healing in salmon

5.1 Impact goals

Although cold temperatures are a known risk factor for ulcerative disease in Norway, millions of fish throughout the country survive winter every year unaffected. While the basic processes of tissue repair and wound healing are well understood in Atlantic salmon[28], only a handful of studies have examined how extrinsic factors such as temperature[29] or fish density[30] affect the tissue repair and wound healing process. Understanding host susceptibility, and how fluctuations in environmental parameters affect tissue repair and wound healing, is key to mitigating the impacts of physical damage sustained by fish during de-lousing and other handling events.

The objective of WP3 is to determine the individual and combined impacts of variations in salinity, dissolved oxygen, daylength and swimming speed on host susceptibility. Specifically, (1) examine how each environmental variable individually influences the rate of tissue repair and wound healing, (2) define the optimal conditions for tissue repair and wound healing, and (3) determine if the combined impacts of sub-optimal conditions for tissue repair interact additively, antagonistically or synergistically.

5.2 Methods

5.2.1 Ethics statement

The following experiments were conducted at the Institute of Marine Research's Matre Research Station (61°N), Norway, and approved by the Norwegian Animal Research Authority according to prevailing animal welfare regulations (FOTS ID 30295).

5.2.2 System preparation

The entire system, including header tanks, pipes, and experimental tanks, was thoroughly cleaned and disinfected prior to the start of the experiment. Also, in addition to the standard drum filtration and water treatment of the facility, ultra-violet lamps were installed in each of the header tanks to provide disinfection throughout the experiment. Tanks were grey, 1 m x 1 m square, 800 L volume. Both experiments were run at 9 °C, a low enough temperature to be relevant for the entire Norwegian coast, but high enough to allow wound healing and tissue repair to occur. Fish in all treatment groups were fed daily, in excess during natural daylight hours, according to identical protocols (Skretting, Norway).

5.2.3 Experiment 1

A total of 1215 mixed sex fish (359 g) were divided equally between 27 experimental tanks (n = 45 fish/tank). Nine different environmental conditions were setup in triplicate tanks according to Table 2. The fish were maintained on these conditions for two weeks prior to 20 fish/tank been sedated on 100mg/L Finquel® and given a standardised circular wound. Wounds were produced using a 5 mm diameter disposable biopsy punch to cut through the dermal layers down to the muscle. Sampling was conducted 3-, 7-, 14-, and 28-days post-wounding (DPW). At the first three samplings, 5 wounded fish/tank were taken (n = 15/environment), with ten fish/tank (5 wounded and 5 unwounded) at the final sampling.

Table 2. Environmental conditions in treatment groups during wound healing experiment 1.

Group name	O_2	Photoperiod	Salinity	Current (TB s^{-1})
Control	100 %	LD 12:12	35 ppt	Low – 1.2
DO-60	60 %	LD 12:12	35 ppt	Low – 1.2
DO-75	75 %	LD 12:12	35 ppt	Low – 1.2
Light-0	100 %	LD 0:24	35 ppt	Low – 1.2
Light-24	100 %	LD 24:0	35 ppt	Low – 1.2
Salinity-22	100 %	LD 12:12	22 ppt	Low – 1.2
Salinity-28	100 %	LD 12:12	28 ppt	Low – 1.2
Speed-med	100 %	LD 12:12	35 ppt	Medium – 1.4
Speed-fast	100 %	LD 12:12	35 ppt	Fast – 1.5

5.2.4 Experiment 2

A total of 1440 mixed sex fish (530 g) were divided equally between 32 experimental tanks (n = 45 fish/tank). Eight different environmental conditions were setup in quadruplicate tanks according to Table 3. The current speed was equivalent to the low group in experiment, 1.2 tailbeats s^{-1}. The fish were maintained on these conditions for two weeks prior to 20 fish/tank being sedated, PIT tagged and given a standardised wound as in experiment 1. Sampling was conducted 7-, 13-, and 22-DPW. At the first sampling, 5 wounded fish/tank were taken (n = 20/environment), while at the second and third it was 10 fish/tank (5 wounded and 5 unwounded).

Table 3. Environmental conditions for the 8 treatment groups in experiment 2. Healing was expected to be the fastest for those in bold.

Group name	DO	Photoperiod	Salinity
DO90 34ppt 24L	**90**	LD 24:0	34
DO90 22ppt 24L	**90**	LD 24:0	**22**
DO90 34ppt 0L	**90**	**LD 0:24**	34
DO90 22ppt 0L	**90**	**LD 0:24**	**22**
DO55 34ppt 24L	55	LD 24:0	34
DO55 22ppt 24L	55	LD 24:0	**22**
DO55 22ppt 0L	55	**LD 0:24**	34
DO55 22ppt 0L	55	**LD 0:24**	**22**

5.2.5 Sampling

Fish were starved for 24 hours prior to each sampling. Immediately prior to collection, fish were lightly sedated by adding 2 mL Aqui-S to each tank. Once the fish were calm and minimally responsive to the presence of the net, they were caught and either euthanized via anaesthetic overdose (0.5 g/L Finquel™), or fully sedated (100 mg/L Finquel). For all fish, the length and weight were recorded, wounds were photographed, and fish welfare scored according to the FISHWELL morphological scoring system (Noble et al. 2018). Additionally, a plasma sample was taken from the caudal vein and a 10 x 10 x 5 mm sample consisting of the wound and surrounding tissue was collected for histological analysis from the euthanized fish with wounds. Histological samples were immediately placed in Davidson's seawater fixative for 48 hours prior to transfer to 70 % ethanol. The sex and maturity status of the fish was recorded based on a macroscopic assessment of the gonads. Females were recorded as either immature (relatively small orange ovary) or sterile (very small ovary, grey in appearance) while males were recorded as either immature (relatively small string-like testes) or pubertal (increase in relative size and change in coloration of the testes).

5.2.6 Gross wound measurement

Photographs of the wounds on all fish were taken with a 1 cm scale at each sampling using a handheld camera (Olympus TG-5). Using the ImageJ software program (U.S. National Institutes of Health, Bethesda, MD, USA), wound area and total affected area of all lesions were measured.

5.2.7 Histological analysis

Tissue samples were dehydrated in a graded ethanol series Thermo Scientific Excelsior tissue processor (Thermo Scientific Excelsior AS), embedded in paraffin Histowax (Histolab, Askim, Sweden) using a Tissue–Tek, TEC 5 (Sakura, Alphen aan den Rijn, The Netherlands) embedding center. Samples were trimmed carefully to the visually estimated center of the lesion, reaching approximately maximum diameter of the ulcer. Embedded tissue was sectioned at 2 µm using a Leica RM 2255 Microtome (Leica Microsystems, Buffalo Grove, IL, USA) and stained with Alcian Blue-Periodic acid-Schiff (AB-PAS). The stained slides were scanned in an Aperio GT-450 scanner and examined using Aperio ImageScope® (Leica microsystems). For all analyses, three areas of the epidermis were established for each sample, 1mm in the affected wound area as well as 1mm either side of the wound as negative controls (unaffected area) from the same fish (Figure 28). In each area, epidermal thickness (µm) and the number of goblet cells were measured. Total wound area (µm2) was quantified as the area with lesions in the subcutis, dermis, and skeletal muscle (Figure 28). An additional set of semi-quantitative parameters were scored from 0 to 4, (0 = nothing, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe), including inflammation, necrosis, bleedings, fibrin, connective tissue, melanin, and infectious agents.

Figure 28. Example section demonstrating the three measurement areas established for each sample (wound – orange, right margin – green, left margin – blue), as well as the total wound area measurement (red).

5.2.8 Data analysis

The data were analysed using R Statistical software (version 4.4.2 “Pile of Leaves”, R Core Team, 2024). Throughout, residual diagnostics were done using the simulation-based functions available in the “DHARMa” package (Hartig, 2025) to identify models that do not meet the expected assumptions of normality and variance, significance was set to p <0.05, and unless otherwise stated, quantitative data are presented as estimated marginal means (EMMs) with their 95% confidence intervals (CI) calculated from the models described below using the “emmeans” function within the “emmeans” package (Lenth 2021).

To assess for environmental effects on quantitative data, the “lmer” from the “lme4” package was used to run linear mixed-effects models (LME). In experiment 1, the fixed effects were DPW (4 levels, 3, 7, 14, and 28), the environmental variable of interest (3 levels/variable. For oxygen saturation, DO 60, DO 75, DO 100; for current speed, low, medium, high; for salinity, 22,ppt, 28 ppt, or 35 ppt; for photoperiod, LD0:24, LD12:12, or LD24:0) with tank included as a random effect on the intercept. The fixed effects were allowed to interact in a 2-way interaction (DPW × environment). Post-hoc tests to assess environmental effects within DPW were done using emmeans with a tukey’s correction for a comparison of 3 estimates. When LME model assumptions were not met, the “rlmer” function within the “robustlmm” package (Koller 2016) was used to perform robust linear mixed-effect models. These assign weights to the model residuals, with the most outlying being the most downweighed.

5.3 Results

5.3.1 Experiment 1

Fish weight & condition

Neither fish weight nor condition factor changed significantly in most treatment groups throughout the 28 day duration of the experiment, with the exception of the 60 % low oxygen and 24 hour light groups. By 28 dpw fish in the 60% DO group were significantly smaller than the 75 and 100% groups, while the fish in 24 hour light had significantly lower condition factor (Figure 29).

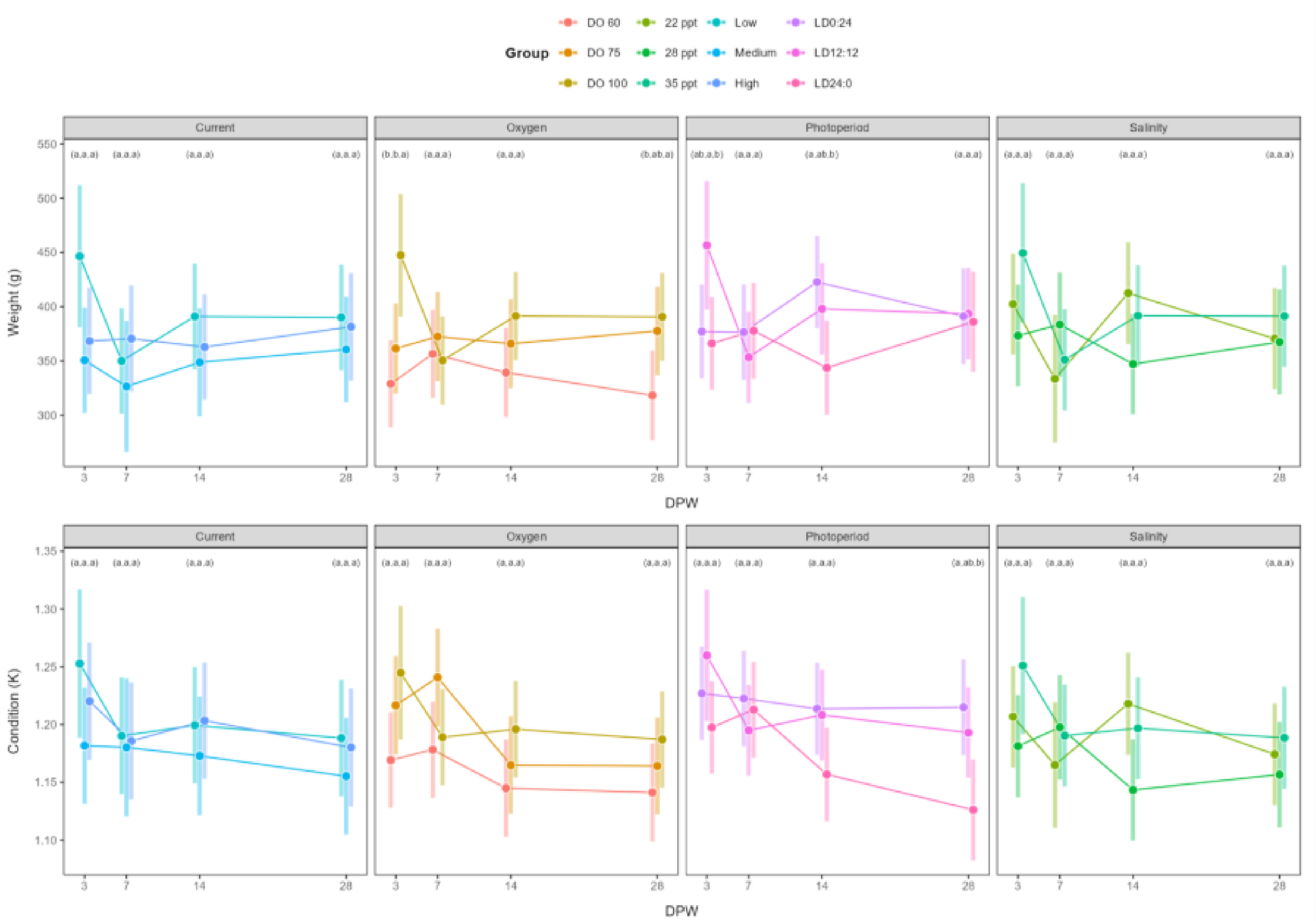

Figure 29. Weight and condition data from experiment 1. Current speed groups are shown in blue, oxygen saturation in orange, photoperiod in pinks, and salinity in greens.

Wound size assessed by photography

In general, wound area in the control increased by ~40% between day 3 and 7, a further ~2% between day 7 and 14, and then decreased by ~70% to the lowest values from day 14 to day 28 (Figure 30). A similar pattern was evident in the other groups, with peak values on days 7 or 14, and the lowest values on day 28.

All the environmental factors explored had significant impacts on wound area (Figure 30). In general, the highest current speed and lowest oxygen saturation led to the largest wound areas of the experiment, which were 37 and 57% greater than in the control on day 7, respectively. However, there were no other significant current or oxygen group differences on any other day. Within the photoperiod comparison, long-day had the largest wound sizes on all days, and the greatest relative difference to the control when the wound was significantly 70% larger on day 28. For salinity, wound area tended to be negatively associated with salinity from day 7 onwards, and on days 7 and 14 this was significant with the 22 ppt group having 20 and 22% smaller wound areas than the controls, respectively.

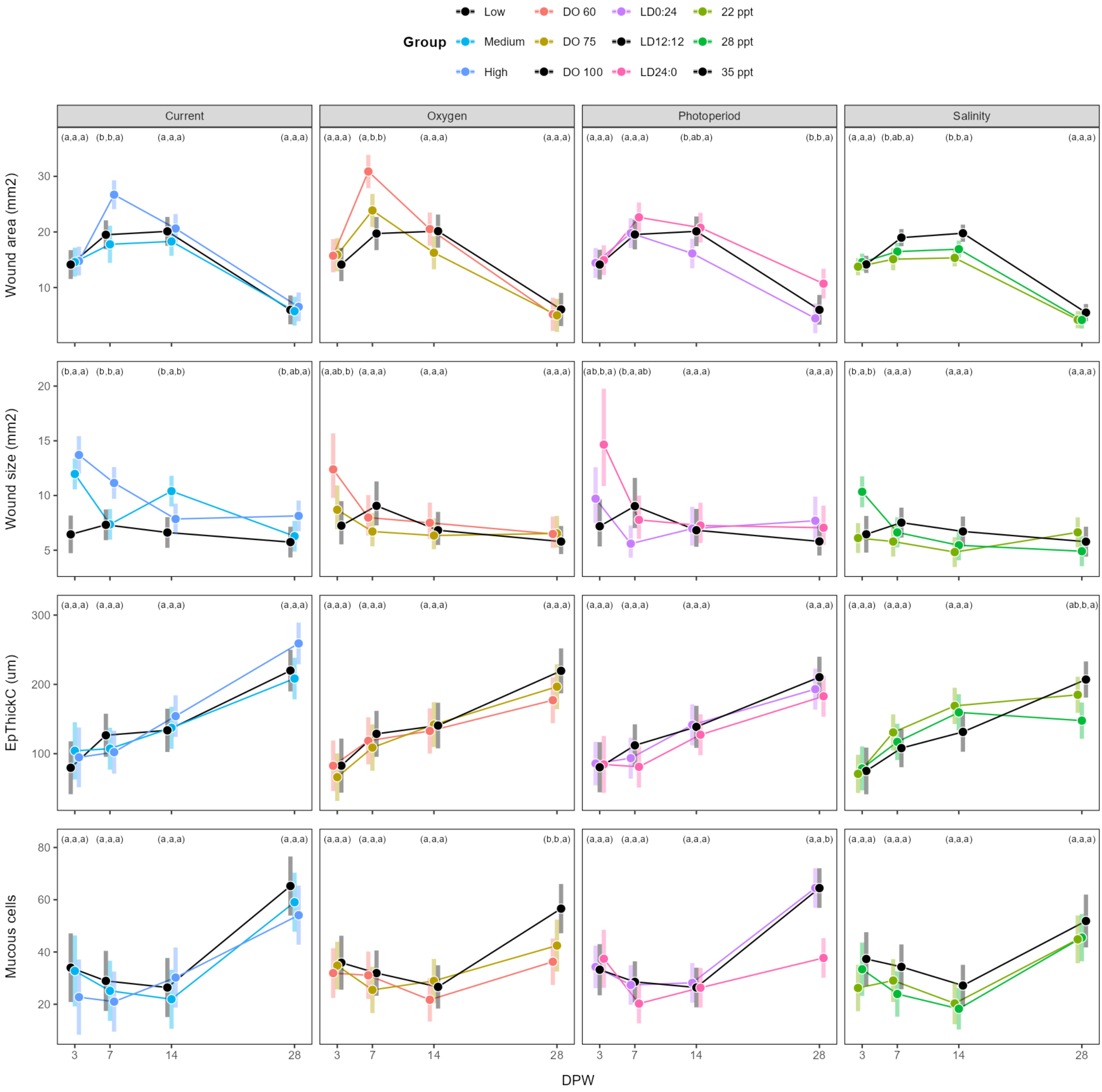

Figure 30. Results for wound area (photographic) and quantitative histology endpoints. Current speed groups are shown in blue, oxygen saturation in orange, photoperiod in pinks, and salinity in greens. The 'control' level in each group is shown in black. Rows display, from top to bottom, wound area as measured from photographic analysis, wound size as measured in histological sections, epidermal thickness mid-wound, and number of mucous cells mid-wound.

Wound histology

In general, total wound area in the controls increased by ~25% from day 3 to its peak on day 7, before decreasing by ~35% to its lowest value on day 28 (Figure 30). In all the other groups the general peak was on day 3, except 28 ppt which peaked on day 28. The medium and high current speeds led to significant increases of 86 and 113% over the controls on day 3, respectively. It remained significantly elevated in the high current environment on day 28, being 42% larger than in the controls. Long-day, 60% oxygen saturation, and 28 ppt, also resulted in significant increases in total wound area on day 3 of 104, 71 and 60%, respectively, but no significant group differences were observed at any other timepoint. A correlation analysis of wound area determined by photography and histology was highly significant (Spearman's rank, $p < 0.001$) but had a rather weak correlation coefficient of 0.23.

Epidermal thickness increased over time (Figure 30). The only significant difference to the control was a 29% reduction in the 28 ppt group on day 28. Mucous cells generally decreased from day 3 to 14 and then increased to their highest levels on day 28 (Figure 2D). The exception being the long-day group where the levels on day 28 were lower than the value on day 3 and significantly 41% lower than the control.

Several semi-quantitative parameters were also scored using an ordinal scale from 0 to 4 (Figure 31). Bleeding initially increased after wounding, peaking at 7 dpw, with bleeding most severe in the DO-60 and fast current speed groups. At the same time, fish in the 22 and 28 ppt groups had considerably less bleeding. Connective tissue was absent until 14 dpw. By 28 dpw the presence of connective tissue was similar in all groups. A large amount of inflammation was present in all groups at 3 and 7 dpw, and began declining at 14 dpw. At 7 dpw inflammation was less severe in the 0 light and 22 ppt salinity groups, while the rest were similar. Melanin was largely absent at 3, 7, and 14 dpw, but ubiquitous in all groups at 28 dpw except 28 ppt salinity. Necrotic tissue was common in all groups at 3, 7 and 14 dpw, and largely absent by 28 dpw. At 14 dpw necrosis was notably more severe in the 24 hour light, 22 ppt and 28 ppt salinity groups compared to the rest.

Plasma

The environmental impact on plasma biochemistry in wounded fish was followed throughout, while a comparison of wounded versus unwounded fish was made on day 28. Nearly all the plasma variables showed variation in relation to time and an interaction with environment. Notably, the DO 60 group had elevated chloride and sodium values on day 7. Based on regression analysis, none of the variables showed any notable association with wound size. In the wounded versus unwounded comparison, plasma lactate and potassium showed the most generic trends with wounded fish having lower values across all environments. Plasma lactate was 10% lower in wounded fish while potassium was 19% lower.

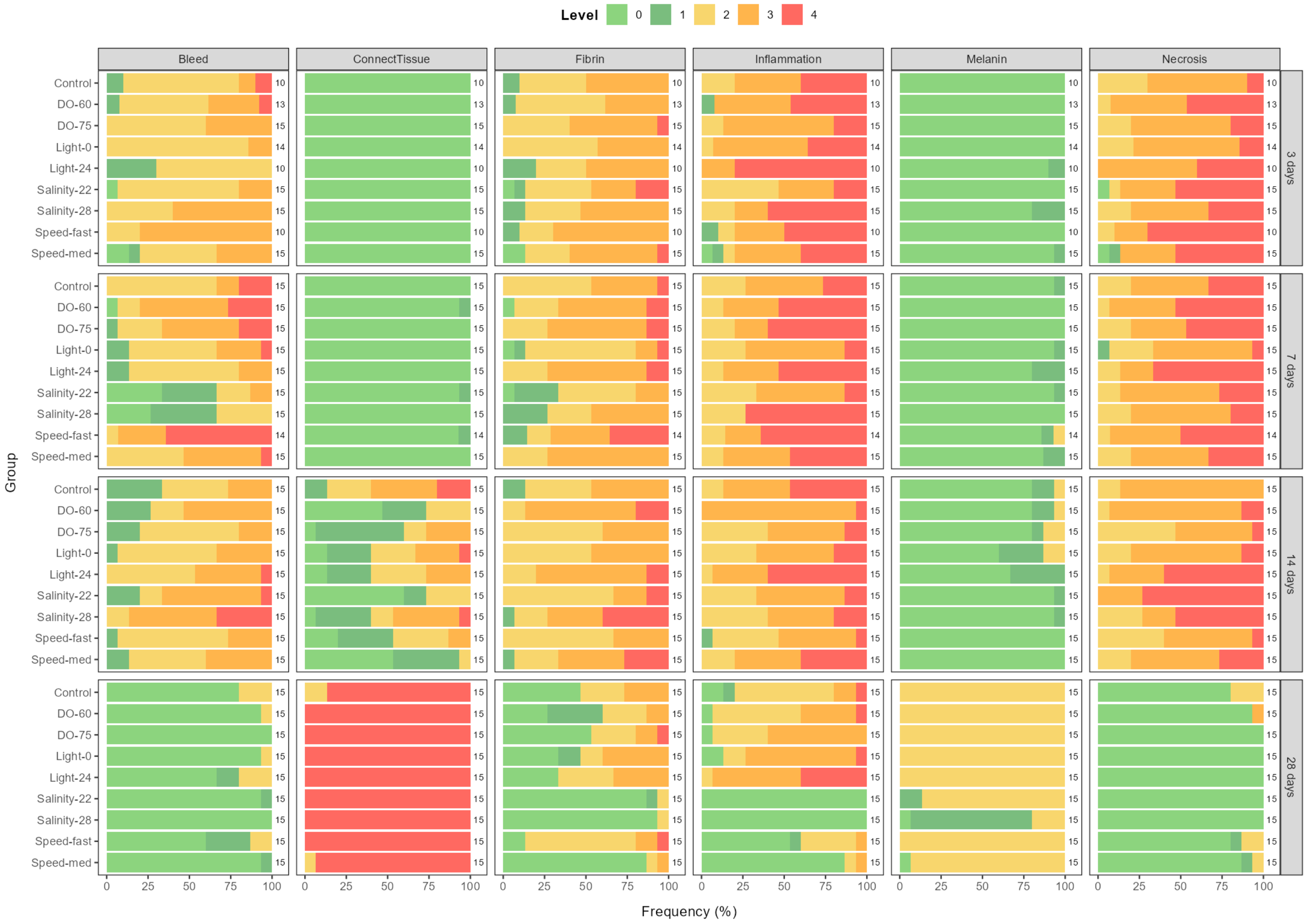

Figure 31. Semi-quantitative (0-4) histological scoring of bleeding, connective tissue, fibrin, inflammation, melanin, and necrosis in wounded fish at four sampling points, 3, 7, 14, and 28 days post-wounding (dpw). Groups are identified on the y-axis by the value of the changed parameter.

Welfare

Backbone deformities, eye bleeding, exophthalmia, jaw deformities, and opercula damage were essentially absent from the fish in this trial. Mild cataracts were present in low quantities in all groups at all sampling points. Similarly, mild to moderate scale loss and fin damage were common in all groups at all sampling points. Gill condition initially worsened with increasing prevalence of light damage between 3 and 14 dpw before peaking and improving again by 28 dpw. Light snout injuries also increased in prevalence from 3 to 14 dpw before stabilizing. Scale lifting was present in all groups at 3 dpw, but mostly gone by 14 dpw. At 3 dpw scale lifting was least common in the control (100% DO, 34 ppt, slow current, 12:12 LD) and 22 ppt groups. Skin haemorrhaging increased from 3 to 7 dpw and was most severe in the DO-60, 24 hour light and fast current speed groups. By 28 dpw there were no differences in welfare scores for any parameter or groups between the wounded and unwounded fish.

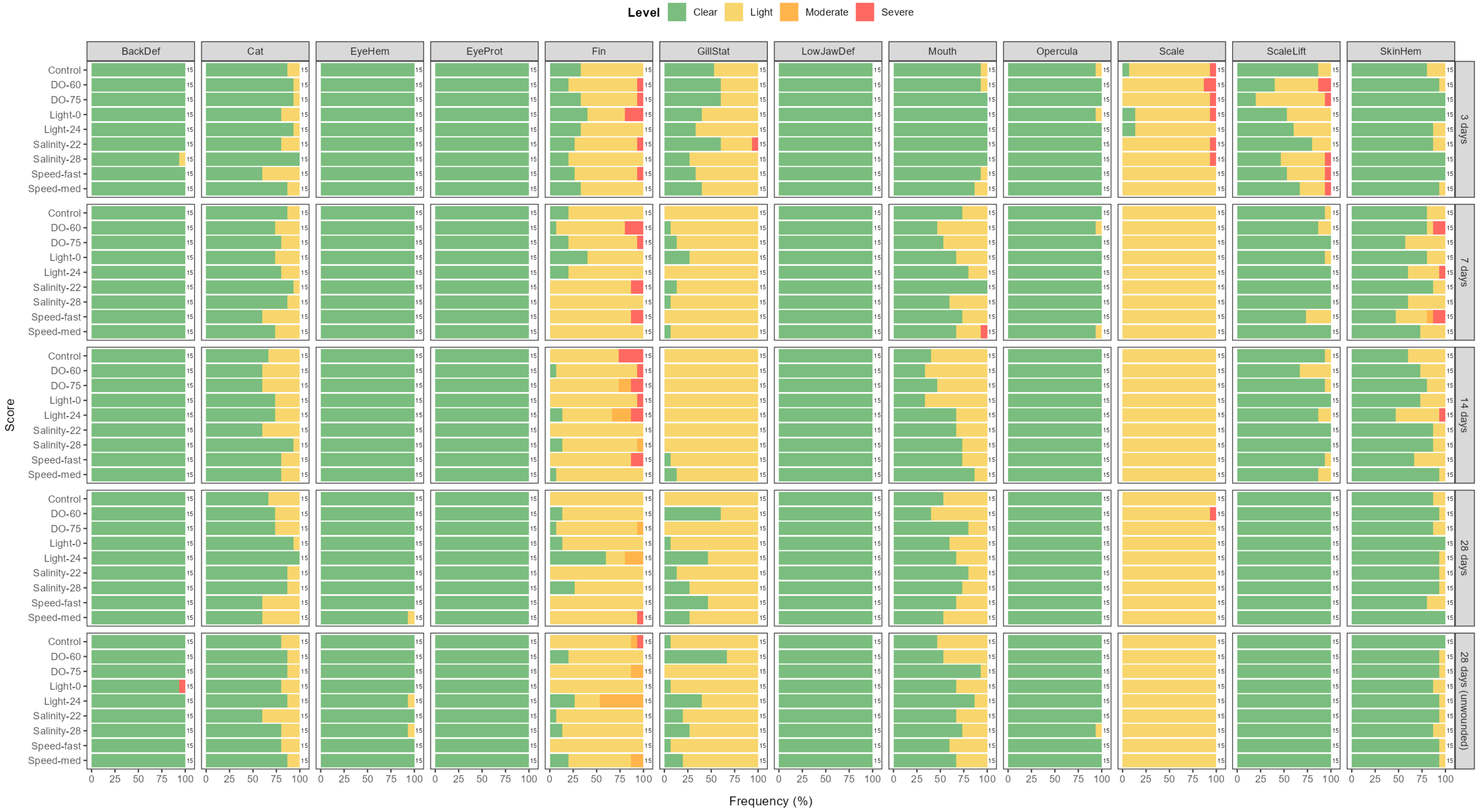
Level
Clear
Light
Moderate
Severe
BackDef
Cat
EyeHem
EyeProt
Fin
GillStat
LowJawDef
Mouth
Opercula
Scale
ScaleLift
SkinHem
Control
DO-60
DO-75
Light-0
Light-24
Salinity-22
Salinity-28
Speed-fast
Speed-med
3 days
7 days
14 days
28 days
28 days (unwounded)
Score
0
25
50
75
100
15
Frequency (%)

Figure 32. Semi-quantitative (0-4) histological scoring of bleeding, connective tissue, fibrin, inflammation, melanin, and necrosis in wounded fish at four sampling points, 3, 7, 14, and 28 days post-wounding (dpw). Groups are identified on the y-axis by the value of the experimental parameter.

5.3.2 Experiment 2

Fish weight & condition

At the start of the experiment the fish weighed 531 g. Over the 22 day course of the trial fish in all groups gained a small amount of weight, with fish in the four 22 ppt salinity groups growing the most, to 654 g on average compared to 606 g in the 34 ppt groups. Fish condition did not change in any groups throughout the experiment.

Wound size assessed by photography

As with experiment 1, wound size increased in all groups between 0 and 7 dpw (Figure 33). At 7 dpw wound area in the 22 ppt salinity groups were on average half the size (12.5 mm^2) of the wound area of fish in the 34 ppt salinity groups (25.7 mm^2). By day 12 when wounds in the 22 ppt groups had begun to shrink but the 34 ppt groups were still expanding, wound area in the 34 ppt groups was 3.5 times larger on average than fish in the 22 ppt groups (Figure 33).

Wound size peaked at 7 dpw for all of the 22 ppt groups, only marginally larger than at day 0. Within the group, the 55 % DO saturation groups were the largest. In contrast, all of the 34 ppt groups increased in wound size up until 13 dpw, at which point the 34 ppt, 55 % DO, 24 hour light group was terminated due to welfare concerns. At 7 dpw both of the 55 % DO groups were significantly larger (x1.6) than the 90 % DO groups in 34 ppt. Interestingly, the 50% DO groups in the 22 ppt treatments were only 1.1x times larger than the 90 % DO groups. By 22 dpw there were no significant differences in wound size between any of the remaining groups.

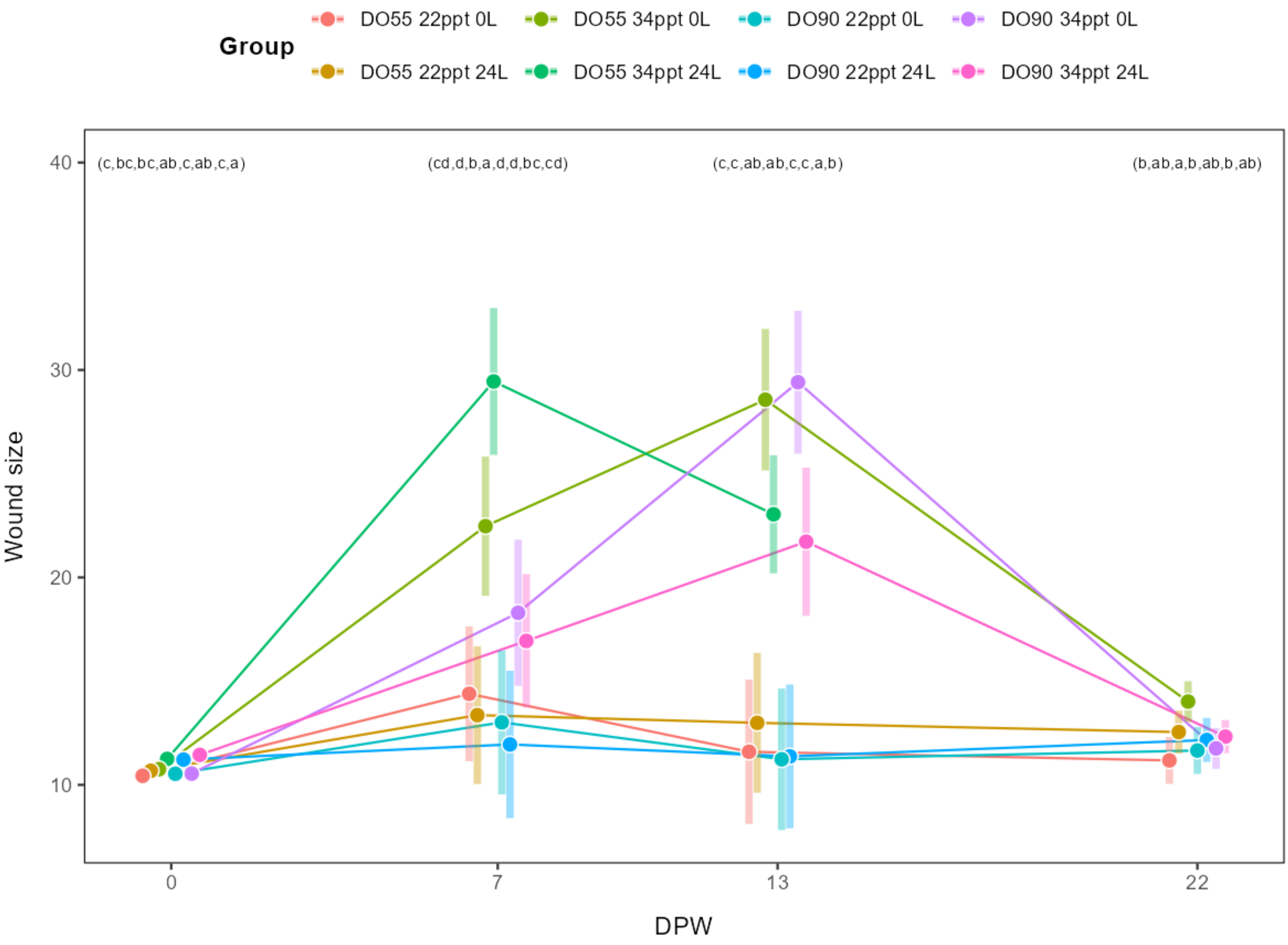

Figure 33. Change in wound area (mm^2) as measured from photographic analysis from 0 to 22 days post-wounding (dpw). Each treatment group is denoted by color.

6 WP4 – Create best practice recommendations for risk-controlled production planning

6.1 Impact goals

Aggregate and summarize the findings from WPs 1-3 into practical, applicable recommendations ready for use by industry.

6.2 Key findings

The costs of winter ulcer disease are substantial and include downgrading of product, increased mortality, reduced growth rate, and reputational impacts. While louse prevention and environmental monitoring require upfront infrastructure investment, the most effective way to reduce the risk of winter ulcer disease after vaccination is minimizing delousing to 3 or less events. As a result, even marginal reductions in louse infestations can provide considerable benefit with regards to winter ulcer disease.

Environmental indicators

- *M. viscosa* only detected at temperatures <10°C and salinities >30 ppt
- Severe ulcers most commonly occur under these combined conditions
- *Tenacibaculum spp*. shows broader tolerance (6-19°C) but severe disease only at <10°C and >30 ppt
- Oxygen saturation of 60% resulted in 1.5x larger wounds at 7 days after injury
- Sub-optimal oxygen synergizes with other stressors (wounds 60% larger at 55% DO in 34 ppt vs only 10% larger at 22 ppt)
- Even low densities of *Apolemia sp.* increase the risk of winter ulcer disease.
- Chlorophyll concentration, turbidity, and phycoerythrin showed NO correlation with pathogen abundance or ulcer severity. These parameters have NO predictive value for winter ulcer risk.

Handling frequency

- **Events 1-2**: Relatively low risk for ulcer development (no increase in wound frequency)
- **Event 3**: Moderate increase in wound frequency begins with all treatment methods
- **Event 4+**: HIGH RISK - significant increase in wound frequency regardless of method or temperature

Delousing method selection

Physical Removal Methods (Thermal & Mechanical)

- **Mortality Risk**: ELEVATED (especially in already compromised individuals)
- **Wound Frequency Impact**: Minimal direct increase
- **Usage Pattern**: Preferentially used when fish are in better condition (earlier events)
- **Mechanism:** Selects for mortality in vulnerable individuals

Chemical Methods (Freshwater, Medicinal)

- **Mortality Risk**: Lower than physical methods
- **Wound Frequency Impact**: Slight increase after repeated treatments
- **Usage Pattern**: Preferentially used when wounds already present or after multiple prior events
- **Mechanism:** Allows survival of compromised individuals but with increased infection susceptibility

Temperature Considerations:

- Regardless of method: repeated handling poses high risk
- NO evidence that any specific method is "safe" at low temperatures or after 3+ events
- In cold water (<8°C), prioritize freshwater or medicinal methods if high-risk delousing is unavoidable

6.3 Risk controlled production principles

These evidence-based guidelines provide actionable procedures for risk-controlled salmon production. Practical implementation will vary by site characteristics and available infrastructure. Start where you can. Environmental monitoring for data-driven decision making is the foundation of prevention.

Monitoring strategy:

- Monitor continuously using real-time sensors INSIDE cages to identify high-risk periods:
    - Temperature
    - Salinity
    - Dissolved oxygen *t*here can be significantly lower oxygen inside cages than outside.*

- If temperature ≤ 12 ˚C, use skin swabs during weekly lice counts to test for *Moritella viscosa* and *Tenacibaculum* (either spp. or *finnmarkense*) using qPCR.

- Monitor for *Apolemia sp.* presence and abundance daily from September through February
    - Optimal method: vertical ROV profile from surface to bottom of cage. Inside the most exposed cage facing the net wall in the primary current direction.
    - Alternate method: Walk the perimeter of the most exposed cage and count all visible *Apolemia* fragments within 3 m of cage wall. Report

Production practices:

1. Minimize Handling: The single most important modifiable risk factor

- Goal: ≤ 3 delousing events during sea phase
- ANY louse prevention strategy is beneficial if it reduces handling to ≤ 3 events

2. Environmental Optimization:

- Maintain oxygen >8 mg/L at 5m depth inside cages
- Attract fish into brackish layers (<28 ppt) using lights and feeding
  * also good for gill health
- Avoid handling when temperature <10°C
- Post-treatment oxygenation for 14 days to support healing after handling events when temp <10°C

3. Risk-Responsive Delousing:

- Freshwater or medicinal methods preferred in high-risk situations (events 3+, temp <10°C)
- Freshwater preferred if pathogen qPCRs are positive and/or *Apolemia* are present
- Physical methods low risk for events 1-2 when fish are in optimal condition

4. Dynamic louse prevention:

- Reduce delousing frequency by 50% and delayed first treatment by 2 months
- Lower wound scores throughout production
- Must be implemented as complete package (skirts + oxygenation + adjustable lights/feeding + environmental monitoring)
- Skirt use without the rest of the strategy can increase pathogen exposure and worsen the cage environment
- Real-time environmental data necessary for responsive decision-making

7 Deliverables

Production related risk analysis manuscript drafted (WP1)

This deliverable was shifted to a news article which will also present key findings from the rest of the project as the available data was insufficient for peer-reviewed publication. This will be released via the HI communications department in early 2026.

Pathogen abundance and distribution throughout production manuscript drafted (WP2)

There are two manuscripts drafted for this task:

Impact of a dynamic lice prevention strategy on gill health in Atlantic salmon. 2026. Submitted to Aquaculture.

Risk factors associated with ulcer outbreaks in farmed Atlantic salmon. 2026. Submtted to Aquaculture.

Additionally, the results of this study were presented at national and international conferences:

Havbruk 2024. Tina Oldham

Havbruk 2024. Jade Sutton

European association of fish pathologists. 2025. Jade Sutton

Influence of environment on wound healing manuscript drafted (WP3)

Impacts of environmental conditions on wound healing in Atlantic salmon.2026. Submitted to Journal of Experimental Biology.

These results were also presented at:

European association of fish pathologists. 2025. Tina Oldham

Submit administrative final report + final project report

This is the final project report, submitted December 2025 to FHF, to be published on the HI website in early 2026.

Administrative final report is in preparation and will be submitted as soon as all budgeting is finalized.

An overview of the entire project was also presented in two webinars organized by NCE Aquaculture:

Tina Oldham. October 2023.

Tina Oldham. November 2025.

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