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Monitoring and optimising the crowding of Atlantic salmon using health and welfare indicators (CrowdMonitor)

Final report

Photo: Frank Gregersen, Nofima

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Report

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Monitoring and optimising the crowding of Atlantic salmon using emerging health and welfare indicators (CrowdMonitor)

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Monitorering og optimalisering av trenging av Atlantis laks ved hjelp av progressive helse og velferdsindikatorer

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Preface

CrowdMonitor received funding from FHF - Fiskeri- og havbruksnæringens forskningsfinansiering (the Norwegian Seafood Research Fund) under grant agreement 901595. The project was carried out between January 2020 and June 2026 and was a collaboration between Norwegian research institutes Nofima (the Norwegian Institute of Food, Fisheries and Aquaculture Research), and the Institute of Marine Research and also commercial partners Cermaq Norway AS and Grieg Seafood AS. All partners are responsible for the methodology and results outlined in this report.

The reference group included Harald Takle (Cermaq Norway), Henrik Trengereid (earlier MOWI, and now SalmonLivingLab) and Margareth Bergersen (Mattilsynet) and we wish to say a big thank you for their inputs and guidance throughout the project. We also wish to say a big thank you for all the help from both research, technical and operational personnel from all the collaborating partners.

The tank experiments were conducted at Nofima's Research Station for Sustainable Aquaculture at Sunndalsøra and the net pen experiments were conducted at the Institute of Marine Research Matre Research farm at Solheim. We would like to thank the staff at both facilities for their assistance and wonderful help and dedication in making these experiments a success under difficult circumstances (Covid-19). We would also like to thank Mattias Bendiksen Lind at HaVet AS, Stian Amble at Nova Sea AS, and the staff at Nova Sea that participated in the ROV-surveys and helped in creating the ROV risk scale for crowding. We also wish to say a big thank you to Sebastian Aker at Sensor Globe for joining us in one of the commercial net pen crowding events and providing us with the loan of some of their crowd monitoring equipment and access to this data.

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1 Executive Summary

English summary

The CrowdMonitor project “Monitoring and optimising the crowding of Atlantic salmon using emerging health and welfare indicators” aimed to update our scientific and operational knowledge on the crowding of Atlantic salmon using a suite of operational- and laboratory-based welfare indicators (OWIs and LABWIs). Research partners were Nofima and the Institute of Marine Research, both Norway and commercial partners were Cermaq Norway AS and Grieg Seafood AS.

The project was built around five work packages:

- Work Package 1 extended and developed a customised OWI and LABWI toolbox for crowding in tanks and net pens.
- In Work Package 2 and 3 a series of experiments were performed to evaluate a commonly used crowding intensity scale for salmon (Mejdell et al., 2009; RSPCA, 2024) held in experimental tanks and medium-scale marine net pens.
- The results from Work Package 2 show that the welfare impact of crowding small scale experimental groups of salmon to four crowding intensities (levels 1-4 of a commonly used crowding intensity scale for salmon, Mejdell et al., 2009; RSPCA, 2024, corresponding to 100- 255 kg/m^3) did not seem to provoke any severe behavioural or welfare related responses when fish were crowded for two hours by partially draining the tank before sideways crowding the fish using a clam shell grader. Fish progessively aligned against the water flow, especially at crowding intensities 3 and 4 after 1-1.5 hours. When fish were later subjected to two different crowding intensities (165 vs 320 kg/m^3) for 1 vs 3 hours, 3 hour crowding had only a marginal effect upon welfare, provided that the conditions for the fish are otherwise good. **NB.** These experimental settings were very different from commercial crowding operations in tanks that normally rely only on tank draining to crowd the fish, in conjunction with pumping fish out of the tank. We advise caution if one were to generalise these findings to commercial settings. Nevertheless, our results show that Atlantic salmon are, at least intermittently, able to adapt behaviourally and physiologically to high density crowding conditions.
- Peracetic acid was shown to be generally safe for use with a limited systemic impact on gill and skin mucosa, but the general state of the fish should be considered during its application.
- We also demonstrated the utility of new approaches to behavioural monitoring during crowding in experimental tanks. Currently published articles from this task are Lazado et al., (2021) https://doi.org/10.1016/j.aquaculture.2020.735830 and Lazado et al., (2022) https://doi.org/10.3389/fimmu.2022.948897. Two further articles are currently in preparation.
- The results from Work Package 3 revealed that crowding in net pens need not be detrimental to Atlantic salmon welfare, provided that the water environment remains satisfactory and the crowding is sufficiently monitored to detect and avoid hazards such as folds or pockets in the net that can trap the fish. Recommended OWIs from the marine net pen trials include surface and subsurface observation by camera or ROV (especially to check that fish are not touching the net, gasping or burrowing), oxygen conditions in the crowd, as well as monitoring for change in prevalence of visible injuries on the fish. This WP has also developed a first version of a ROV risk scale for crowding. Two articles from this WP are now published and are published open access, see Stien et al., (2024) https://doi.org/10.1016/j.aqrep.2025.103109 and Stien et al., (2025) https://doi.org/10.1016/j.aqrep.2024.102211.
- In Work Package 4 OWI and LABWI crowding toolboxes were then subjected to on-farm evaluations around i) smolt transfer from a commercial hatchery, and ii) crowding in relation to a commercial delousing event. These evaluations showed that the majority of suggested welfare indicators outlined in WPs 2 and 3 were suitable for documenting commercial scale crowding of Atlantic

salmon in commercial scale tanks and net pens. One article is under preparation for submission in May 2026.

- Work Package 5 has produced simple operational recommendations and summaries based upon the CrowdMonitor project results and other scientific state of the art to enable the translation of the project results from science into practice, building upon the format of the FISHWELL Welfare Indicator handbook for Atlantic salmon (Noble et al., 2018). These recommendations are outlined in two separate CrowdMonitor Handbooks for both tanks and net pens, see Noble, Stien et al., (2025a) https://doi.org/10.21357/edhh-5736 and Noble, Stien et al., (2025b) https://doi.org/10.21357/68na-zn54, have been summarised in posters, brochures, an industry webinar in October 2025, and will be the subject of a public workshop in May 2026 to possibly refine their recommendations based upon initial usage.
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Norsk sammendrag

CrowdMonitor-prosjektet «Monitorering og optimalisering av trenging av Atlantisk laks ved hjelp av progressive helse og velferdsindikatorer» hadde som mål å oppdatere vitenskapelig og operativ kunnskap om trenging av atlantisk laks ved hjelp av operative- og laboratoriebaserte velferdsindikatorer (OVIer og LABVIer). Forskningspartnere var Nofima og Havforskningsinstituttet, og næringspartnere var Cermaq Norway AS og Grieg Seafood AS.

Prosjektet var organisert i fem arbeidspakker:

- Arbeidspakke 1 utvidet og utviklet OVI- og LABVI-verktøykasser for velferdsvurdering av fisk under trenging i kar og merder.
- I arbeidspakke 2 og 3 ble det gjennomført en rekke eksperimenter for å evaluere en mye brukt trengings-intensitetsskala for laks holdt i eksperimentelle kar og mellomskala merder.
- Resultatene fra arbeidspakke 2 viste at trenging av småskala eksperimentelle grupper av laks til fire intensiteter (100–255 kg/m³) ikke så ut til å utløse alvorlige atferds- eller velferdsrelaterte responser når fisken ble trenget i to timer ved delvis reduksjon av vannvolum før sideveis trenging med en "clam shell"-grinder. Fisken orienterte seg gradvis mot vannstrømmen, spesielt ved intensitet 3 og 4 etter 1–1,5 time. Da fisken senere ble utsatt for to ulike trengingsintensiteter (165 vs. 320 kg/m³) i 1 vs. 3 timer, hadde 3-timers trenging kun marginal effekt på velferden, forutsatt at forholdene ellers var gode. **NB:** Disse eksperimentelle forholdene var svært forskjellige fra kommersielle trenging-operasjoner i kar, som normalt kun baserer seg på å senke vannstanden i forbindelse med pumping av fisk ut av tanken. Vi anbefaler derfor forsiktighet ved generalisering av disse funnene til kommersiell praksis. Likevel viser resultatene våre at atlantisk laks, i hvert fall periodevis, er i stand til å tilpasse seg atferdsmessig og fysiologisk til trenging ved høy tetthet.
- Pereddikksyre (PAA) viste seg generelt å være trygt å bruke, med begrenset systemisk påvirkning på slimlaget på gjeller og hud, men fiskens helsetilstand bør vurderes før bruk. Vi demonstrerte også nytteverdien av nye tilnærminger til atferdsovervåkning under trenging i forsøkskar. Publiserte artikler fra denne oppgaven er Lazado mfl. (2021) https://doi.org/10.1016/j.aquaculture.2020.735830 og Lazado mfl. (2022) https://doi.org/10.3389/fimmu.2022.948897. To ytterligere artikler er under utarbeidelse.
- Resultatene fra arbeidspakke 3 viste at trenging i merder ikke nødvendigvis er skadelig for laksens velferd, forutsatt at vannmiljøet forblir tilfredsstillende og trengingen overvåkes godt nok til å oppdage og unngå farer som folder eller lommer i nota der fisk kan sette seg fast. Anbefalte OVIer fra merdforsøkene inkluderer overflate- og undersjøiske observasjoner med kamera eller ROV (særlig for å sjekke at fisk ikke berører nota, snapper etter luft eller graver seg ned), oksygenforhold under trengingen, samt overvåkning av endringer i forekomst av synlige skader på fisken. I denne arbeidspakken har vi også utviklet en første versjon av en ROV-basert

risikoskala for trenging. To artikler fra denne arbeidspakken er publisert som åpent tilgjengelige: Stien mfl. (2024) https://doi.org/10.1016/j.aqrep.2025.103109 og Stien mfl. (2025) https://doi.org/10.1016/j.aqrep.2024.102211.

- I arbeidspakke 4 ble OVI- og LABVI-verktøykasser evaluert i fullskala ved to kommersielle operasjoner: i) smolt-utsett fra et kommersielt settefiskanlegg, og ii) trenging i forbindelse med en kommersiell avlusingsoperasjon. Evalueringene viste at majoriteten av velferdsindikatorene foreslått i arbeidspakkene 2 og 3 var egnet til å dokumentere trenging av atlantisk laks i kommersielle kar og merder. En artikkel er planlagt innsendt i mai 2026.
- Arbeidspakke 5 har produsert enkle operative anbefalinger og oppsummeringer basert på CrowdMonitor-resultatene og øvrig vitenskapelig kunnskap, for å muliggjøre oversetting av prosjektresultater fra vitenskap til praksis, med utgangspunkt i formatet til FISHWELL-veilederen for atlantisk laks (Noble et al., 2018). Disse anbefalingene er presentert i egne CrowdMonitor-håndbøker for både kar og merder, se Noble, Stien mfl. (2025a) https://doi.org/10.21357/edhh-5736 og Noble, Stien mfl. (2025b) https://doi.org/10.21357/68na-zn54. De er også oppsummert i plakater, faktaark, et bransjewebinar i oktober 2025, og vil være tema for en offentlig workshop i mai 2026 der anbefalingene muligens vil bli justert basert på tidlig brukererfaring.

2 Extended Summary of Main findings

Some of the main findings of the project regarding tank crowding and net pen crowding have already been outlined in Lazado et al., (2021), Lazado et al., (2022), Stien et al., (2024) and Stien et al., (2025) and the CrowdMonitor handbooks (Noble, Stien et al., 2025a and 2025b).

WP2 – small scale crowding of salmon post-smolts in experimental tanks using a clam shell sideways crowding method

- **Effect of crowding intensity** on salmon post-smolts (ca. 530 g) crowded in triplicate tanks for 2 hours to crowding intensities 1-4 (after Mejdell et al., 2009; RSPCA, 2024) that corresponded to ca. 100, 130, 180, 255 kg/m^3.
  - Growth: Fish subjected to crowding intensity 1 (ca. 100 kg/m^3) grew marginally but significantly better than fish subjected to crowding intensities 2,3, and 4 after the crowding event.
  - Acute stress responses: In the fish sampled 1 hour after crowding, significant increases in blood cortisol and magnesium were observed. These had returned to pre-crowding levels in fish sampled after 24 hours.
  - Injury-based OWIs: no systematic and significant differences were observed between crowding intensities. However, a tendency toward increased OWI score levels in pectoral and dorsal fins was seen intermittently. Likewise, a tendency toward more scale loss was seen intensities 2, 3, and 4, as compared with intensity 1.
  - Skin and gill histology: Minor skin changes were observed, including changed pigmentation, an increase in epidermis fragmentation and a decrease in thickness, as well as increased mucus production after 168 hours. No differences were observed in the gills.
  - Heart rate: increased to about 65 BPM during crowding.
  - Behavioural analyses showed some differences between intensities.
    - Contact between fish: higher at level 4 compared to intensities 1-3.
    - Swimming activity: minor changes during level 1 crowding, more activity at levels 2-4.
    - Fish alignment against the water flow became particularly pronounced after 1-1.5 hours at the front of the crowd in crowding intensities 3 and 4 and fish generally avoided the rear of the crowder aside from at intensity level 1.
  - **The fairly gentle method of partially reducing water volume before sideways crowding the fish using a clam shell grader type crowder allowed the fish to always have some space to move and not be forced above the water surface. This meant crowding did not seem to provoke any severe behavioural or welfare related responses when fish were crowded for two hours. After initial disruption at the start of the crowd, fish became more organized within the crowding volume, holding station against the current, especially at higher crowding intensities after 1-1.5 hours.**
  - **NB.** Commercial crowding operations in tanks usually rely on just lowering the water level, in conjunction with pumping fish out of the tank. Nevertheless, our results show that Atlantic salmon are, at least intermittently, able to adapt behaviourally and physiologically to high density crowding conditions.
- **Effect of crowding duration (1 vs 3 hours)** on salmon post-smolts (ca. 1340 g) crowded in triplicate tanks at either crowding intensity 2 vs 4 (after Mejdell et al., 2009; RSPCA, 2024) that corresponded to ~160 vs 320 kg/m^3.
  - Growth, Acute stress responses, Injury-based OWIs, Skin and gill histology: no significant effects of crowding duration or intensity
  - Heart rate: increased to about 60 BPM during crowding. In the 3 hour crowding duration it decreased after about 2 hours.
  - Behaviour: no or minor differences between durations and intensities.

    - **Increasing the crowding duration to 3 hours did not seem to have any major effects upon fish welfare when partially reducing water volume before sideways crowding the fish using a clam shell grader type crowder in our studies, provided that the conditions for the fish are otherwise good.**
- **Effects of Peracetic acid (PAA):** PAA was shown to be generally safe for use with a limited systemic impact on gill and skin mucosa, but the general state of the fish should be considered during its application, See Lazado et al., (2021) https://doi.org/10.1016/j.aquaculture.2020.735830 and Lazado et al., (2022) https://doi.org/10.3389/fimmu.2022.948897.

WP3 – crowding of adult salmon in research scale net pens at differing crowding intensities and developing an ROV underwater crowd monitoring scale

- **Effect of crowding intensity** on adult salmon (ca. 1.5-5 kg) crowded in triplicate net pens for 2 hours to crowding intensities 2-4 (after Mejdell et al., 2009; RSPCA, 2024) across different seasons and fish sizes.
    - Growth: No effect of crowding intensity upon final weight of the fish
    - Mortality: No effect of crowding intensity upon mortality, except in one net pen at level 4 in January. This may illustrate that even though crowding does not inherently lead to overt poor welfare outcomes, the risk increases with intensity.
    - Acute stress responses: increased with crowding intensity.
    - Injury-based OWIs: no systematic and significant differences were observed between crowding intensities.
    - Skin histology: increased damage immediately after crowding. The crowding operation may weaken skin integrity which could be problematic for the fish when they are subjected to further handling.
    - **Steady, slow and controlled crowding limits risk.** Crowding pen-held salmon at the intensities outlined in this project under experimental conditions does not necessarily negatively impact upon fish welfare in the long-term.
    - Recommended OWIs from the marine net pen trials include surface and subsurface observation by camera or ROV (especially to check that fish are not touching the net, gasping or burrowing), oxygen conditions in the crowd, as well as monitoring for change in prevalence of visible injuries on the fish.
    - See Stien et al., (2024) https://doi.org/10.1016/j.aqrep.2024.102211 for a more detailed summary of these results
- **Developing an underwater risk scale for ROVs** based upon video clips collected from commercial crowding operations**.**
    - The preliminary risk scale (termed version 0.1) goes from level 1-5 (e.g. calm swimming, low risk – fish pressed or trapped against the net, high risk), see Stien et al., (2025) https://doi.org/10.1016/j.aqrep.2025.103109 and is operationally outlined in the CrowdMonitor net pen handbook, see Noble, Stien et al., (2025) https://doi.org/10.21357/68na-zn54

WP4: On-farm evaluation of the CrowdMonitor toolbox

OWI and LABWI crowding toolboxes were then subjected to on-farm evaluations around i) smolt transfer from a commercial hatchery, and ii) crowding in relation to a commercial delousing event.

- When documenting **commercial crowding around smolt transfer from large scale tanks:**
    - Crowding intensity increased as the operation progressed and this intensity can be reduced by pumping extra water into the tank.

    - Injury based OWIs: no systematic differences were observed with the exception of minor increases of sampled fish exhibiting level 2 scale loss in the last 30 minutes of the crowd.
    - Acute stress response: Plasma markers showed a distinct stress response over time and fish stress levels progressively increased during crowding
    - Other physiological markers: a gradual increase in plasma lactate and glucose levels during crowding.
    - Crowding also induced changes in the fish plasma osmoregulatory balance.
    - Migration assays with scale explants showed increased migration from 24h to 96 h and stages had normal migration from the scales, and few differences between crowding stages could be detected.
    - Behaviour: a level 1 crowding intensity (after Mejdell et al., 2009; RSPCA, 2024) was maintained for the first hour of crowding as the water was being drawn down and only increased to level 2 at the start of pumping. Increases in intensity to level 3 were due to more white sides being visible in the crowd and this was reduced by pumping in more water by the crowding operator.
    - Surface foam can limit over-water visibility but some behavioural metrics were still detected.
- When documenting **commercial crowding operation in relation to delousing in net pens:**
    - Crowding intensity increased as the operation progressed.
    - Injury based OWIs: no systematic differences were observed with the exception of minor increases sampled fish exhibiting level 2 gill damage in the last 30 minutes of the crowd when crowding levels increased from level 2 to level 3.
    - Acute stress response: Plasma markers showed a distinct stress response over time and fish stress levels progressively increased during crowding
    - Physiological markers: a gradual increase in plasma lactate and glucose levels during crowding.
    - Migration was characterized as normal in all but the fish exposed to crowding for 1h and 40 min. Time is probably not essential here, rather that the fish had previously been exposed to 30 minutes of level 3 crowding intensity, as data from 1h and 20 min of crowding (still level 2 intensity) showed similar response as the 7 min crowding data.
    - Behaviour: a level 2 crowding intensity (after Mejdell et al., 2009; RSPCA, 2024) was maintained for 1 hour and 20 minutes. Increases in intensity to level 3 were primarily due to more white sides being visible in the crowd around the proximity of the floating line. Relationships between surface observations and underwater state were sometimes asynchronous. For example, surface activity along the floating line was sometimes observed, but corresponding footage from the ROV at this time showed there was still space between the net and the fish and e.g., no fish trapped in net pockets. At other times, the water surface was stable, with no surface activity or white sides of the fish, but underwater fish were touching the net and there was a potential risk of fish being pressed against it. We recommend that where possible, underwater observations e.g., from an ROV and surface observations are used together to monitor the crowd.
- **These evaluations showed that the majority of suggested welfare indicators outlined in WPs 2 and 3, involving the monitoring of water quality, surface and sub-surface observations of behaviour and the state of the group, mortalities, appetite and the health status of the fish before handling, and fish injury levels are recommended for steering crowding. As are LABWIs associated with stress monitoring (cortisol) and skin integrity.**

WPs 1-4 formed the basis of the CrowdMonitor Handbooks for both tanks and net pens, see Noble, Stien et al., (2025a) https://doi.org/10.21357/edhh-5736 and Noble, Stien et al., (2025b) https://doi.org/10.21357/68na-zn54

3 Introduction

Welfare is a key sustainability driver for aquaculture production in Norway. In 2023 over 427 million fish were transferred to Norwegian grow out facilities (Norwegian Directorate of Fisheries, 2024) and these fish face numerous operations which can pose health and welfare challenges (Sommerset et al., 2024). Crowding, a process where fish are subjected to a reduced volume to expedite their removal or transfer from a given tank or net pen is a central component of several salmon farming procedures, including i) vaccination, ii) transport, iii) manual grading, iv) medicinal and non-medicinal delousing that involves handling, and v) lice counting, amongst others (see Noble et al., 2018). This operation has been identified as a potential welfare risk in Atlantic salmon (see Espmark et al., 2015; Erikson et al., 2016; Roth, 2016). Fish subjected to crowding may be unable to express certain behaviours or lose behavioural control (Noble et al., 2018), can become stressed (Erikson et al., 2016), become injured (especially with regard to the skin, gills and fins, see Noble et al., 2018), lose mucous cells (Vatsos et al., 2010) and have their immunity suppressed (Veiseth-Kent et al., 2006). Damage to the epidermis and mucosal barrier can also lead to secondary infections such as winter ulcers and fish may also be subjected to suboptimal water qualities such as low oxygen levels, especially if water exchange within the crowd is limited (Noble et al., 2018).

To identify welfare risks and their drivers, we need a set of fit for purpose tools to measure and monitor fish welfare. These tools are called welfare indicators (WIs) which can be applied to the animal itself (originating on or from the fish, Outcome-based WIs) or can be applied to the resources or environment the fish is subjected to (Input-based WIs). These indicators can be further classified as being suitable for farm use (Operational Welfare Indicators, OWIs) or more complex indicators that are sampled on the farm and analysed elsewhere (Laboratory-based Welfare Indicators, LABWIs), see Noble et al., (2018). The suite of OWIs and LABWIs can be tailored to differing species, life-stages, rearing systems and operations, and a fit-for-purpose toolbox of WIs is essential to help stakeholders document crowding risks in order to circumvent them or devise mitigation strategies.

Numerous authors, welfare standards and the industry themselves state that a range of indicators should be closely followed during crowding (e.g., Mejdell et al, 2009; RSPCA, 2024; K.F. Ottem, M. Åsli, Cermaq Norway and B. Seljestokken, Grieg Seafood, pers. comm.) and an OWI toolbox has previously been developed specifically for crowding that utilises a wide range of Input and Outcome-based OWIs (Noble et al., 2018). This toolbox also incorporates a widely used behavioural crowding scale, based on surface observations of crowding intensity that has been developed for Atlantic salmon (Mejdell et al., 2009; RSPCA, 2018, see Table 1).

Although this crowding intensity scale is widely used, it is not without limitations. For example:

i. it only monitors surface activity (giving a limited overview of the fish group and it assumes behavioural challenges identified at the water surface are an indicator for potential problems elsewhere in the crowd),

ii. it was designed for net pen crowding of fish at slaughter and may not be applicable to crowding in other situations (e.g. tanks/wellboats) or potentially other fish sizes,

iii. it is somewhat opinion based,

iv. it has not been evaluated using a wide range of fit for purpose OWIs and LABWIs.

Table 1 An observational scale for quantifying the crowding intensity of Atlantic salmon in marine net pens (developed for fish at slaughter). Classification terminology for salmon is in accordance with the RSPCA (2024) and Mejdell et al., (2009), reproduced with permission.

Level	Atlantic salmon crowding behaviour
1	Fish in the side of the crowd swimming slowly. Normal swimming behaviour, not all in same direction. No dorsal fins on surface. No white sides on surface.
2	Normal swimming behaviour at suction point, low stress. Few dorsal fins on surface. No white sides on surface.
3	Over-excited swimming behaviour (different directions). More than 20 dorsal fins on surface. Some white sides constantly on surface.
4	Over-excited swimming behaviour (different directions). Some fish decreasing activity. Pumping rate: Not possible to keep a constant rate. Many fish stuck up against the crowd net. Many dorsal fins on surface and numerous white sides on surface. A few very lethargic fish.
5	Whole crowd boiling. Potential for large fish kill without rapid release.

This crowding intensity scale is used as the basis for handling regulations and welfare standards. For example, the Norwegian Food Safety Authority (Mattilsynet) and the RSPCA (UK) states a crowding level 1 is the goal and any crowding that is more severe than level 3 is unacceptable (Mattilsynet, 2014; RSPCA, 2024). However, this scale is just one of the existing and emerging welfare documentation tools available for monitoring fish welfare during crowding. For example, there have been dramatic developments in the range, precision and scope of OWIs and LABWIs in recent years. New tools for monitoring cardiac activity such as heart rate loggers (and associated physiological stress indicators) can give us an indicator of the fish's stress response beyond what has currently been measured. Correlating this data with simpler indicators of fatigue e.g. lactate, can help optimise the operational feasibility of the toolbox. Other LABWIs such as semi-*in vivo* skin models can be used to determine if there is any innate and adaptive immune suppression associated with crowding, or skin damage that may reduce healing capacity (see Karlsen et al., 2012; Ytteborg et al., 2025). The tools will give us much more information than we currently have regarding handling.

4 Objectives

The main objective of the CrowdMonitor project was to update our state of the art on the crowding of Atlantic salmon using a suite of OWIs and LABWIs, to provide crowding operators with more objective tools to facilitate and monitor crowding operations.

CrowdMonitor also had several sub-objectives including:

i. giving the farming community and other stakeholders a more detailed evaluation of the existing and well-established behavioural crowding intensity scale that has been developed for salmon (Mejdell et al., 2009; RSPCA, 2024) using data from e.g., echosounders, sub surface cameras, fish tags and a range of additional LABWIs in relation to tank production (Work Package 2) and net pen production (Work Package 3).

ii. examining how a crowding stressor influences the responses of AGD-infected fish to the chemotherapeutic agent peracetic acid (PAA) in synergy with the FHF-funded project PERAGILL (FHF project #901472) in WP2.

iii. the development of a semi-autonomous computer vision tool for continuously monitoring surface behaviour during a crowd (WP2).

iv. evaluating various crowding situations such as differing crowding durations (WP2), fish sizes (WP2 and WP3) and seasons (WP3), using the suite of OWI and LABWI tools.

v. the development of a qualitative observational toolbox for crowding in tanks and net pens (WP2, WP3 and WP5)

vi. the development of a sub-surface intensity scale for marine net pens (WP3 and WP5).

vii. the testing of these OWI and LABWI toolboxes on-farm with the commercial project partners Grieg Seafood and Cermaq Norway in relation to a) the tank-based crowding of smolts in relation to smolt transfer and b) the crowding of pen-held post-smolt salmon in relation to non-medicinal delousing that involves handling (WP4).

viii. the creation of operational recommendations regarding crowding to all interested stakeholders (WP5), see the CrowdMonitor handbooks Noble, Stein et al., 2025a,b).

The outcomes of CrowdMonitor are relevant to all Atlantic salmon farmers (nationally and internationally), fish health services, aquaculture support services, system and technology suppliers, regional and national legislatory agencies (e.g. Mattilsynet), NGOs and other interested stakeholders. The CrowdMonitor toolbox can also be applied to all handling treatments that crowd fish in tanks by tank draining or utilize sweep nets or net lifting to crowd fish in net pens. Results of the project can help farmers define bottlenecks and help the end-user find work arounds or solutions that may eliminate these bottlenecks altogether. It can also be used to inform technology development and farming trends, e.g. by providing an updated OWI and LABWI toolbox for assessing the crowding challenges that fish face in aquaculture, building on the OWI toolbox that has already been developed for crowding (Noble et al., 2018). Outcomes were realised by creating new knowledge about:

- The range of existing and emerging OWIs and LABWIs that can be suitable for crowding.
- The crowding process and different levels of its intensity.
- The effects of changes in crowding and duration on fish welfare.
- The effects of repeated crowding on fish welfare at different fish sizes and under potentially different seasons.
- The effects of crowding on the responses of AGD-infected fish to the chemotherapeutic agent peracetic acid (PAA).

5 Project execution

CrowdMonitor was a collaboration between Norwegian research institutes Nofima (the Norwegian Institute of Food, Fisheries and Aquaculture Research), the Institute of Marine Research and also commercial partners Cermaq Norway AS and Grieg Seafood AS. It was led by Chris Noble of Nofima. Nofima led four work packages in the project: WP1 (David Izquierdo Gomez), WP2 (René Alvestad, formerly led by Åsa M. Espmark) and WP's 5 and 6 (Chris Noble). The Institute of Marine Research led two work packages in the project: WP's 3 and 4 (Lars Helge Stien). Other participants from Nofima included Gunhild S. Johansson, Amritha Johny, Jelena Kolarevic, Carlo Lazado, Gerrit Timmerhaus and Elisabeth Ytteborg, and from IMR included Angelico Madaro and Jonatan Nilsson. Cermaq Norway (Karl F. Ottem, Magnus Åsli, Tirril Slettjord, Vilde C. Alsos) and Grieg Seafood (Berit Seljestokken, Sigrun N. Johannessen) also participated in WP's 4 and 5.

The reference group included Harald Takle (Cermaq Norway), Henrik Trengereid (earlier MOWI, currently SalmonLivingLab) and Margareth Bergersen (Mattilsynet).

CrowdMonitor was organised into six work packages (WPs).

- WP1 evaluated the range of OWI and LABWI tools that were potentially suitable for crowding operations.
- WP2 evaluated the existing Atlantic salmon crowding intensity scale for tanks and consisted of five tasks that investigated: (a) how different crowding intensity levels (1-4 from Table 1) impacted upon fish welfare in small scale experimental flow through tanks, (b) how different crowding durations at different intensities affected fish welfare, (c) how crowding affects the efficacy of chemotherapies for amoebic gill disease (AGD), (d) the development of a semi-autonomous computer vision tool for continuously monitoring surface behaviour during a crowd and (e) the development of a qualitative observational toolbox for crowding in tanks.
- WP3 evaluated the existing Atlantic salmon crowding intensity scale for marine net pens and consisted of one long-term experiment that investigated: (a) how different crowding intensity levels (2-4 from Table 1) impacted fish welfare in nine 12 x 12 m research scale net pens at IMR's marine based research facilities, over (b) different fish sizes and (c) differing seasons. Further objectives of WP3 included (d) the development of a qualitative observational toolbox for crowding in net pens, and (e) the development of a ROV risk scale for crowding in collaboration with HaVet AS and Nova Sea AS.
- WP4 addressed the on-farm evaluation of the CrowdMonitor toolbox at farm partners Grieg Seafood and Cermaq Norway to ensure a critical appraisal of the toolbox in relation to i) different rearing systems and ii) commercial crowding operations
- WP5 generated operational recommendations form the project and had three tasks (a) to update the surface crowding intensity scale for tanks and net pens, (b) develop a new sub-surface crowding risk assessment scale for net pens, and (c) update the existing FISHWELL OWI toolbox for crowding with data from CrowdMonitor. The dissemination format for the recommendations is built upon the FISHWELL handbook (Noble et al., 2018) and is released as separate CrowdMonitor handbooks for tank and net pens (see Noble, Stien et al., 2025a,b). Posters outlining recommendations from the handbooks have also been produced for operational use out on the farm, in addition to operational brochures. A webinar (October 2025) and an in-person workshop (May 2026) will be used to disseminate and refine these handbooks after their initial usage.
- WP6 was responsible for management and dissemination in the project.

The general approach adopted by the CrowdMonitor project was to conduct and scale up in-depth experimental studies to commercial settings to ensure the commercial feasibility and applicability of the results the project generated. To adhere to the 3R principles (reduce, refine, replace), experiments included both *in vitro* and semi-*in vivo* aspects and we paid full attention to an additional potential 4th R, relevance (Sneddon et al., 2017) throughout the lifecycle of the project. Results and approaches developed in WPs 1-3 were discussed with the farm partners, especially with regard to on-farm testing in WP4 and the development of operational recommendations in the CrowdMonitor handbooks (WP5).

6 Findings, discussion, and conclusion

WP1: CrowdMonitor toolbox development

WPL: David Izquierdo Gomez, Nofima. Participants: René Alvestad, Åsa M. Espmark, Gunhild S. Johansson, Jelena Kolarevic, Carlo Lazado, Angelico Madaro, Jonatan Nilsson, Chris Noble, Lars Helge Stien, Gerrit Timmerhaus, Elisabeth Ytteborg.

OWI toolboxes for monitoring operational crowding operations are already available (Mejdell et al., 2009; Noble et al., 2018; RSPCA, 2024). However, these toolboxes do not include LABWIs and technologies that may be suitable for crowding. WP1 involved a literature/web-based search of state of the art on potential OWIs and LABWIs that are suitable for crowding, in addition to technologies and protocols for monitoring OWIs and LABWIs (it also utilised ongoing project portfolios). This resulted in a large set of potential OWIs, both established and potential new OWIs, as a foundational toolbox for crowding. This toolbox was the basis of the OWIs and LABWIs tested for tanks in WP2 and for net pens in WP3. A summary of the foundational toolbox is outlined in Figure 1 below.

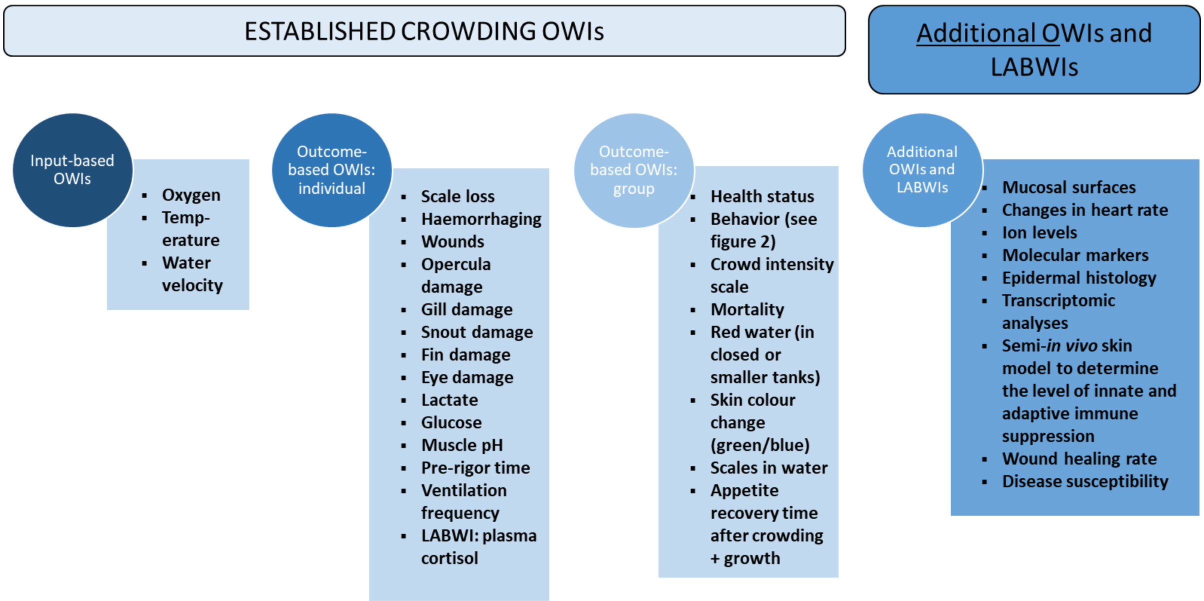

Figure 1 An overview of established crowding OWIs and potentially applicable emerging OWIs and LABWIs for crowding (adapted with permission from Noble et al., 2018).

An updated framework for its application, including potential stages in how to apply the toolboxes, whether the indicators are OWIs or LABWIs and whether they are non-, minimally or invasive for the fish is outlined in the CrowdMonitor handbooks (Noble, Stien et al., 2025 a,b).

WP2: Evaluating the Atlantic salmon crowding intensity scale for tanks

WPL: René Alvestad, Nofima. Participants: Åsa M. Espmark, Gunhild S. Johansson, Jelena Kolarevic, Carlo Lazado, Angelico Madaro, May Britt Mørkedal, Kristin Nerdal, Jonatan Nilsson, Chris Noble, Britt Kristin Reiten, Lars Helge Stien, Rita Storslett, Gerrit Timmerhaus, Elisabeth Ytteborg.

FOTS ID: 25304 and 20/37233

The objective of WP2 was primarily to evaluate the Mejdell et al., (2009) and RSPCA (2024) surface crowding intensity scale for Atlantic salmon in tanks for different fish sizes. It had three key tasks examining: (2.1) how crowding intensity levels 1-4 (see Table 1) impacted upon fish welfare and also correlated each intensity to fish crowding density in the tanks, (2.2) how different crowding durations at different intensities affected fish welfare, (2.3) how crowding affected the efficacy of chemotherapies for amoebic gill disease (AGD). Further tasks included (2.4) the development of a semi-autonomous computer vision tool for continuously monitoring surface behaviour during a crowd and (2.5) the development of a qualitative observational toolbox for crowding in tanks. Task 2.1, 2.2 and 2.4 will be submitted to an aquaculture journal as an open access article during 2026 and results are only briefly outlined here.

WP2.1 evaluating the welfare effects of the existing surface crowding intensity scale for Atlantic salmon in tanks for fixed crowding durations

Objective:
To evaluate the welfare effects of crowding Atlantic salmon in tanks to intensities corresponding to the crowding intensities described by the surface crowding intensity scale (e.g. Mejdell et al., 2009; RSPCA, 2024).

Brief materials and methods:
An experiment was performed in which Atlantic salmon post-smolt were subjected to four different crowding intensities. The crowding intensities were intended to reproduce crowding intensities 1 through 4, as described in the aforementioned crowding intensity scale. The fish were kept in flow-through tanks with full strength seawater (PSU ~32), at 10 degrees Celsius. The fish were about 530 grams when crowding commenced and feed was withdrawn from 2 days prior to the crowding, until 2 days after. Crowding was induced by lowering the water level in the tanks down to 30 cm water height, followed by reducing the tank area and volume available to the fish using a clamshell crowder. The resulting biomass densities during crowding, for each level on the intensity scale, were 1: ~100 kg/m^3, 2: ~130 kg/m^3, 3: ~180 kg/m^3, and 4: ~255 kg/m^3. The crowding lasted for 2 hours. Triplicate tanks were used for each crowding intensity. Samples were collected prior to crowding, 1 hour after crowding, 24 hours after crowding, and about 168 hours after crowding. Length and weight measurements were recorded for each sampled fish. Morphological operational welfare indicators (Noble et al., 2018) were recorded for 15 fish per tank per sampling. Blood samples were taken from 6 fish per tank per sampling for the analysis of cortisol, lactate, glucose, and magnesium concentrations. Gills and skin samples were resected for histological analysis from 9 fish per treatment, at T0, and 24 and 168 hours following crowding. In each tank, 4 fish had sensors (Star-Oddi DST micro-HRT) to record heart rate throughout the experiment. Scales were collected from the outlet of each tank during crowding, to measure the total dry weight of scales lost. HD video data was recorded using a camera placed above each tank. The video data was used for behavioural analysis using a qualitative behavioural analysis toolbox (see WP2.5 below).

Results:
Fish subjected to crowding intensity 1 grew marginally but significantly better than fish subjected to crowding intensities 2,3, and 4. In the fish sampled 1 hour after crowding, significant increases in blood cortisol and magnesium were observed. These had returned to pre-crowding levels in the fish sampled

24 hours after crowding. For morphological OWIs, heart rate, and blood chemistry parameters no systematic and significant differences were observed between treatments. However, a tendency toward increased OWI score levels in pectoral and dorsal fins was seen intermittently. Likewise, a tendency toward more scale loss was seen intensities 2, 3, and 4, as compared with intensity 1. Minor changes were observed in skin samples subjected to histological analysis, including changed pigmentation, an increase in epidermis fragmentation and a decrease in thickness, as well as increased mucus production after 168 hours. No differences were observed in the gills. The heart rate increased to about 65 BPM during crowding. Behavioural analysis from video recordings showed some differences between intensities. For example, with respect to the degree of contact between the fish, crowding intensity 4 provoked a much greater degree of contact than what was seen for intensities 1, 2, and 3. With respect to swimming activity, fish subjected to intensity level 1 displayed only minor changes during the crowding, whilst a greater degree of activity was seen in the other treatments.

Discussion:
The fairly sensitive crowding methods applied, which allowed the fish to always have some space to move and not be forced above the water surface, were seemingly insufficient to provoke any severe behavioural or welfare related responses within the space of two hours. Where differences were seen, they were most pronounced with respect to behaviour. The results should be considered as indicative of the effect of crowding fish to high densities *per se* and are not necessarily indicative of the aggregate effects of crowding in commercial tanks, as these crowding operations are more complex, involving larger tanks and flow rates, greater forces, and more fish. Commercial crowding operations in tanks usually rely on lowering the water level, exclusively, as the crowding is done in conjunction with pumping fish out of the tank (for transport, grading, or vaccination). Nevertheless, the results show that Atlantic salmon are, at least intermittently, able to adapt behaviourally and physiologically to high density crowding conditions.

WP2.2 evaluating the welfare effects of the existing surface crowding intensity scale for Atlantic salmon in tanks for differing crowding durations

Objective:
To evaluate the welfare effects of two different durations of crowding fish in tanks to intensities corresponding to the crowding intensities 2 and 4 in the surface crowding intensity scale (Mejdell et al., 2009; Noble et al., 2018; RSPCA, 2024).

Brief materials and methods:
As a continuation of the experiment in WP2.1, the same fish were kept in the same tanks over 12 weeks before being restocked for the purpose of the WP2.2 experiment. The fish subjected to crowding intensities 1 and 2 in the first experiment were pooled and randomly distributed across 6 tanks. This group of fish were now subjected to a reduction in area and volume corresponding to crowding intensity 2, resulting in a biomass density of about 165 kg/m^3 during crowding. Likewise, the fish subjected to crowding intensities 3 and 4 were pooled and randomly distributed across 6 different tanks. This group of fish were now subjected to a reduction in are and volume corresponding to crowding intensity 4, resulting in a biomass density of about 320 kg/m^3 during crowding. For each of the crowding intensities now applied, 3 tanks were crowded for 1 hour and 3 tanks for 3 hours. Rearing conditions and sampling protocols were the same as before, with the exception that no samples were collected 24 hours after crowding during this experiment.

Results:
For growth, morphological OWIs, blood chemistry parameters, collected scales dry weight, and skin and gill histology no significant differences were seen between treatments. We saw the same patterns in blood glucose and magnesium as in WP2.1. The temporal pattern of the heart rate was different between crowding durations. The heart rate in the fish subjected to 3 hours of crowding seemed to decrease

somewhat after 2 hours, before increasing intermittently when the crowder was removed. The heart rate increased to about 60 BPM upon crowding, somewhat lower than what was observed in the first experiment. There were no or minor differences between treatments with respect to behaviour.

Discussion:
The same points apply as for WP2.1. Increasing the crowding duration to 3 hours did not seem to have any major effects upon the welfare of the fish. There are some indications that the fish somewhat adapt to the new situation, at least intermittently, but this should be investigated further. It should be noted that crowding operations in commercial farms, especially when fish are taken out of the tank to be vaccinated, can last for several more hours, with crowding intensities varying throughout the procedure.

WP2.3 the effect of crowding on the efficacy of chemotherapies for amoebic gill disease (AGD)

This task is published as:
Lazado, C. C., Sveen, L. R., Soleng, M., Pedersen, L. F., & Timmerhaus, G. (2021). Crowding reshapes the mucosal but not the systemic response repertoires of Atlantic salmon to peracetic acid. Aquaculture, 531, 735830 https://doi.org/10.1016/j.aquaculture.2020.735830
Lazado, C. C., Strand, D. A., Breiland, M. W., Furtado, F., Timmerhaus, G., Gjessing, M. C., Hytterød, S., Merkin, G. A., Pedersen, L.F., Pittmann, K., & Krasnov, A. (2022). Mucosal immune and stress responses of Neoparamoeba perurans-infected Atlantic salmon (*Salmo salar*) treated with peracetic acid shed light on the host-parasite-oxidant interactions. Frontiers in immunology, 13, 948897 https://doi.org/10.3389/fimmu.2022.948897

Objective:
To investigate the effects of crowding on the responses of salmon to peracetic acid.

Brief materials and methods:
Trial 1 investigated the influence of crowding prior to exposure to PAA in salmon, and responses were analysed using transcriptomics, histology, and metabolomics. Post-smolts were subjected to crowding by reducing the water volume for 1 h before being exposed to 4.8 ppm PAA for 30 min. Samples were collected at 4 h and 2 w post-exposure. There were four treatment groups: i) no crowding/control, ii) no crowding/PAA, iii) crowding/control, and iv) crowding/PAA. The physiological changes were documented at the mucosal (i.e., skin and gills) and systemic (i.e., plasma) levels. For more detailed information see Lazado et al., (2021).

In trial 2, amoebic gill disease (AGD) was induced in smolts. AGD-affected fish were treated with PAA following two treatment protocols (5 ppm for 30 mins; or 10 ppm for 15 mins). Responses to the treatment were followed at 24 h, 2 weeks and 4 weeks. At week 4, the remaining fish were exposed to a crowding stress by increasing the density 5X for 1 hr. Thereafter, stress parameters were determined at 1-, 3- and 6h after crowding (for more detailed information see Lazado et al., 2022).

Results:
In Trial 1, the physiological changes were documented at the mucosal (skin and gills) and systemic (plasma) levels, with all experimental groups showing a good external welfare score. Treatments had minimal impact on mucous cell numbers in the skin and gills. Branchial histomorphology was generally healthy, though epithelial lifting increased in crowded groups at 2 weeks post-exposure. The gill transcriptome, more affected by crowding and PAA than the skin, showed significant transcriptional responses at 4 hours post-exposure. Crowding altered transcriptional responses to PAA, with more DEGs in gills without crowding history and more skin DEGs in previously crowded groups. Plasma metabolomics identified 639 compounds, with changes influenced mainly by crowding and sampling time, not PAA.

In trial 2, behavioural changes occurred during PAA exposure, and post-treatment mortality was higher in infected, PAA-treated groups, especially at 10 ppm for 15 min. Plasma indicators showed that AGD impacted liver health, though PAA did not worsen the effects of infection. Gill transcriptome profiling revealed significant changes from AGD and PAA, with notable effects 24 h post-treatment, including downregulation of immune and protein metabolism genes, particularly in the 10 ppm group. While AGD pathologies persisted, the 5 ppm for 30 min treatment reduced parasite load at 4 weeks. Mucous cell size and density increased significantly post-treatment, especially in AGD-affected fish. Infection and treatments caused oxidative stress, with early effects in gill mucosa and later systemic ROS dysregulation, prompting infected fish to boost antioxidant production. Crowding stress exposure further altered post-stress responses, with AGD-affected groups showing lower cortisol levels.

Discussion:

The first study enhances understanding of PAA's physiological impact on fish, showing that salmon post-smolts activate mucosal and systemic defense, structural, oxygen transport, and oxidative stress molecules in response to PAA. The gills were more reactive than the skin, especially at the molecular level. Crowding notably influenced mucosal responses, particularly in the gills, but had limited effect on the circulating metabolome. The findings highlight the mucosal impact of PAA at the tested concentration and temperature, with minimal systemic disruption, supporting its safe use in aquaculture. Managing fish crowding protocols can further minimise adverse effects, and future research could explore the roles of fish size and temperature in response to PAA.

The second study provides new insights into the physiological effects of AGD and its interaction with oxidative chemotherapeutics. PAA treatment increased toxicity risk in AGD-affected fish, underscoring the need for careful consideration in its use. AGD impacted liver function and caused systemic oxidative stress, but PAA did not worsen these effects. The gill transcriptome highlighted immune responses to infection, with higher PAA doses briefly modulating these responses. Although PAA did not fully resolve AGD-related pathologies, it reduced parasite load in the 5 ppm, 30 min group. The findings emphasise the need for objective methods to assess disease state and response, as AGD and PAA altered stress response dynamics without impeding the fish's resilience to secondary stressors. Overall, the results contribute valuable insights for the evidence-based use of PAA in aquaculture, with further research needed to optimise treatment protocols for AGD.

WP2.4 the development of a semi-autonomous computer vision tool for continuously monitoring surface behaviour during a crowd

Objective:

To explore computer vision as a potential cost-effective tool to help automate behavioural monitoring during crowding in experimental studies.

Brief Materials and Methods:

Task 2.4 utilised footage collected in the earlier experimental Task 2.1 that monitored a total of four crowding intensities in triplicate for a 2-hour crowding event. GoPro Hero 4 cameras were used to obtain video footage of the entire surface of the crowd at 30 fps for 15 minutes before the crowd, the entire crowd and up to 15 minutes after the crowd. This footage was then subsampled in five-minute segments i) prior to crowd, ii) during the reduction in water volume, and iii) every half an hour during the crowding event, starting immediately after the clamshell-type crowder was installed. The subsamples were used to explore both the aggregated and single frame footage of swimming behaviour of fish in each of the five-minute sub-sampling periods for each tank and crowding intensity.

To analyse aggregated swimming behaviour, each of the video subsamples was condensed into a median frame and were transformed into grey scale values, then into numerical matrixes ranging from

0 to 255 (i.e., white to black colour respectively). With regard to the analysis of swimming behaviour using single frame footage, key points corresponding to the tail peduncle, dorsal fin and snout were annotated for every fish which showed visible keypoints in a total of 5 frames (i.e., the first frame of every one-minute segment of the five-minutes of footage), using the open-source software ImageJ. Swimming orientation was then calculated for each of the annotated fish using the angle between the water flow vector and the vector from the dorsal fin to the snout keypoints in that direction.

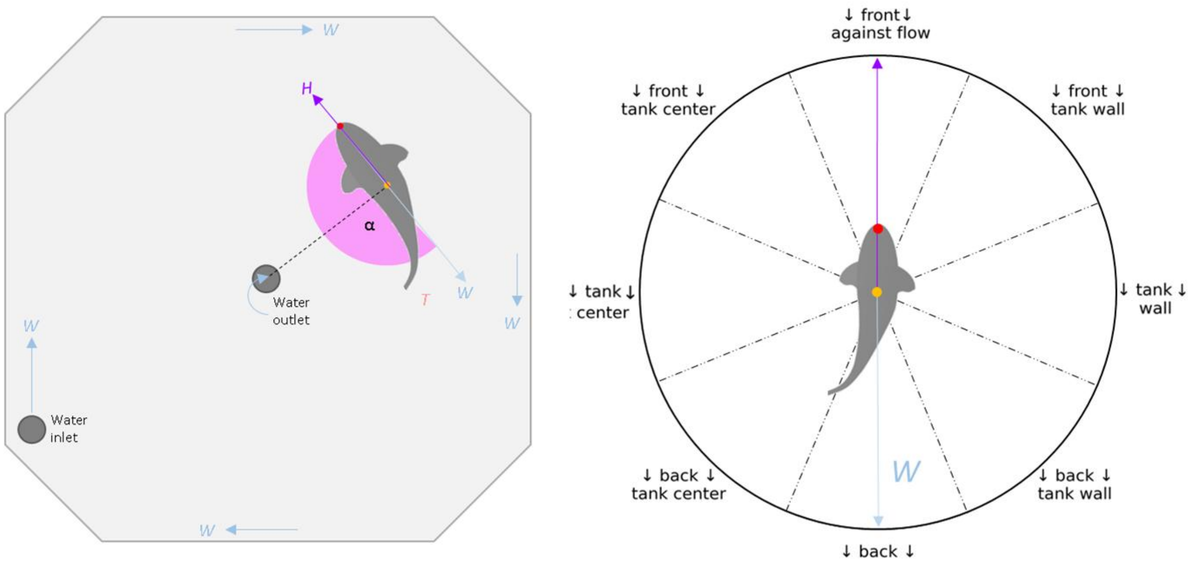

Figure 2 Calculation of fish orientation within the tank in relation to the circular water flow based on the angle formed between the vector H (dorsal key point in yellow to snout in red colour) and the vector W (water flow direction, orthogonal to the radius of the tank passing by the tank center and the dorsal fin key point; left figure). Eight potential fish orientations within the tank (right figure).

Results:

A total of 47097 key points corresponding to 15699 fish were annotated for all tanks and crowding intensities. On average, a total of 72.7 ± 6.8 (mean ± SD), 62.5 ± 3.5, 57.5 ± 1.9 and 45.8 ± 4.4 fish per frame were annotated from frames of crowding intensities level one to four, respectively. During crowding, median frames differed over time, especially in the front and rear parts of the crowd. Overall, the results showed consistent trends between triplicates in each crowding intensity. Interestingly, silhouettes of fish aligning against the water flow became progressively more evident in the front of the crowd after one hour of crowding, becoming increasingly apparent, especially during the last half an hour of the crowding for intensities 3 and 4. Fish also avoided the rear of the crowder for all crowding intensities with the exception of level 1. Fish also orientated themselves more towards the water flow over time and orientation increased with increasing crowding intensity.

Discussion:

Fish maintained structured swimming against the water current in all replicates prior to crowding. The installation of the clam shell crowder initially disrupted this structure, but the fish group gradually regained it and again swam more stationary within the crowd with a preference for the front of the crowd and orientated against the water flow. Interestingly, this organized structuring of the group was higher in crowding intensity levels 3 and 4 and this occurred after one hour of crowding. At levels 1 and 2 fish changed positions and swam more widely within the greater crowding volume that was available to them. A reduction in the number of fish in proximity to the rear of the crowd may be due to them avoiding the crowder to reduce potential risk of damage to their caudal region or due to micro level differences in water quality parameters such as flow rate or oxygen levels.

WP2.5 the development of a qualitative observational toolbox for crowding in tanks

A key output of WP2 is to develop and propose an observational toolbox that stakeholders and operators can use to monitor and potential steer their tank-based crowding operations. Observations are primarily behavioural and metrics consider how fish interact with the surface, each other and the clam shell crowder (Table 2). We suggest that visual analogue scales are then used to score each metric from 0 (absent) to 10 (completely dominating the observer's impression of the footage) after Stien et al., (2024).

Table 2 Observational metrics and descriptions applied to the visual assessment of Atlantic salmon behaviour during crowding in tanks (adapted from Alvestad et al., in prep for submission).

	Observational metric	Description
1	Burrowing	"when the fish burrow into the bottom of the holding [..] tank" [1]
2	Gasping	Proportion of fish exhibiting respiratory distress and gasping at the water surface [47]
3	Lethargy	Number of fish that lose buoyancy/behavioural control/equilibrium
4	White sides at surface	Proportion of white sides (belly side) observed at the surface (although this can also be observed when fish are sedated [38])
5	Contact with tank and equipment	Proportion of fish touching or being pressed into the net or equipment
6	Space between fish	The amount of space between fish in the crowd (after [48])
7	Distance to water surface	Distance between the water surface and the majority of the fish group (fish backs may also be exposed if there is no distance to water surface in a shallow crowd). NB. Sometimes fish will choose to be close to the surface, so a small gap (level 2 in the intensity scale below) isn't always a sign of risk. This metric should be compared to the depth preferences of the fish before crowding.
8	Dorsal fins/backs/heads out of water	Proportion of dorsal fins/backs/heads sticking out of the water
9	Water surface state	Amount of visible activity from fish at the surface. Including ripples in the water and various surface breaks by the fish (rolling, jumping etc.)
10	Swimming speed	Relative swimming speed, as activity can increase during crowding in other production systems e.g. net pens [19,49]

WP3: Evaluating the Atlantic salmon crowding intensity scale for net pens

WPL: Lars Helge Stien, IMR. Participants: David Izquierdo-Gomez, Angelico Madaro, Jonatan Nilsson, Chris Noble, Gerrit Timmerhaus, Elisabeth Ytteborg. FOTS ID: 24315

The objective of WP3 was to evaluate the existing surface crowding intensity scale for Atlantic salmon in net pens for different fish sizes and seasons, refine it and assess the feasibility of drawing up a potential sub-surface salmon crowding scale. We investigated (3.1a) how important factors such as differing fish sizes and seasons impact upon crowd monitoring using the crowding intensity scale, (3.1b) how the crowding scale relates to other OWIs and LABWIs, and (3.1c) how different crowding levels affect fish health and welfare in the weeks after crowding. Further objectives of WP3 included the development of a qualitative observational toolbox for crowding in net pens (3.2a) and the development of an intensity risk scale for monitoring the crowding of Atlantic salmon in commercial marine net pens using an ROV camera (3.2b).

WP3.1 Evaluating a crowding intensity scale and welfare indicators for Atlantic salmon in net pens

This task is published open access as Stien, L. H., Nilsson, J., Noble, C., Izquierdo-Gomez, D., Ytteborg, E., Timmerhaus, G., & Madaro, A. (2024). Evaluating a crowding intensity scale and welfare indicators for Atlantic salmon in sea cages. Aquaculture Reports, 37, 102211. https://doi.org/10.1016/j.aqrep.2024.102211

Summary of the experiment and results:

In this study, we followed 55,000 Atlantic salmon distributed in nine marine net pens during a standard production from release to harvest. The salmon were crowded for 2 hours at three different occasions, once in October, once in January and once in May. On each occasion three of the pens were crowded to level 2, three to level 3 and three to level 4 on the crowding intensity scale. The fish were about 1.5 kg at the October crowding. At this crowding it soon became apparent that we could not use the crowding intensity scale to decide on crowding intensity. The fish avoided the surface, and there were little to no visible surface activity when we pulled in the net, until the fish were absolutely forced up into the surface, and the fish at the surface suddenly expressed level 4 activity, bypassing level 2 and 3. We therefore had to rely on underwater observations to create what we perceived as three different levels of crowding. In January the fish had reached a size of about 3 kg and in May a size of about 5 kg. At both these crowding events, the fish gradually increased their surface activity as we pulled the nets in, and we were therefore able to use the crowding intensity scale as intended.

There was a correlation between blood cortisol and crowding intensity and skin histology showed increased skin damage with crowding intensity. There were, however, no detected increases in the prevalence of external damages visible to the naked eye, nor mortality after the crowding events. The reason that the crowding operations went so well may be that the nets were pulled in relatively slowly, giving the fish time to adapt to the net the crowd, and that underwater cameras and a ROV was used to monitor that no risk factors such as folds in the net or pockets trapping the fish occurred. There was, however, one case of increase in mortality. This was for one of the pens crowded to level 4 in January. This may illustrate that even though crowding does not inherently lead to overt poor welfare outcomes, the risk increases with intensity.

Based on this experiment we concluded that a toolbox of OWIs for crowding should include surface observations, subsurface observations, oxygen conditions in the crowd, and the prevalence and change in severity of external injuries on the fish. For the underwater observations it is important to look for risk factors such as the fish coming too close to the net, folds and pockets in the net. Recommended LABWIs include plasma cortisol and skin histology. For more details and results from the study see the open access published article Stien, L. H., Nilsson, J., Noble, C., Izquierdo-Gomez, D., Ytteborg, E., Timmerhaus, G., & Madaro, A. (2024). Evaluating a crowding intensity scale and welfare indicators for

Atlantic salmon in sea cages. Aquaculture Reports, 37, 102211. https://doi.org/10.1016/j.aqrep.2024.102211.

WP3.2 the development of (a) a qualitative observational toolbox for crowding in net pens and (b) an intensity risk scale for monitoring the crowding of Atlantic salmon in commercial marine net pens using ROV cameras

WP3.2a. developing a qualitative observational toolbox for crowding in net pens

This task is published open access as Stien, L. H., Nilsson, J., Noble, C., Izquierdo-Gomez, D., Ytteborg, E., Timmerhaus, G., & Madaro, A. (2024). Evaluating a crowding intensity scale and welfare indicators for Atlantic salmon in sea cages. Aquaculture Reports, 37, 102211. https://doi.org/10.1016/j.aqrep.2024.102211.

Summary of outputs:

A key output of WP3 is to develop and propose an observational toolbox that stakeholders and operators can use to monitor and potential steer their net pen-based crowding operations. Observations are primarily behavioural, and metrics consider how fish interact with the surface, each other and the crowding equipment and the net (Table 3). We suggest that visual analogue scales are then used to score each metric from 0 (absent) to 10 (completely dominating the observers' impression of the footage) see below.

Table 3 Observational metrics (overwater metrics: 1-3; sub-surface metrics: 4-10) and descriptions applied to the visual assessment of Atlantic salmon behaviour during crowding in net pens (reproduced with permission from an open access article under the CC BY license: Stien, L. H., Nilsson, J., Noble, C., Izquierdo-Gomez, D., Ytteborg, E., Timmerhaus, G., & Madaro, A. (2024). Evaluating a crowding intensity scale and welfare indicators for Atlantic salmon in sea cages. Aquaculture Reports, 37, 102211. © 2024 The Authors. Published by Elsevier B.V).

#	Observational metric	Description
1	Surface activity	Degree of visible activity from fish in the surface. Including ripples in the water and various surface breaks by the fish (rolling, jumping etc.)
2	Fins out of water	Degree of fins sticking out of the water across the crowding surface
3	White sides	Degree of white sides (belly side) observed at the surface
4	Space to surface	Degree of space between the surface and the fish group
5	Queuing	Degree of fish being hindered and having to slow down and swim slowly due to other fish
6	Structured	Degree of the fish managing to maintain a structured school with fish swimming in the same general direction.
7	Swimming speed	Relative swimming speed.
8	Space to net	Degree of closeness between the fish group and the net
9	Touching the net	Degree of fish touching the net
10	Pressed into net	Degree of fish being pressed into the net

WP3.2b An intensity risk scale for monitoring the crowding of Atlantic salmon in commercial sea cages using ROV camera

This task is published open access as Stien, L. H., Noble, C., Izquierdo-Gomez, D., Bui, S., Amble, S., & Lind, M. B. (2025). An underwater risk scale for monitoring the crowding intensities of Atlantic salmon in commercial net pens using Remotely Operated Vehicles (ROVs), v0. 1. Aquaculture Reports, 45, 103109. https://doi.org/10.1016/j.aqrep.2025.103109.

Summary of the experiment and results:
In this study underwater footage (8 films from anonymous crowding operations undertaken in relation to delousing in commercial net pens) was collected from ROV's during the duration of the project. Crowding operations covered a range of estimated fish sizes from 0.5 – 5 kg. Each film was divided into 100 clips of 10-second duration. Fifty of the 10-second clips were then randomly selected for inclusion in a survey addressed to farmers, welfare scientists and fish health students to help devise and determine risk levels for crowding (1-5) according whether the observer perceived little risk to the fish (level 1) or an extreme risk to the fish (level 5). Two further categories were added to the scale: level 0 (no crowding risk as these fish do not appear to be crowded), and level 6 (where there is a catastrophic risk for the fish). These additional categories were used to allow participants to refine how they defined either minimal or extreme risk. Survey results were then analysed to help determine which video clips were most consistently representative of each risk level.

WP4: On-farm evaluation of the CrowdMonitor toolbox

WPL: Lars Helge Stien, IMR. Participants: René Alvestad, David-Izquierdo-Gomez, Gunhild S. Johansson, Amritha Johny, Angelico Madaro, Chris Noble, Elisabeth Ytteborg

The objective of WP4 was to test and evaluate the CrowdMonitor OWI and LABWI toolbox under commercial large-scale conditions. The farm partners (Cermaq Norway AS and Grieg Seafood AS) provided the research partners with access to crowding situations at their farm facilities allowing for a critical appraisal of the toolbox in relation to i) different rearing systems and ii) commercial crowding operations.

WP4.1 Smolt crowding in commercial tanks prior to wellboat transfer to net pens

Objective:
The goal of this task was to test the feasibility of using the CrowdMonitor tank-based WI toolbox in commercial settings.

Brief materials and methods:
Crowding was followed in a single commercial tank over the 7^{th}-8^{th} December at one of the farm partners. 193 114 fish, mean weight ca. 209g were held in an 804 m^3 circular conical tank (16m diameter, tank wall 4.5m, max water height at time of sampling, 4.05m) at a density of ca. 50 kg/m^3. Water temperature at the time of sampling was 7.6 ^oC. Five to ten fish were sampled i) before the crowding operation started, ii) at various points when the water level was dropped and iii) after the start of pumping until the end of the crowding and fish transfer procedure according to Figure 4. Fish were sampled using a dip net close to the tank wall, euthanised and sampled for: length; weight; morphological OWIs (Laksvel, Nilsson et al., 2022); blood samples were taken for plasma physiological markers and stress indicators (plasma cortisol, plasma osmolality and other plasma parameters, pH, lactate, glucose, Na^+, Cl^-, and Ca^{2+}), and scale explant cultures (Karlsen et al., 2018). Scale explants were used for migration assays of skin epithelia, where the migration of keratocytes moving from the scales and onto the culture dish were measured. Skin tissue pieces were sampled on site and stored in culture media before transported to the lab. Tubes with tissue and media were kept on ice and delivered to the lab within 24h. In brief, scales from 6 fish were sampled at T1-T4 and cultures in cell culture dishes, 3 scales per well, 3 wells per fish, n=54 scales per treatment. Cultures were kept in incubators at 8 ^oC. Migration rate was measured 24, 48, 72 and 96h after cultivation, according to a 0-4 scoring system where: 0=no migration, 1=keratocytes start to migrate, 2=migrating keratocytes makes sheets corresponding to 1/2 the scale size, 3: migrating keratocytes makes sheets corresponding to 3/4 the scale size, 4: confluent sheets of keratocytes covers large area of culture dish. Fish behaviour was documented throughout the crowding operation using a GoPro 4 camera attached to the wall of a gangway next to the tank that allowed for the whole tank surface to be included in the field of view. Fish behaviour prior and during the crowding operation was assessed by means of an adapted version of the observational toolbox from WP2 (Table 4). Assessments were carried out based on the first five-minutes of each of the 12 minutes GoPro camera files. Visual analogue scales were again used to score each metric from 0 (absent) to 10 (completely dominating the observer's impression of the footage) after Stien et al., (2024). There was a degree of foam (sulte-skum) at the water surface before and during the crowding that had to be considered in the application of the observational toolbox. We also documented crowding in relation to the established crowding intensity scale (Mejdell et al., 2009; RSPCA, 2024) throughout the crowd.

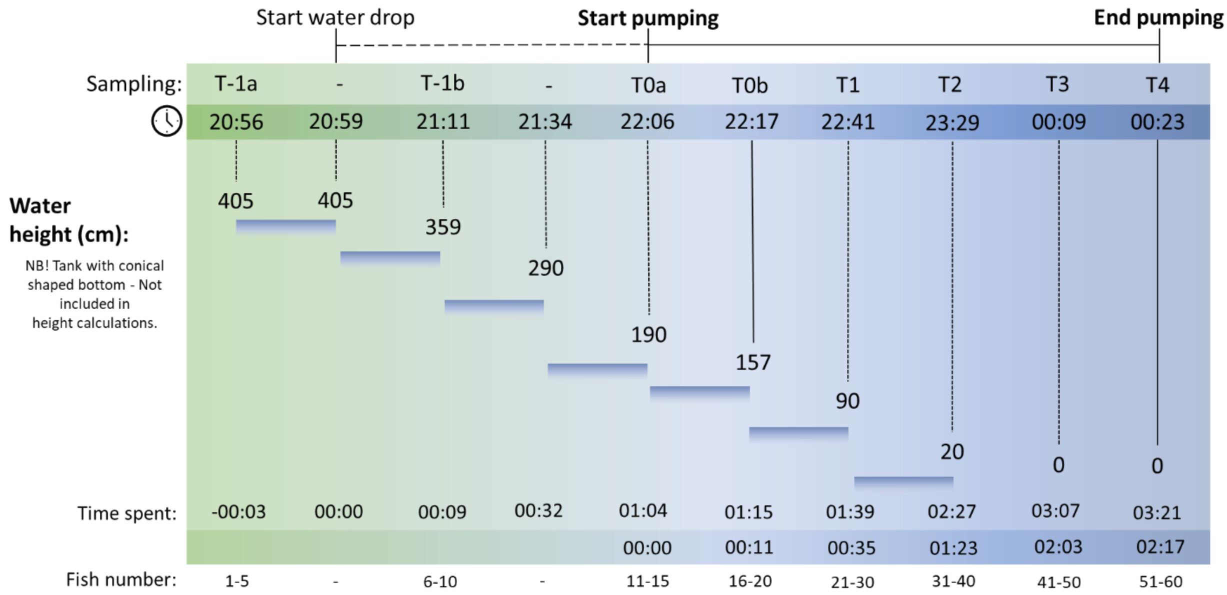

Figure 3 Outlining the timing of sampling in relation to differing stages of the crowding operation. Figure: Gunhild S. Johansson.

Table 4 Toolbox of observational metrics used to assess smolts' behaviour in the RAS tank prior seawater transfer.

Metric	Description
Overall Surface Activity	Fish jumps, rolls and/or breaks of the water surface/foam
Distance to the surface	Fish group distance to the water surface of fish swimming next to the tank wall
Cohesion (BW)	Degree of closeness the fish group together
Swimming structure	Degree of synchrony within the swimming activity of the fish group
White sides	Fish oriented on one of their sides and/or with signs of lethargy/equilibrium loss

Results:

Laksvel morphological OWI scoring showed no clear trends over time, with the possible exception of increased numbers of sampled fish exhibiting level 2 scale loss in the last 30 minutes of the crowd. Plasma markers showed a distinct stress response over time. Fish stress levels progressively increased during crowding, as indicated by rising cortisol levels in the plasma and there was also a gradual increase in plasma lactate and glucose levels. Crowding also induced changes in the fish plasma osmoregulatory balance. Osmolarity, K^+, Na^+ Ca^{2+} increased over time. Migration assays with scale explants showed increased migration from 24h to 96 h, as expected (Figure 5). All stages had normal migration from the scales, and few differences between crowding stages could be detected. Most stages had normal migration from the scales, and few differences between crowding stages could be detected.

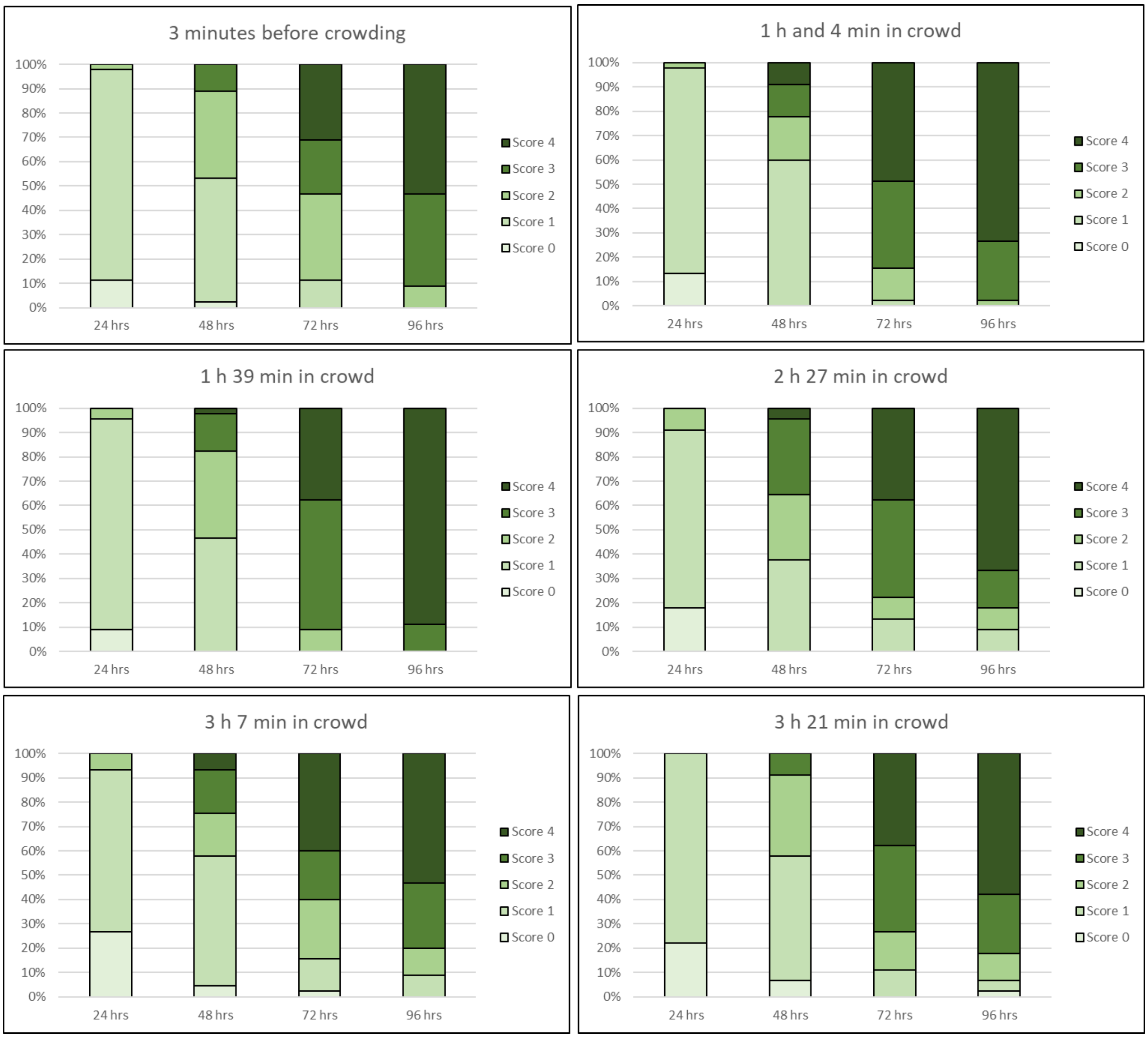

Figure 4 Migration assays showing scoring of migrating keratocytes from scales picked from fish exposed to different stages of the crowding operation. Scoring is as described in the text, with 0 showing no migration and 4 confluent cell sheets covering the culture plate.

From a behavioural perspective: prior to the onset of crowding waterflow was constant, foam coverage was maximal, fish exhibited a high degree of structured swimming with most of the fish schooling oriented towards the water flow with a degree of cohesion, with fish at the surface swimming ca. 3-4 body widths from each other (see Figure 6). Surface activity was also low as only single fish were observed jumping or breaking the surface. At the start of crowding, there was a reduction in foam coverage, so most of the water surface was free of foam. The distance of the fish to the surface also decreased as well as decreases in synchrony in swimming structure, and cohesion increased as fish started swimming closer to each other. The crowding intensity scale increased from level 1 to level 2 as the pumping process started around one hour into the crowding, due to asynchrony in swimming orientation. Crowding intensity then increased to level 3 around 30 minutes after pumping onset (1.5 hours into the crowd) and this is due to an increase in the number of fins visible on the surface. Crowding operators pumped extra water into the tanks as needed and this reduced the levels of the RSPCA intensity scale after this point. As the end of the crowding operation approached, and fish number decreased, crowding intensity levels again dropped to level 1.

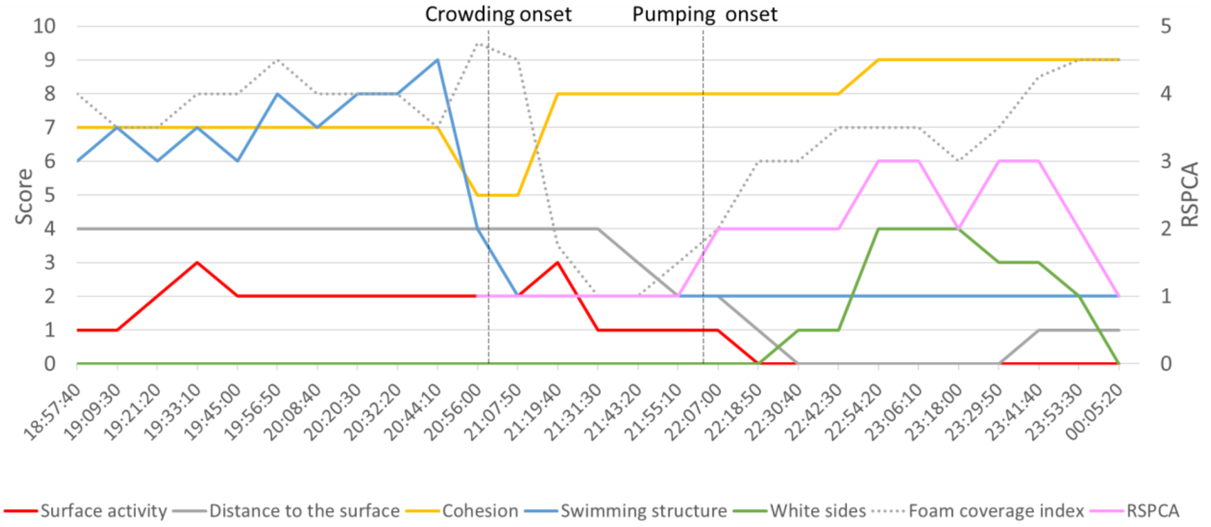

Figure 5 Observational metrics of smolts behaviour in a RAS tank during crowding prior to wellboat transfer. The abiotic conditions of the tank influencing the water surface are also included as water flow speed index, foam coverage index of the tank surface and the crowding onset (vertical dashed line). The RSPCA crowding intensity levels are also shown (pink line, secondary y-axis, levels 1-5).

Discussion:

No clear trend in Laksvel OWI scoring was apparent at any stage of the crowding process with the exception of minor increases in numbers of sampled fish exhibiting level 2 scale loss in the last 30 minutes of the crowd. Plasma markers showed a distinct stress response over time and fish stress levels progressively increased during crowding, and there was a gradual increase in plasma lactate and glucose levels. Crowding also induced changes in the fish plasma osmoregulatory balance. Migration assays with scale explants showed increased migration from 24h to 96 h and stages had normal migration from the scales, and few differences between crowding stages could be detected. From a behavioural perspective, the RSPCA level 1 crowding intensity was maintained for the first hour of crowding as the water was being drawn down and only increased to level 2 at the start of pumping. Increases in intensity to level 3 were due to more white sides being visible in the crowd and this was reduced by pumping in more water by the crowding operator. When water level decreased until the conical bottom of the tank became visible, the group distance to the surface increased slightly and crowding intensity level dropped as the operation ended, possibly linked to reduced fish number or density.

WP4.2 Crowding in commercial net pens prior to delousing

Objective:

The goal of this task was to test the feasibility of using the CrowdMonitor net-pen based WI toolbox in commercial settings.

Brief materials and methods:

Crowding was followed in a single commercial pen on the 28th September at one of the farm partners. An initial number of 187 900 salmon were crowded and treated using Hydrolicer delousing in two steps, 92 452 fish in the first treatment (during the night) and the remaining 95 448 fish in the second treatment (during the day). Results from the second treatment will be outlined here. Fish had an estimated mean weight of 3700 g at the time of treatment and had previously been subjected to three thermal delousing treatments (between December 2022 and August 2023) prior to our documentation trial at the end of September 2023. Five to ten fish were sampled i) before the crowding operation started, and ii) at various points until end of the crowding procedure according to Figure 7. Fish were sampled using a dip net close to the pen wall, euthanised and sampled for: length; weight; morphological OWIs (Laksvel, Nilsson et al., 2022); blood samples were taken for plasma physiological markers and stress indicators (plasma cortisol, plasma osmolality and other plasma parameters, pH, lactate, glucose, Na^{+}, Cl^{-}, and Ca^{2+}), and scale explant cultures (Karlsen et al., 2018). Scale explants were used for migration assays of skin epithelia, where the migration of keratocytes moving from the scales and onto the culture dish were measured. Scale explant cultures were carried out as described in 4.1, except that scales were picked from fish on site and not transported in cell culture media. The same scoring system was used to describe migration. Fish behaviour was documented throughout the crowding operation using a GoPro 4 camera attached to a fence on the upper deck of the wellboat that allowed for the whole pen surface to be included in the field of view. Additionally, an ROV was used to monitor the whole procedure underwater and such footage was also collected and used to document fish behaviour during the whole crowding operation from below water (described below). Fish behaviour during the crowding operation was examined by means of an adapted version of the observational toolbox from Stien et al., 2024 (Table 5) which included both surface and underwater observational metrics including: fins out of the water, surface activity, white sides, fish touching the net, queueing, fish pressed against the net, swimming structure and swimming speed. Both for underwater and surface behaviours, a score was assessed every 10 seconds since the ROV was rapidly moving between distant positions around the net pen in short intervals. Visual analogue scales were again used to score each metric from 0 (absent) to 10 (completely dominating the observers impression of the footage) after Stien et al., (2024). We also documented crowding in relation to the established crowding intensity scale (Mejdell et al., 2009; RSPCA, 2024) throughout the crowd.

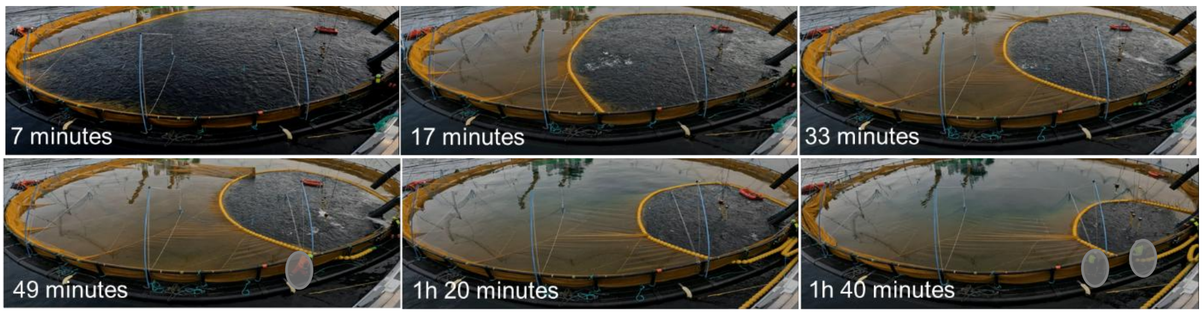

Figure 6 Outlining the timing of sampling in relation to differing stages of the crowding operation. Translucent grey overlays on the images at 49 minutes and 1h 40 minutes are used to anonymize farm and project staff. Figure: David Izquierdo Gomez.

Table 5 Observational metrics outlined in Stien et al. (2024) and applied to the evaluation of underwater (white) and surface footage (light gray shaded) during a commercial net-pen crowding operation.

#	Metric	Description
1	surface activity	Degree of visible activity from fish in the surface. Including ripples in the water and various surface breaks by the fish (rolling, jumping etc.)
2	fins out the water	Degree of fins sticking out of the water across the crowding surface
3	white sides	Degree of white sides (belly side) observed at the surface
4	space to the surface	Degree of space between the surface and the fish group
5	queueing	Degree of fish being hindered and having to slow down and swim slowly due to other fish
6	swimming structure	Degree of the fish managing to maintain a structured school with fish swimming in the same general direction.
7	swimming speed	Relative swimming speed
8	closeness	Degree of closeness between the fish group and the net
9	fish touching the net	Degree of fish touching the net
10	fish pressed against the net	Degree of fish being pressed into the net

Results:
Laksvel morphological OWI scoring showed no clear trends over time, with the possible exception of a minor increase in the numbers of sampled fish exhibiting level 2 gill damage in the last 30 minutes of the crowd. Plasma markers showed a distinct stress response over time. Fish stress levels progressively increased during crowding, as indicated by rising cortisol levels in the plasma and there was also a gradual increase in plasma lactate and glucose levels. With regard to migration assays from scale explants: as expected, migration from scales in cultures showed increasing migration from observations done after 24h of incubation compared to 96h of incubation (Figure 8). The best migration was observed in scales picked from fish kept in crowding conditions only for 7 minutes at the onset of level 2 crowding, and lowest migration in scales from fish exposed to the crowding environment for 1h and 40 minutes and this lowest migration occurred when fish had been subjected to a crowding intensity of level 3 for 30 minutes (see Figure 12). After 96 h in culture, more than 70% of the scales had migration in the 7min group, whereas only around 30% had migrating keratocytes in the 1h 40 group.

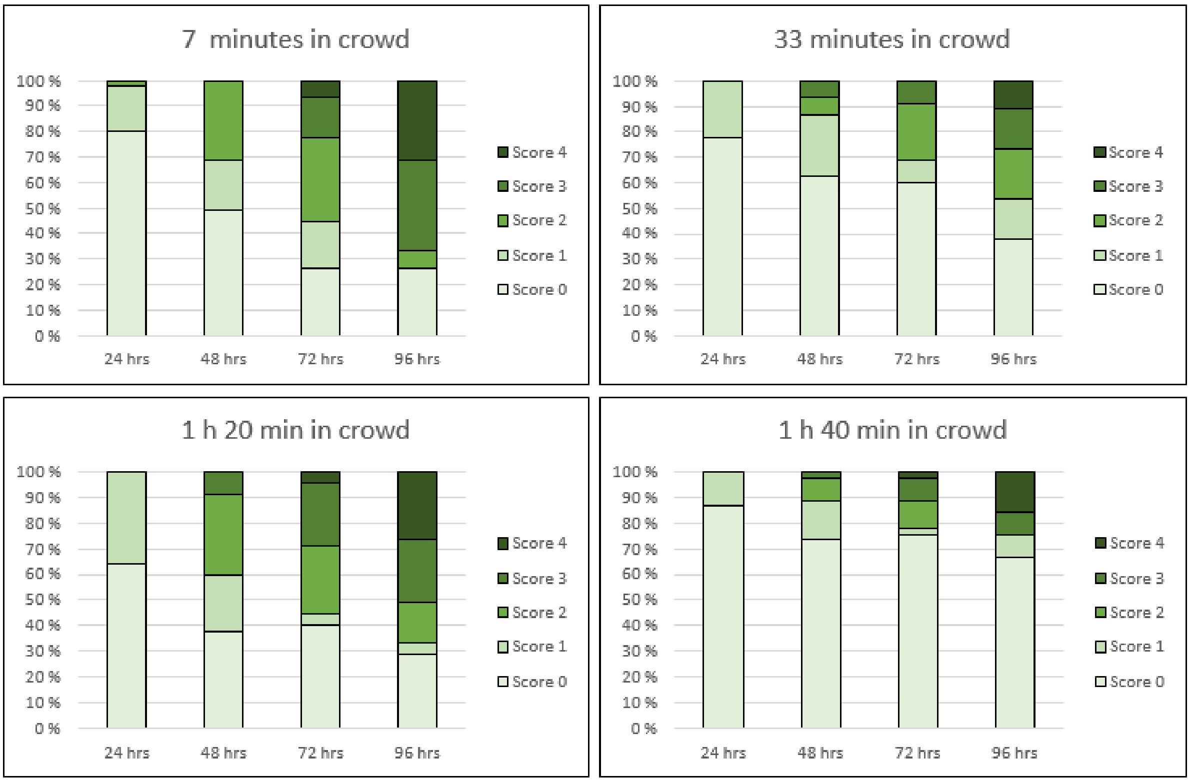

Figure 7 Migration assays showing scoring of migrating keratocytes, where 0 Is no migration and 4 is confluent cell sheets covering the culture plate. The best migration was seen in scales picked from fish exposed to crowding for 7 minutes (start of crowding at intensity level 2), and worst migration from scales picked from fish after 1h and 40 min (after fish had been subjected to 30 minutes of level 3 crowding intensity).

To test the observational metrics for monitoring fish behaviour during crowding, the net pen was divided into zones of interest throughout the crowding operation. These corresponded to different areas of the floating line (kulerekke) the non-mobile net collar, the centre of the net pen, and the pumping area (see Figure 9). For the analysis of the ROV footage, the net pen was also segmented clockwise as a pie-chart (Figure 9), so the ROV position could be systematically tracked over time while visualizing both the surface and underwater footage.

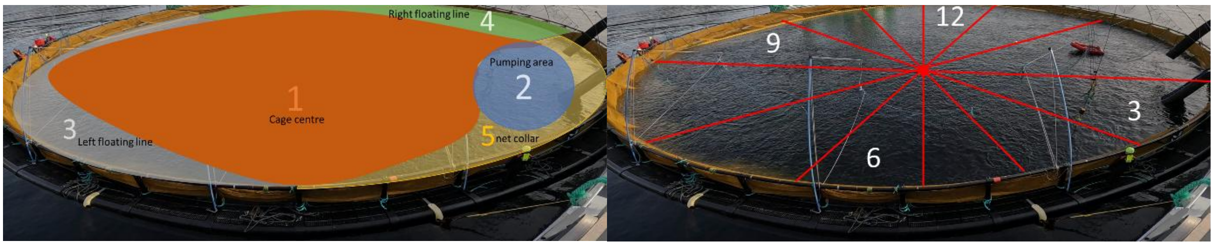

*Figure 8 Segmentation of the surface area of the net pen. **Left picture**: five main zones of interest, namely, the cage centre (1), the pumping area (2), the left-hand side floating line, the right-hand side floating line (3 and 4) and the net collar close to the well-boat (5). **Right picture**: pie-chart type segmentation of the net pen surface in 12 clockwise segments for analysis of the ROVs position.*

Slightly over two hours of continuous footage were obtained from the surface camera, and similarly for the ROV camera with the exception of fifteen unrecorded minutes due to ROV issues ca. 1.5 hours after the crowding onset. The crowding operation lasted a total of 1 hour and 50 minutes (Figure 10). The ROV operator mostly deployed the ROV around the far end of the net pen from the wellboat (segment 10) at a mean depth between 4 and 5 meters. Isolated trips to deeper waters than 10 meters were carried out for e.g., checking the shackle, located in segment 10, until circa one hour after the onset of crowding when the shackle was lifted over the floating line. Hereafter, ROV surveillance was more focused on areas close to the stern of the boat where the oxygen hoses were deployed in the crowd and also in the area next to the bow of the boat checking the crowd from below the boat around the net collar area (segments from 2 to 5). Within the ROV-based metrics, all metrics showed low values apart from *fish touching the net* which reached intermediate levels (i.e., score 6) at some points of the crowding operation, in segments that corresponded to the right-hand side of the floating line and net-collar. In terms of *surface activity*, the whole surface showed ripples including fish splashes consistently higher in the left floating line compared to the right side of it. Overall, the net pen centre and pumping area showed lower surface activity values, followed by the net collar zone, which overall showed the lowest surface activity (Figure 11). Concerning *fins out of the water*, the pattern was similar to surface activity and the higher values were observed around the floating line, especially the left-hand side, followed by the net pen centre, pump area and net collar area.

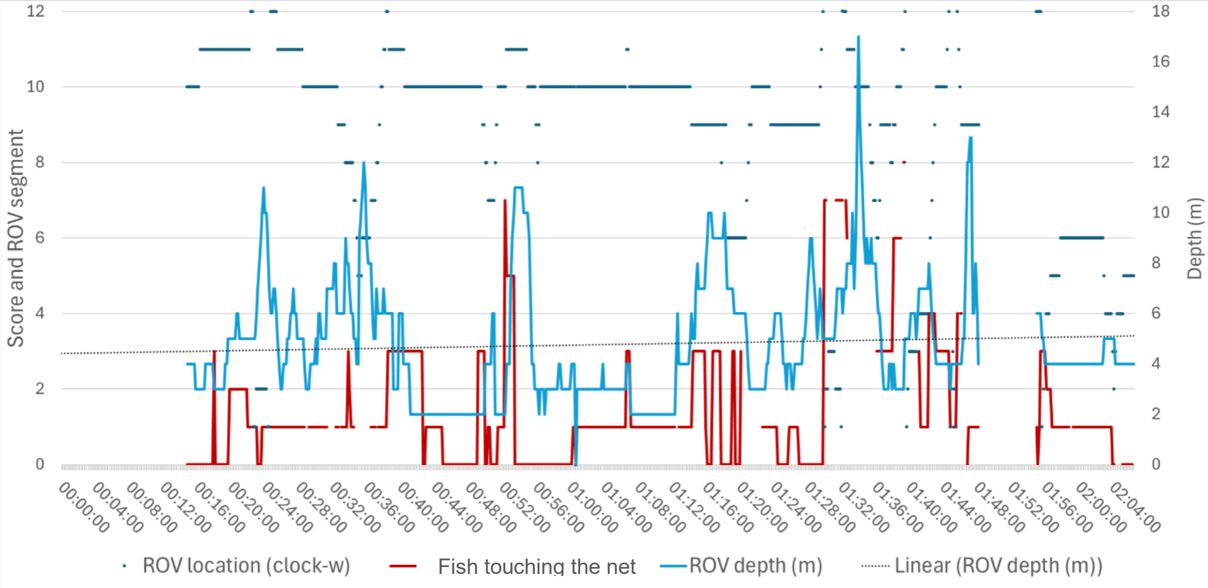

Figure 9 Temporal variation of the ROV depth (blue line) and location around the pen, together with the score of the ROV-based metric fish touching the net (red line). There is a 15-minute data gap at 1h and 40 minutes due to technical problems with the ROV recording system.

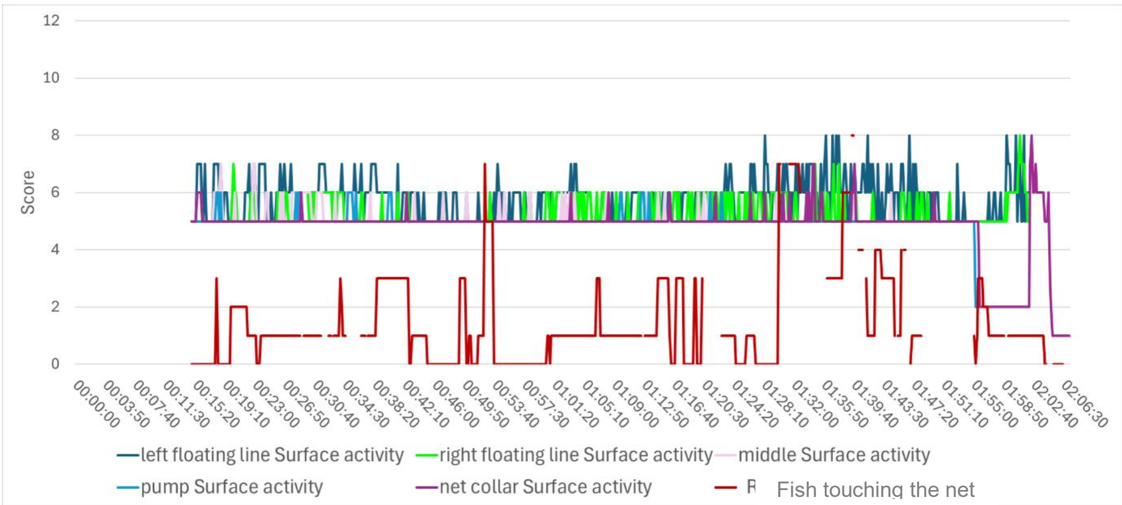

Figure 10 Surface activity scores during the crowding trial per in each of the surface zones, namely, left floating line, right floating line, net pen centre, pumping area and net collar area. The temporal variation of the ROV-based metric fish touching the net is also shown for comparison.

White sides were barely observed during the first half of the crowding and as observed for the other metrics, the higher numbers occurred next to the floating lines, especially the left-hand side followed by the net pen centre. Nearly no white sides were observed in the pump area or near the net collar (Figure 12).

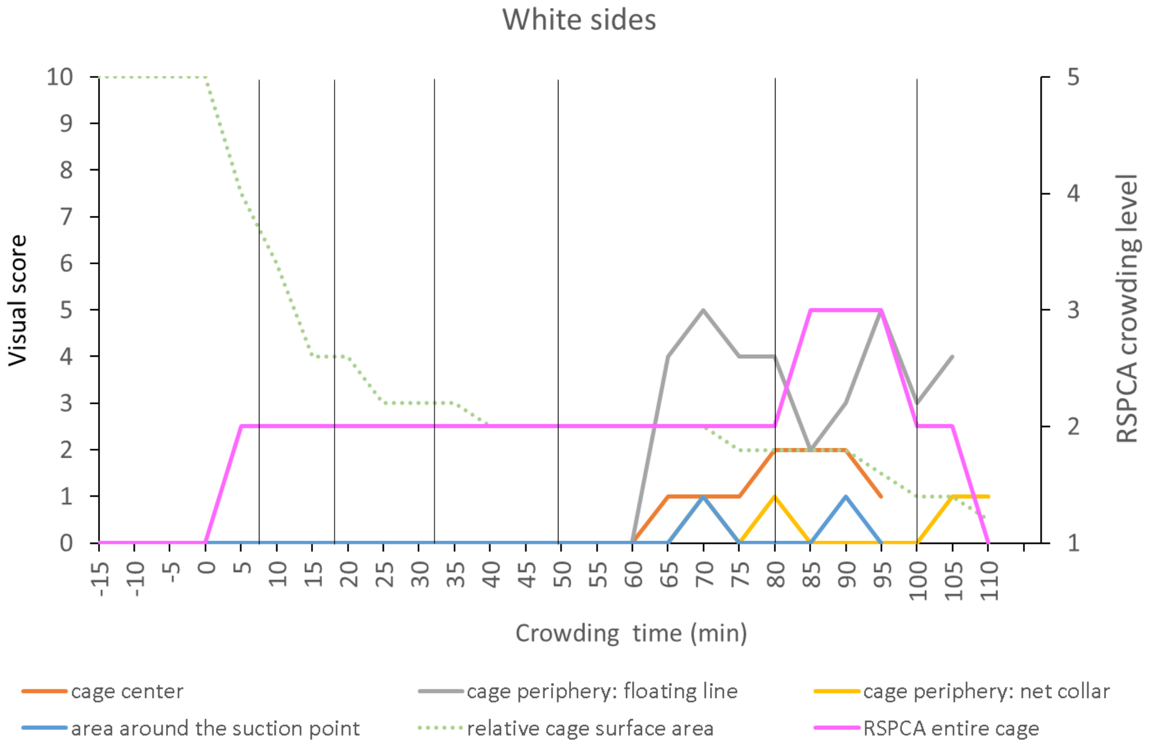

Figure 11 White side metrics during the crowding trial in each of the surface zones, namely, floating line, net pen centre, pumping area and net collar area. The RSPCA crowding intensity levels are also shown (pink line, secondary y-axis, levels 1-5).

Discussion:

No clear trend in Laksvel OWI scoring was apparent at any stage of the crowding process with the exception of minor increases in numbers of sampled fish exhibiting level 2 gill damage in the last 30 minutes of the crowd when the overall crowding intensity increased from level 2 to level 3. Plasma markers showed a distinct stress response over time and fish stress levels progressively increased during crowding, and there was a gradual increase in plasma lactate and glucose levels. As with WP4.1, these data demonstrate that plasma biochemistry is a valuable tool for assessing the physiological condition of fish and evaluating the severity of aquaculture procedures on fish. Migration assays proved to be useful for evaluations of crowding intensity levels. Migration was characterized as normal in all but the fish exposed to crowding for 1h and 40 min. Time is probably not essential here, rather that the fish had previously been exposed to 30 minutes of level 3 crowding intensity, as data from 1h and 20 min of crowding (still level 2 intensity) showed similar response as the 7min crowding data. These findings correspond to what we found in Stien et al., (2024). This indicates that increased intensity induces micro-damage to keratocytes that may lower their migration capacity. This may further have impact on the skins ability to heal. How long it takes for the keratocytes to recover after an intensive crowding event is not known and not included in this study, but precautions should be taken to protect the skin further after crowding events, if crowding more than level 2 is needed. From a behavioural perspective, the crowding intensity scale was applicable to the observed crowding operation. Crowding intensity level 2 was maintained for the first hour and twenty minutes of crowding. Increases in intensity to level 3 were primarily due to more white sides being visible in the crowd around the proximity of the floating line. Relationships between surface observations and underwater state were sometimes asynchronous. For example, surface activity along the floating line was sometimes observed, but corresponding footage from the ROV at this time showed there was still space between the net and the fish and e.g., no fish trapped in net pockets. At other times, the water surface was stable, with no surface activity or white sides of the fish, but underwater fish were touching the net and there was a potential risk of fish being pressed against it. We recommend that where possible, underwater observations e.g., from an ROV and surface observations are used together to monitor the crowd.

WP5: Operational recommendations – going from science into practice.

WPL: Chris Noble, Nofima. Participants: Vilde C. Alsos, René Alvestad, Åsa M. Espmark, David Izquierdo Gomez, Sigrun N. Johannessen, Gunhild Seljehaug Johansson, Amritha Johny, Jelena Kolarevic, Carlo C. Lazado, Angelico Madaro, Jonatan Nilsson, Karl F. Ottem, Berit Seljestokken, Tirril Slettjord, Lars Helge Stien, Gerrit Timmerhaus, Linda Tschirren, Elisabeth Ytteborg, Lucas Zena and Magnus Åsli

The objective of WP5 and a key output of the project was to summarise the CrowdMonitor toolbox and draw up recommendations relation to differing crowding procedures in an operational and informative format. This involved three tasks: (a) to develop an updated surface crowding intensity scale for tanks and net pens, (b) develop a new sub-surface crowding intensity scale for net pens, and (c) update the existing FISHWELL OWI toolbox for crowding with data from CrowdMonitor. These recommendations are outlined in separate CrowdMonitor Handbooks for both tanks and net pens, see Noble, Stien et al., (2025a) https://doi.org/10.21357/edhh-5736 and Noble, Stien et al., (2025b) https://doi.org/10.21357/68na-zn54. Results have been summarised in posters, brochures, an industry webinar in October 2025, and will be the subject of a public workshop in May 2026 to possibly refine their recommendations based upon initial usage.

7 Main findings

Some of the main findings of the project regarding tank crowding and cage crowding have already been outlined in Lazado et al., (2021) https://doi.org/10.1016/j.aquaculture.2020.735830, Lazado et al., (2022) https://doi.org/10.3389/fimmu.2022.948897, Stien et al., (2024) https://doi.org/10.1016/j.aqrep.2024.102211 and Stien et al., (2025) https://doi.org/10.1016/j.aqrep.2025.103109.

To summarise the whole project:

- Crowding fish to high densities for durations up to 3 hours in small scale experimental tanks using a clam shell crowder had a marginal effect upon fish welfare in our studies, provided that the conditions for the fish are otherwise good.
- Peracetic acid was shown to be generally safe for use with a limited systemic impact on gill and skin mucosa, but the general state of the fish should be considered during its application.
- Crowding pen-held salmon at the intensities outlined in this project under experimental conditions does not necessarily negatively impact upon fish welfare in the long-term.
- The crowding operation may weaken skin integrity which could be problematic for the fish when they are subjected to further handling.
- A comprehensive OWI toolbox, involving the monitoring of water quality, surface and sub surface observations of behaviour and the state of the group, mortalities, appetite and the health status of the fish before handling, and fish injury levels is recommended for steering crowding. As are LABWIs associated with stress monitoring (cortisol) and skin integrity. See the CrowdMonitor Handbooks for both tanks and net pens, see Noble, Stien et al., (2025a) https://doi.org/10.21357/edhh-5736 and Noble, Stien et al., (2025b) https://doi.org/10.21357/68na-zn54.

Noen av hovedfunnene i prosjektet angående trening i kar og merd er allerede skissert i Lazado mfl. (2021) https://doi.org/10.1016/j.aquaculture.2020.735830, Lazado mfl. (2022) https://doi.org/10.3389/fimmu.2022.948897, Stien mfl. (2024) https://doi.org/10.1016/j.aqrep.2024.102211 og Stien mfl. (2025) https://doi.org/10.1016/j.aqrep.2025.103109.

For å oppsummere hele prosjektet:

- Å trenge fisk ved høye tettheter og varigheter opptil 3 timer i småskala forsøkskar ved bruk av en trengegrind har en begrenset effekt på fiskens velferd, forutsatt at forholdene ellers var gode.
- Pereddikksyre (PAA) viste seg generelt å være trygt å bruke, med begrenset systemisk påvirkning på slimlaget på gjeller og hud, men fiskens helsetilstand bør vurderes før bruk.
- Å trenge laks i merder med de intensitetene som er skissert i dette prosjektet har ikke nødvendigvis en negativ innvirkning på fiskevelferden på lang sikt.
- Trenging kan svekke hudens integritet, noe som kan være problematisk for fisken når den utsettes for ytterligere håndtering.
- En omfattende verktøykasse for å vurdere operative velferdsindikatorer anbefales brukt for å styre trenging. Denne inkluderer overvåking av vannkvalitet, observasjoner av atferd og gruppens tilstand, både på og under vannoverflaten, dødelighet, appetitt, skadenivåer på fisken, samt fiskens helsetilstand før håndtering. Laboratoriebaserte velferdsindikatorer knyttet til stressovervåking (kortisol) og hudintegritet bør også vurderes. Se CrowdMonitor-håndbøkene for kar og merder, se Noble, Stien mfl. (2025a) https://doi.org/10.21357/edhh-5736 og Noble, Stien mfl. (2025b) https://doi.org/10.21357/68na-zn54.

8 Deliverables

Final results report:

Noble, C., Alvestad, R., Espmark, Å.E., Izquierdo Gomez, D., Johny, A., Kolarevic, J., Lazado, C.C., Madaro, A., Nilsson, J., Stien, L.H., Ytteborg, E. (2026) Monitoring and optimising the crowding of Atlantic salmon using emerging health and welfare indicators (CrowdMonitor). Nofima report. 21 (2026).

Handbooks:

Noble, C.*, Stien, L.H.*, Alsos, V.C., Alvestad, R., Espmark, Å.E., Izquierdo Gomez, D., Johannessen, S.N., Johansson, G.S., Johny, A., Kolarevic, J., Lazado, C.C., Madaro, A., Nilsson, J., Ottem, K.F., Seljestokken, B., Slettjord, T., Timmerhaus, G., Tschirren, L., Ytteborg, E., Zena, L., Åsli, M. (2025a). The CrowdMonitor handbook for commercial tanks v0.1: an updated welfare indicator toolbox and crowding intensity scale for measuring and monitoring Atlantic salmon welfare during crowding operations. Nofima rapport nr. 31/2025 https://doi.org/10.21357/edhh-5736

Noble, C.*, Stien, L.H.*, Alsos, V.C., Alvestad, R., Espmark, Å.E., Izquierdo Gomez, D., Johannessen, S.N., Johansson, G.S., Johny, A., Kolarevic, J., Lazado, C.C., Madaro, A., Nilsson, J., Ottem, K.F., Seljestokken, B., Slettjord, T., Timmerhaus, G., Tschirren, L., Ytteborg, E., Zena, L., Åsli, M. (2025b). The CrowdMonitor handbook for commercial net pens v0.1: an updated welfare indicator toolbox and crowding intensity scales for measuring and monitoring Atlantic salmon welfare during crowding operations. Nofima rapport nr. 30/2025 https://doi.org/10.21357/68na-zn54

Peer reviewed publications:

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