

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

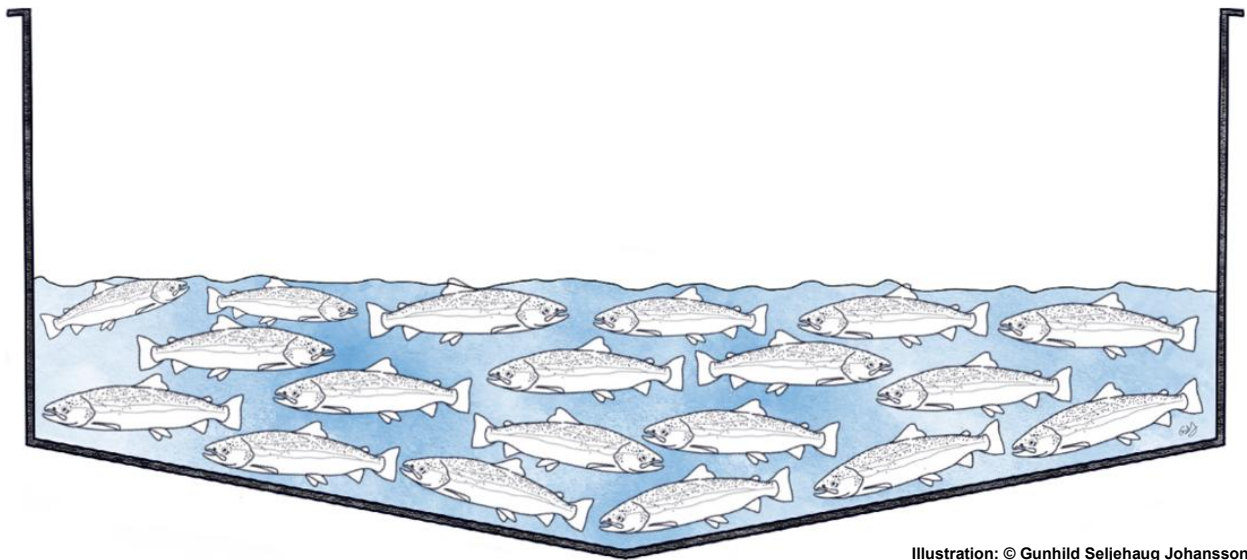


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The CrowdMonitor handbook for commercial tanks v0.1: an updated welfare indicator toolbox and crowding intensity scale for measuring and monitoring Atlantic salmon welfare during crowding operations

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 CrowdMonitor-håndbok v0.1: Oppdatert verktøykasse for vurdering og overvåkning av velferd og trengingsintensitet under trenging av laks i kar (i kommersielt oppdrett)

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Summary/Recommendation:
 This handbook is an output of the FHF - Fiskeri- og havbruksnæringens forskningsfinansiering (the Norwegian Seafood Research Fund) funded project "CrowdMonitor" (grant agreement number 901595). 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 in net pens and tanks using an updated suite of operational and laboratory-based welfare indicators (OWIs and LABWIs). It involved two Norwegian research partners (Nofima and the Institute of Marine Research) and two commercial partners (Cermaq Norway AS and Grieg Seafood ASA). This handbook builds upon and expands existing welfare indicator toolboxes, suggests new crowding intensity scales and recommendations for aiding the crowding of Atlantic salmon in tanks based upon the results of the CrowdMonitor project, other relevant projects, other information sources, and an updated scientific state of the art. The suggestions we provide are no guarantee of crowding outcomes and are meant as guidance and support for personnel involved in monitoring and managing the crowd.

Sammendrag på norsk:

Denne håndboken inneholder resultater fra det FHF-finansierte prosjektet CrowdMonitor (prosjektnummer 901595). Prosjektet har hatt som formål å oppdatere den vitenskapelige og operative kunnskapen om trengingsoperasjoner i lakseoppdrett, både i kar og merder, ved bruk av en oppdatert samling av operative og laboratoriebaserede velferdsindikatorer. Prosjektet ble utført som et samarbeid mellom to norske forskningsinstitusjoner, Nofima og Havforskningsinstituttet, og to kommersielle aktører, Cermaq Norway AS og Grieg Seafood AS. Håndboken bygger på og utvider eksisterende verktøykasser for velferdsvurdering og foreslår nye vurderingsskalaer for trengningsintensitet, og anbefalinger for hvordan å trenge Atlanterhavslaks i kar. Dette med utgangspunkt i resultatene fra CrowdMonitor-prosjektet, andre relevante prosjekter og informasjonskilder, samt oppdatert vitenskap. Anbefalingene i håndboken er ingen garanti for gode utfall ved trengning, men er ment som en veiledning og støtte for operatører som utfører og overvåker slike operasjoner.

Preface

This handbook is an output of the FHF - Fiskeri- og havbruksnæringens forskningsfinansiering (the Norwegian Seafood Research Fund) funded project “CrowdMonitor” (grant agreement number 901595). 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 in net pens and tanks using an updated suite of operational and laboratory-based welfare indicators (OWIs and LABWIs). It involved two Norwegian research partners (Nofima and the Institute of Marine Research) and two commercial partners (Cermaq Norway AS and Grieg Seafood ASA).

This handbook builds upon and expands existing welfare indicator toolboxes, suggests new crowding intensity scales and recommendations for aiding the crowding of Atlantic salmon in tanks based upon the results of the CrowdMonitor project, other relevant projects, other information sources, and an updated scientific state of the art. The handbook utilises and further develops the format of the existing crowding welfare indicator toolbox outlined in the FISHWELL Welfare Indicator handbook for Atlantic salmon ¹.

NB: The suggestions we provide are no guarantee of crowding outcomes and are meant as guidance and support for personnel involved in monitoring and managing the crowd.

We wish to say a big thank you for all the fantastic help from research, technical and operational personnel from all the collaborating partners, especially the farm partners when we tested the toolboxes. Some of the recommendations in this handbook are based on the CrowdMonitor tank experiments, which were conducted at Nofima’s Research Station for Sustainable Aquaculture at Sunndalsøra. Other recommendations are based on the CrowdMonitor net pen experiments, which 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 wonderful help and dedication to making these experiments a success. 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 remotely operated vehicle (ROV) surveys and helped in creating the ROV risk scale for crowding. We also wish to say a big thank you to Sebastian Gjertsen 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 the data that was generated.

Front cover image © Gunhild Seljehaug Johansson.

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1 Introduction

Crowding is an operation where fish are temporarily subjected to increased density and a reduced available water volume. It is usually carried out to expedite the removal or transfer of fish from a tank or net pen. It is a central component of several Atlantic salmon farming procedures throughout a tank-based production cycle, including but not limited to i) transfer between production units, ii) vaccination, iii) transport, and iv) grading ¹. This handbook focuses on tank crowding in relation to the seawater transfer of smolts or post smolts from hatchery facilities. Crowding operations involve hazards that are a welfare risk for Atlantic salmon (see ¹⁻⁵) and all other farmed fish species. These risks are dependent upon the crowding method, rearing system, water environment, life stage and fish health and welfare status. Their threat can be minimised if tools are available for detecting them at an early stage and mitigation strategies are available.

1.1 A welfare indicator framework for crowding

A range of welfare indicators (WIs) are needed to measure and document fish welfare before, during and after crowding operations. These WIs can be classified as originating on or from the fish (Outcome-based WIs) or address the resources or environment the fish are subjected to (Input-based WIs). The indicators can be further broken down into those that are farm friendly (Operational Welfare Indicators, OWIs) or more complex indicators that are sampled on the farm and sent away for further laboratory analysis (Laboratory-based Welfare Indicators, LABWIs), see ¹. To facilitate the targeted use of each WI, they can be organised in a framework.

The FISHWELL welfare indicator handbook ¹, a further book chapter on assessing fish welfare ⁶ and the LAKSVEL protocol ⁷ have suggested a three-step framework for measuring and monitoring farmed Atlantic salmon welfare with different OWIs and LABWIs (see a refined version of this, Figure 1). The complexity of the WIs increase from the first to third stage of the framework. The first stage of the framework utilises non-invasive OWIs, e.g. simple water quality indicators (e.g. dissolved oxygen saturation) and basic observations of e.g. fish behaviour and appetite. In case one or more of these first stage outcome-based indicators suggest that something may be wrong with the fish, or there is not enough information, the fish farm personnel can move to the second stage of the framework that still involves non- or minimally invasive monitoring. Depending on the nature of the problem they can then perform more extensive monitoring of e.g. oxygen variability, fish behaviour and/or sample fish for scoring of external injuries according to the LAKSVEL protocol ⁷ to get a more precise and detailed overview of the problem. If the fish farm personnel still do not have enough information for an informed decision (in the case of crowding, on whether to commence, proceed with, or how to best manage the crowd), fish health personnel or other experts should be called in to carry out even more complex monitoring and collect samples for later auditing. This third stage is typically the stage where one can start using the more complex LABWIs. In this handbook, we have refined this framework to consider the complexity of measuring and monitoring welfare and clarify whether the WIs are non-invasive, minimally invasive or invasive and then applied it exclusively for crowding.

Although OWIs are the most commonly used welfare indicators, their ease of use and general applicability should not distract from the numerous existing and emerging LABWIs that are becoming increasingly used by the R&D community, fish health services and also the farmers themselves. For example, assessing mucosal health is driven by emerging knowledge on the sensitivity and responsiveness of mucosal surfaces to the stressors fish can face during crowding, and new instruments can detect changes in the responses of this WI to existing and emerging threats with considerable resolution e.g. ⁸. Further LABWIs to consider are tools for monitoring cardiac activity such as electrocardiograms, which can give information on cardiac health, including wave morphology, durations, and intervals that can serve as indicators of the fish's ability to tolerate crowding operations

⁹. Heart rate loggers—along with associated physiological stress indicators—may provide important information on the cardiac and physiological status of the fish. However, they require surgical implantation ¹⁰. Other LABWIs, such as skin models for assessing wound healing, can be used to determine if there is any innate and adaptive immune suppression associated with the crowding operation ^{11,12}. Each OWI and LABWI has various benefits and challenges for documenting fish welfare and there are various technologies and methods for measuring and monitoring each WI ranging from non-invasive to invasive (see Box 1).

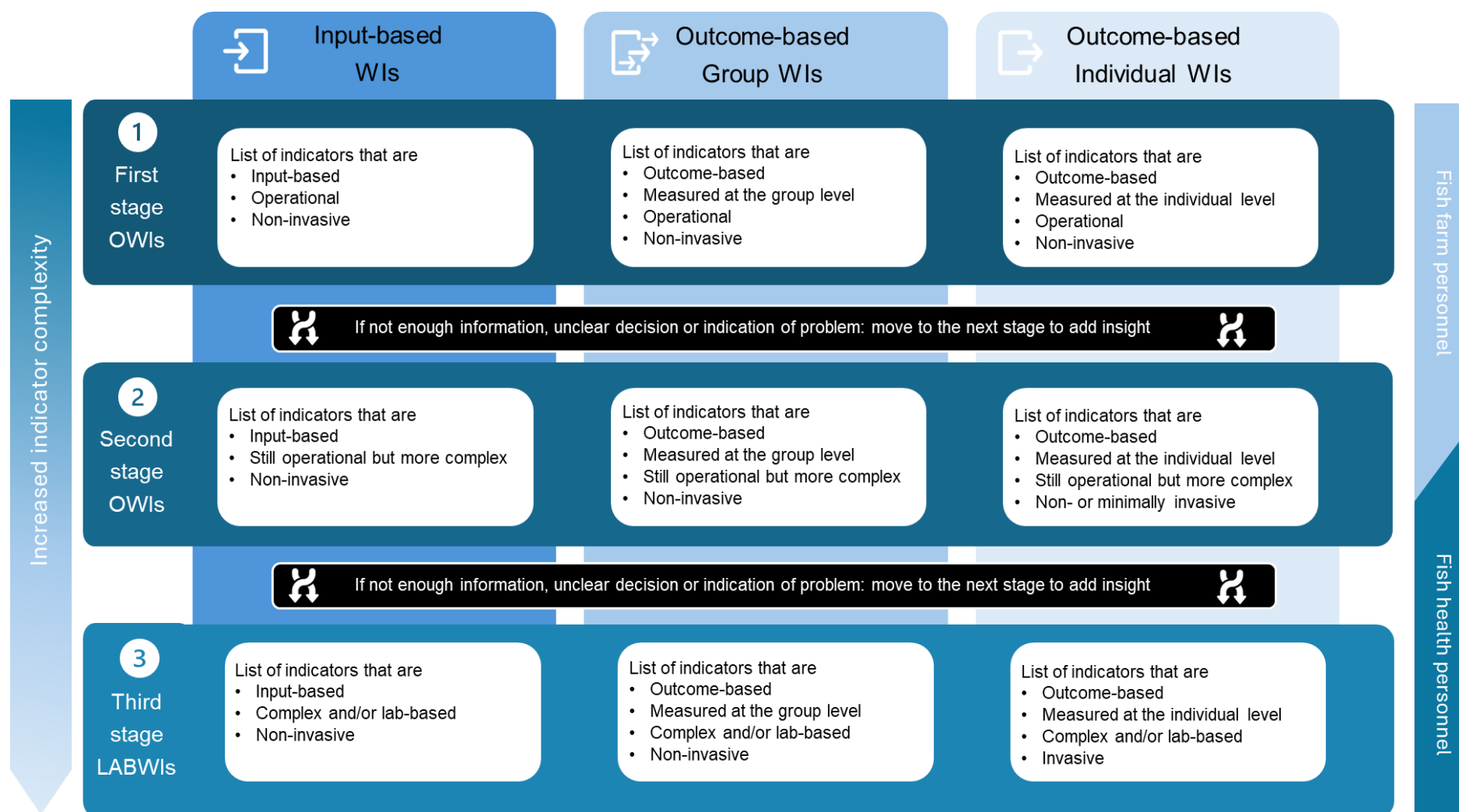


Figure 1 An updated OWIs and LABWI framework for crowding utilising i) Input-based WIs (water quality parameters in this instance), ii) Outcome-based WIs at the group and individual level. Figure © Noble, C. Stien, L.H., Tschirren L. and Johansson, G.S. Adapted from figures outlined in ^{1,6,7}.

Box 1: Levels of invasiveness of the OWIs and LABWIs within the presented framework

Non-invasive monitoring technologies can be used to:

- Measure and monitor water current and water quality in and around the crowd ¹. This can help identify potential water-based risks and to detect deteriorating conditions before they become serious.
- Monitor the behaviour of the fish either above or below water, preferably both ⁵ to especially identify behaviours associated with any potential welfare problem that pose a danger for the fish.
- Observe and monitor mouth opening frequency or gill beat rate as an indicator of potential stress, exhaustion or stimulation ¹³.
- Observe the speed of the crowding operation and irregularities.
- Measure and monitor changes in mechanical forces towards the crowding equipment or system infrastructure to detect and monitor the frequency and severity of impacts that may harm the fish.
- Observe the frequency and severity of external injuries on the fish before, during and after a crowding operation ^{5 14}.

Minimally invasive monitoring methods (on anaesthetized fish, e.g. during lice counting) can be used to:

- Measure and monitor the health and welfare of individual fish, e.g. by using the LAKSVEL injury scoring scheme ⁷ or AGD scoring of the gills ¹⁵.
- Assessing cardiac health in anesthetized fish through real-time electrocardiography using either minimally invasive subcutaneous needle placement ^{9,16} or non-invasive flat electrode ECG recordings (Lucas Zena, personal communication).

Invasive monitoring methods can include:

- Histological approaches to evaluate the status of the skin, heart and gills before, during and after a crowding operation to understand how the operational procedures affect cellular integrity ⁵.
- Assessing heart morphology using qualitative and quantitative methods ¹⁷.
- Molecular and cellular analytical tools such as transcriptomics and proteomics, which may give insight into potential damage at the micro-level ⁸. This may help a stakeholder understand the impact of different levels of stress and to identify high risk elements of the crowding process.
- Indicators of wound healing potential, such as migration assays that utilise scale explants, can add further functional information for understanding how the fish will respond to operational procedures in the long run ⁵.
- Physiological markers such as glucose and lactate take some time to peak after the initiation of a stressor, which limits their utility for real-time risk monitoring and Gismervik et al. (in ¹) state that *“the values are dependent on the condition/state of the fish in addition to the event itself.... Measuring lactate and pH can give an indication of stress if the measurements are repeated during the crowding procedure or carried out before, during and after. They can also help direct future best practice procedures but are not a good “stop signal” concerning welfare during ongoing operations.”* Similarly, cortisol is a well-established LABWI in many operational settings and has utility in crowding situations if samples can be obtained in a standardised and rapid manner.
- Biosensors for measuring changes in heart rate to detect increases in potential stress and arrhythmic heart beats ^{14,18–20}.

2 Targeted welfare indicator toolboxes for crowding

It is important that staff have a plan on how to measure and monitor welfare before, during and after a crowding operation and any subsequent handling the fish are subjected to and act upon any problems they discover ^{e.g. 21,22}. From the updated OWI and LABWI framework (Figure 1), specific sets of WIs – so-called toolboxes – can be put together to monitor fish welfare in a targeted situation.

The main goal of this CrowdMonitor handbook is to suggest toolboxes with fit-for-purpose indicators when Atlantic salmon are subjected to crowding in tanks, specifically in relation to the seawater transfer of smolts or post smolts from hatchery facilities. To further facilitate their use, specific toolboxes are proposed for the time before, during and after the crowding operation following the structure proposed by ^{23,24} (and unlike the FISHWELL welfare indicator handbook ¹ which proposes a WI toolbox for crowding that only applies for the crowding event itself). The proposed toolboxes can help stakeholders in their selection of appropriate welfare indicators and in identifying anomalies that can be an early warning of emerging risks or draw their attention to an ongoing problem.

A major part of crowd management is based on surface and sub-surface observations of the fish to gauge the intensity of the crowding operation. Various crowding intensity scales have been produced to help the farmer steer a crowd using surface observations such as one outlined by ²⁵, adopted by the RSPCA welfare standards for farmed Atlantic salmon ²⁶ and adopted in the FISHWELL handbook ¹. This specific crowding intensity scale is widely used in the industry, by retailers and by the Norwegian Food Safety Authority (Mattilsynet ²⁷), but it is not without its limitations: i) it only monitors surface activity (giving a limited overview of the whole group), ii) it was designed for net pen crowding of fish at slaughter and may not be very applicable to crowding in other situations (e.g. tanks) or potentially other fish sizes, iii) it is somewhat opinion based. These limitations were some of the main focus areas for the CrowdMonitor project. It should also be noted that fish can still be stressed and swim calmly ⁴ and behaviour/observation metrics should be considered as part of an integrated welfare indicator toolbox.

In the CrowdMonitor handbook we propose the first version of an updated crowding intensity scale for tanks based upon surface observations, increasing the range of observational metrics included in the scale compared to previous works, whilst also attempting to standardise the terminology for each metric.

We also aim to format it in a way that can be integrated into Standard Operating Procedures (SOPs) and have assigned it five levels (1-5).

We propose the following:

- **level 1** addresses any potential acclimation and inter-crowd period and can be classified as **minimal risk - the crowding can proceed as planned**
- **level 2** can be classified as **low risk** and the crowding can **proceed as planned**
- **level 3** has **increasing risk** but the crowding can **proceed as planned (with increasing levels of diligence** from the crowding staff with **corrective actions being readied and applied if needed)**
- **level 4 poses even greater risks** and the crowding staff should **consider intervening with a corrective action/pausing/releasing the crowd**
- **level 5 is high risk, clearly unacceptable, and a corrective action should be immediately implemented/the crowd should be released** (this also equates to many of the metrics for level 5 crowding in the previous intensity scales ^{1,25,26}).

NB: The suggestions we provide are no guarantee of crowding outcomes and are meant as guidance and support for personnel involved in monitoring and managing the crowd.

3 Before crowding

The following section outlines a brief WI toolbox for monitoring fish welfare and managing welfare risks before a crowding operation.

3.1 Risks to consider before crowding

Crowding is a stressful operation for the fish and optimal outcomes are expected when stress is minimised and welfare maximised before, during and after the operation. **Awareness of potential risks before the operation** (Box 2) helps a stakeholder select a range of relevant welfare indicators from the WI framework and put together a useful toolbox.

Fish with compromised health or welfare may not be resilient enough to withstand the potential challenges they can encounter during the crowding procedure and any subsequent handling it is associated with. A particular factor to consider is **gill health**, as fish with damaged or diseased gills may struggle to meet their oxygen demand under stressful and/or reduced oxygen conditions. **Heart health** is a critical concern, particularly as underlying heart conditions—such as abnormalities in heart morphology and cardiovascular diseases—can be prevalent in farmed salmon. These issues can stem from infections, including viral infections that cause heart inflammation, such as Heart and Skeletal Muscle Inflammation (HSMI) and Cardiomyopathy Syndrome (CMS). Additionally, coronary arteriosclerosis is becoming increasingly common and severe in farmed salmon (Heidi Mortensen, Lucas Zena, personal communication). This condition can impair heart oxygenation (i.e., myocardial ischemia) thereby weakening the heart's ability to cope with exposure to a stressor such as crowding and any potential subsequent handling. Engdal et al. ¹⁷ have refined and developed quantitative (addressing 5 traits) and qualitative (picture guide addressing 42 morphological traits) methods for auditing heart morphology, in addition to producing a pictorial guide for operational use as an appendix ¹⁷. **Skin condition** is also important, as existing skin infections and/or skin damage can increase the risk of and be exacerbated by the crowding operation. Further, factors such as **abnormal behaviour**, **reduced appetite and/or increased mortality** (which all indicate that something is wrong with part or all of the population), should be considered indicators of underlying problems that can pose a risk to the crowding.

Fish should also undergo **fasting** before crowding so they are not digesting food, and thereby have lower metabolism and lower oxygen demand, which increases their stress tolerance. Additionally, fasting prevents the risk of fish evacuating their gastric tract and defaecating if stressed, which can decrease water quality ²⁸.

Water quality risks include those associated with **dissolved oxygen (DO) saturation levels**, **water temperature** and **water current speed**. Low dissolved oxygen saturation levels are a welfare risk. High water temperatures lower the amount of oxygen available to the fish. Crowding at low water temperatures can increase the risk of winter ulcers in the days and weeks after the operation e.g. ^{1,27}. With regard to non-medicinal delousing treatments that involve handling, the Norwegian Food Safety Authority have stated that risk is not just linked to absolute temperatures but also whether it is expected to rise or fall during the period after the operation ²⁴. This approach is relevant for evaluating whether fish should be crowded in tanks. Information on water velocity helps the farmer assess how fast the water is replaced inside the crowd and if the fish will be under added stress due to high water speed or be at risk of low dissolved oxygen levels if speeds are too low.

With regard to **number of previous treatments/crowding operations**, and the **time since/severity of the last crowding operation**, each crowding or handling operation poses a risk for fish welfare, and this risk can accumulate as the number of crowds/handlings increases, e.g. ²⁴. However, there is a high

level of uncertainty around this risk as it is influenced by an array of confounding factors related to e.g. the severity of previous events and their effect upon fish welfare.

Box 2: Risks BEFORE crowding include, but are not limited to:

- Compromised health and welfare status:
 - Poor gill health
 - Poor heart health
 - Poor skin condition
 - Abnormal behaviour
 - Reduced appetite
 - Increased mortality
 - Disease status and history
- Non-fasted, or insufficiently fasted, fish
- Suboptimal water quality:
 - Low dissolved oxygen saturation levels
 - High temperature (and whether it is expected to rise or fall post handling)
 - Low temperature (and whether it is expected to rise or fall post handling)
 - Water velocities that are too high or too low
- Management factors
 - Time since last crowding/handling
 - Severity of last crowding/handling

3.2 A Welfare Indicator Toolbox to use before crowding

The overall goal of this toolbox is to suggest indicators for evaluating whether the status of the fish, the equipment and the environment will promote a low risk crowding operation. These will be simple OWIs, mostly outcome-based, but also a selection of input-based OWIs related to the rearing environment and e.g. handling history.

3.2.1 OWIs to consider before the crowding operation

The CrowdMonitor project has identified six key sets of welfare indicators and factors that a stakeholder should consider to gain a holistic insight into fish health and welfare status **before crowding**. These indicators can be used to support and inform decisions on e.g. whether the fish can handle the operation. The grading of the welfare indicators follows the LAKSVEL protocol ⁷, where the indicators are divided into four levels from 0 to 3 (Table 1). The six welfare indicators are outlined in Table 2. NB: water quality parameters should also be considered in the toolbox and monitored as close to the start of crowding as possible, but these are not assigned levels 0-3.

Table 1 General definition of some of the outcome-based welfare indicator levels used in the CrowdMonitor handbook (adapted from the LAKSVEL protocol ⁷)

Level	Fish status
0	Free from injuries, disease or deviation.
1	Indicates a minor injury, disease or deviation, that normally is assumed to have little impact on fish welfare but still indicates that something is not optimal.
2	Indicates a clear injury, disease or deviation.
3	Indicates a severe injury, disease, or deviation that is assumed to have large consequences for the welfare of the fish.

Table 2 A suggested welfare indicator toolbox for monitoring the health and welfare status of the fish **before crowding**. The toolbox focuses predominantly on outcome-based WIs. NB: water quality parameters should also be considered in the toolbox, but these are not assigned levels 0-3 ²⁹

Before crowding	Level 0	Level 1	Level 2	Level 3	
Behaviour	"Normal group shoaling behaviour (outside feeding times) with no or very few individuals with deviating behaviour"	"Normal shoaling behaviour by the majority of fish, but small groups of individuals isolate themselves from the general fish group ("loser fish")"	"Clear deviating behaviour of large parts of the group or an increasing or significant part of the group with disease related or other abnormal behaviour"	"Very clear deviating behaviour, or large part of the group showing behaviour that indicates bad environmental conditions or disease, often connected with increased mortality"	Nilsson et al. ⁷
Appetite	Fish show normal feeding motivation and have eaten as much as expected over the past week based on fish size, water temperature and time of year	Fish show slightly lower feeding motivation and have eaten somewhat less than expected over the past week based on fish size, water temperature and time of year	Fish show markedly lower feeding motivation and have eaten noticeably less than expected over the past week based on fish size, water temperature and time of year	Fish are eating very little and have shown little feeding motivation over the past week based on fish size, water temperature and time of year	Adapted from Nilsson et al. ⁷
Mortality	Monthly: 0.0-0.3% Weekly: 0.0-0.7% Daily: 0.0-0.1%	Monthly: 0.3-0.7% Weekly: 0.7-1.7% Daily: 0.1-0.25%	Monthly: 0.7-2.0% Weekly: 1.7-4.9% Daily: 0.25-0.7%	Monthly: >2.0% Weekly: >4.9% Daily: >0.7%	Nilsson et al. ⁷
Gill health status	Free from injuries or deviations, no evidence of e.g. amoebic gill disease (AGD)	Minor injury or deviation	Clear injury or deviation	Severe injury or deviation	Nilsson et al. ⁷
Heart health status	No evidence of HSMI/CMS. No deviation in autopsy findings/histology	HSMI/CMS suspected. Minor deviation in autopsy findings/histology	HSMI/CMS confirmed. Clear deviation in autopsy findings/histology	HSMI/CMS outbreak ongoing. Severe deviation in autopsy findings/histology	Histology scoring adapted from Fritzvold et al. ²⁹
Scale loss, body wound/ulcer, skin haemorrhaging, snout damage, fin damage	Free from injuries or deviations	Minor injury or deviation	Clear injury or deviation	Severe injury or deviation	Nilsson et al. ⁷

Other input based OWIs to consider not assigned a 0-3 score: Dissolved oxygen saturation levels, water temperature and changes in water temperature, water flow and velocity, see section on input-based indicators during crowding for more information on these parameters

Monitoring welfare indicators before crowding and handling can, to a large extent, follow some of those that are monitored during the operation, but with some specific information to consider for certain input- and outcome- based welfare indicators:

Behaviour. Observational metrics developed in the LAKSVEL project ⁷ are appropriate to follow before crowding and are outlined in Table 2 above.

Appetite. Metrics for monitoring fish appetite developed in the LAKSVEL project ⁷ are appropriate to follow before crowding and adapted versions are also outlined in Table 2 above.

Mortality. A non-specific but appropriate indicator ⁷ to use around crowding operations. The mortality status of the fish group (stable, decreasing or increasing) and potential expected developments in mortalities should be considered before crowding, in addition to mortality causes (after ^{23,24}). Differing mortality levels are outlined in Table 2, after ⁷.

Health Status. An ongoing overview of any changes in gill, heart and skin health status before the operation will help an operator get a better overview of the potential health and welfare risks it will pose to the fish. See Table 2 for guidelines on how to follow health status before the crowding event. Engdal et al. ¹⁷ have refined and developed quantitative (addressing 5 traits) and qualitative (picture guide addressing 42 morphological traits) methods for auditing heart morphology, in addition to producing a pictorial guide for operational use as an appendix ¹⁷. With regard to non-medicinal delousing treatments that involve handling, the Norwegian Food Safety Authority ²⁴ have stated that it is good practice to undertake a thorough assessment of fish health status before the treatment, and that fish health status at the time of treatment is up to date (using data < 3 weeks old) and used as the foundation for the treatment plan. The health status of the fish should also be updated immediately before the operation ²⁴. Nygaard et al. ²³ also suggest clinical diseases/disorders should be considered in relation to whether it is early or late in their development, mortality levels (high or low) and the expected development of the disease/disorder. This information is also relevant for tank crowding.

The majority of the **LAKSVEL ⁷ external injury-based OWIs** are appropriate for monitoring fish welfare status before the operation, especially the ones relating to i) the first impression of the fish, ii) scale loss, iii) skin haemorrhaging, iv) body wounds, v) snout injuries, vi) eye opacity, vii) eye injuries, viii) opercular injuries, ix) gill damage and x) fin damage. With regard to non-medicinal delousing treatments that involve handling, the Norwegian Food Safety Authority ²⁴ have stated that with regard to injury levels amongst the fish group, the proportion of fish with body wounds that correspond with LAKSVEL ⁷ level 2 should not exceed 5% of the group before handling. They also state that the proportion of fish with level 3 body wounds according to LAKSVEL should be very low, i.e. close to 0%. The future healing prognosis will affect any potential risk appraisal and should also be included in an overall assessment. Small snout wounds, "white snouts", such as Level 1 snout wounds according to LAKSVEL ⁷ are excluded from Norwegian Food Safety Authority guidelines and no upper limit has been set for the proportion of fish with Level 1 LAKSVEL wounds, scale loss and skin haemorrhaging bleeding ²⁴.

NB. For further potential consideration:

Handling history. As each crowding or handling operation poses a risk for fish welfare, handling history and the welfare consequences of previous handling events should be taken into account before crowding and/or handling the fish in the future and should be judged on a case-by-case basis.

Time since last handling. This can affect e.g. the ability of fish to tolerate a further handling event and should be considered in any pre-crowding welfare evaluation on a case-by-case basis.

Number of previous crowdings. With regard to non-medicinal delousing treatments that involve handling, the Norwegian Food Safety Authority ²⁴ have stated that they do not take a position on the number of treatments associated with high risk, stating risk must be evaluated on a case-by-case basis. We believe this information is also relevant for tank crowding.

Frequency of crowding. For information, crowding frequency is addressed in various welfare standards and guidelines. For example, the RSPCA ²⁶ state “No enclosure must be crowded more than twice in any one week or three times in any month, unless this is required by the designated veterinary surgeon for fish welfare reasons” and “After transfer to sea, smolts must not be handled for at least 120 days, for example not crowded, except for veterinary treatments.” Global Animal Partnership’s (G.A.P’s) 5-step Animal Welfare Standards for Farmed Atlantic Salmon v1.0 ³⁰ state “Newly transferred smolts must not be pumped, crowded or graded for their first 90 days in seawater unless immediate medical intervention or culling is required.” Global Animal Partnership standards ³⁰ also state “Salmon must not be crowded more than 3 times in any 30-day period”. Compassion in World Farming: Food Business ³¹ state in their humane slaughter of salmon article “repeated crowding should be avoided. Where unavoidable there should be a period of 24-48 hours between subsequent crowds”.

NB. Water quality samples can also be collected before the operation for later retrospective LABWI analysis if needed, see e.g. ³².

4 During tank based crowding

The following section outlines a tank-specific WI toolbox for monitoring fish welfare and managing welfare risks during the crowding operation. The tank section of this handbook applies specifically in relation to the seawater transfer of smolts or post smolts from hatchery facilities, not earlier or later in the production cycle (although it can be adapted by the user for tank crowding earlier in the hatchery phase).

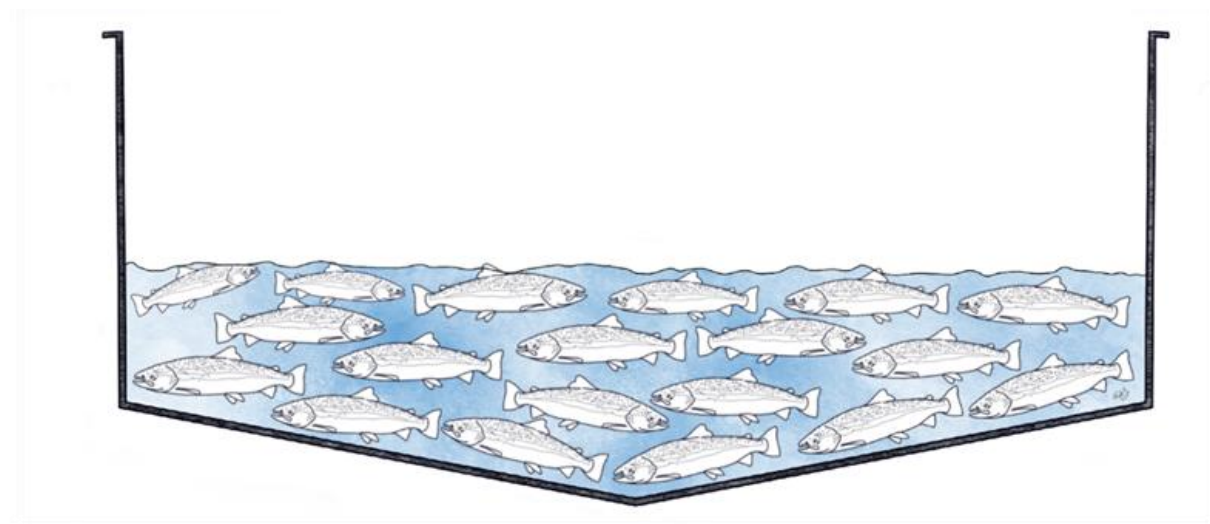


Figure 2 A tank-based crowding operation that involves draining the tank and decreasing water levels to reduce the available water volume and increase fish density. © Gunhild Seljehaug Johansson

4.1 Risks to consider during tank crowding

Tank-based crowding often involves draining the water to reduce water volume before the fish are pumped and/or transported out of the tank, especially for e.g. smolt transport, in Norway, the UK and Ireland ³³ and this will be the primary focus of the following section. However, tank crowding can also involve a combination of water draining and/or the adjustment of mobile crowding screens that create a narrow but deep crowd by reducing lateral water volume without overtly reducing depth ^{34,35} and this is a method often used in tank crowding of rainbow trout, European seabass and gilthead seabream in e.g. some European countries ³³. For crowding Atlantic salmon using this method, we refer the reader to the CrowdMonitor tank experiment results ¹⁴ and also ³⁶. Sweep nets (or seines) can also be used for crowding in large tanks in some situations. In such cases, we recommend that the operator follows the majority of WIs recommended for net pens in the associated CrowdMonitor net pen handbook. Each of these approaches may have a differing behavioural effect upon the fish, and existing crowding guidelines may not account for this.

As outlined earlier in section 3 (before crowding), fish with compromised health or welfare may not be resilient enough to withstand the potential challenges or risk they can encounter during the crowding procedure and any subsequent handling it is associated with. A particular factor to consider is **gill health**, as fish with damaged or diseased gills may struggle to meet their oxygen demand under stressful and/or reduced oxygen conditions. **Heart health** is a critical concern, particularly as underlying heart conditions can impair heart oxygenation (i.e., myocardial ischemia) thereby weakening the heart's ability to cope with exposure to a stressor such as crowding and any potential subsequent handling. **Skin condition** is also important, as existing skin infections and/or skin damage can increase the risk of problems and also be exacerbated by the crowding operation. The crowding operation can also lead to

various external injuries (e.g. scale loss ³) as the fish come into contact with the tank, tank equipment or each other. Any form of struggle or panic can make this worse. **Abnormal behaviour** or **increased mortality** indicate something is wrong with part or all of the population and the fish are **stressed**. Stress and high activity increase **oxygen demand**.

It is advisable to ensure that the **water quality** in the tanks and inlet water is good prior to starting the crowding operation, especially when crowding fish in systems with some degree of water recirculation. See the FISHWELL handbook ¹ for water quality recommendations. If the fish are pumped or otherwise transported out of the tank through closed pipes, with no water replenishment except from the tank itself, one should take into account that the worst water quality will likely be experienced by the fish near the end of those pipes.

In addition to water quality risks associated with **Dissolved Oxygen (DO) saturation levels**, **water temperature**, and **water current speed**, crowding fish in tanks is vulnerable to **gas supersaturation** and **changes in temperature or pH**. Low dissolved oxygen saturation levels lower the amount of oxygen available to the fish. Crowding at **low water temperatures** can increase risks for skin ulcers in the days and weeks after the operation e.g. ^{1,27}. Increased water flows can reduce the risk for poor oxygen conditions, but at the same time, increase risks of the fish being stuck against the tank and its equipment if they become exhausted or if flows are too strong for them to swim against. **Gas supersaturation** of water, e.g. due to the use of emergency oxygenation systems, inadequately sealed pressurised pipes or pumps, or the mixing of water with different temperatures can also pose a welfare risk to the fish. **Changes in water temperature or pH** can alter the speciation of compounds or ions that are potentially toxic to the fish (e.g. TAN, Al, Fe) or change the solubility of gases.

With regard to crowding equipment and crowd management factors, it is important to note that although crowding risk increases with **crowding duration** ³, risk also increases with **crowding intensity** ¹⁴. The farmer must therefore find a balance between these two risks and steer the crowding process at a speed that both expedites the operation and exposes the fish to as little stress and risk as possible. **Equipment limitations** and a **shortage of trained staff** can increase the risks associated with crowding operations. **Inadequate process monitoring set up**, e.g. improperly calibrated sensors or poor camera coverage, can result in delayed or erroneous information processing and decision making. **Poor water clarity** can negatively impact observations of the fish and the crowd and may mean a risk goes undetected ¹. **Hazards** such as sharp edges or poorly designed tank infrastructure (that the fish can get trapped around or harm themselves against) are also a welfare problem. **Logistical pressures** regarding recipient tank/boat availability may drive a need for finishing the operation quickly and can mean the operation is rushed. If the tank crowd is associated with a wellboat transfer, poor weather, such as high winds or currents, can delay the loading of the fish or delay/terminate the operation.

It is important to evaluate these risks and others (Box 3) and come up with suitable mitigations. For example, some water quality and operational risks may be alleviated by supplementary oxygenation or assigning extra personnel to monitor the crowd, while in other cases the added risk may be so high that the crowding operation should be delayed for a few hours to potentially days, until the risk levels decrease.

Box 3: Risks to consider DURING tank crowding include, but are not limited to:

- Compromised health and welfare status:
 - Poor gill health
 - Poor heart health
 - Poor skin condition
 - Abnormal behaviour
 - Increased mortality
- Water quality:
 - Low dissolved oxygen saturation levels and/or high temperature
 - Low water temperatures
 - Gas supersaturation of water
 - Changes in water temperature or pH
- Equipment and crowd management factors:
 - High crowding intensity
 - Long crowding duration
 - Equipment limitations
 - Shortage of trained staff
 - Inadequate process monitoring set up
 - Poor water clarity – turbid water or surface foam hindering visual observations
 - Tank hazards – e.g. sharp edges
 - Logistical pressures
 - If the tank crowd is associated with a wellboat transfer, poor weather

4.2 Managing the crowd during tank crowding

Crowding fish in tanks is a complex operation requiring skilled personnel who have a thorough knowledge on features of the rearing system and how to manage the crowding process (Box 4). Poor management can cause fish to be pressed against the tank, tank equipment or other fish, resulting in physical damage, compressive asphyxiation, or even death if gill ventilation is impaired. This can occur even when the starting point for the crowd involves good water quality, sufficient available space, healthy fish and fully checked and maintained crowding equipment.

Tanks come in a large variety of shapes and sizes, and water inlets and outlets also have a variety of designs that can affect tank hydrodynamics (before and during crowding) depending on e.g. their placement. Mismatches between the pumping and the speed of the crowding process can lead either to overcrowding or to a crowd that goes too fast or too slow, which would overexpose the fish to potential risks. Water levels in the tank might drop too fast for the fish to adapt to the reduced water volume and depth and increasing crowding intensity. Personnel must therefore know how fast to steer the crowd, and it is especially important that personnel can quickly recognise warning signs of poor welfare, so they can then adjust the operation accordingly and minimise risk. For example, if the fish begin expelling bubbles through the mouth/gill openings it can be a sign that fish are potentially stressed ³⁷ and there are problems in the crowd ³² but this might be challenging to observe with e.g. small fish and a high density crowd.

Box 4: Managing a crowd in a tank involves managing the equipment, water quality, behaviour of the fish and logistics of the operation. Factors to consider include, but are not limited to:

- Slowly introducing any crowd monitoring equipment and personnel around the tank to allow the fish to adapt to their presence.
- Adjusting water volume and depth gradually when draining the tank to prevent stressing the fish and to ensure that the fish have time to adapt.
- Avoiding situations that can lead to poor water quality for the fish in the crowd.
- Maintaining water quality and adequate flow through fish transfer pipes during operations.
- Avoiding instances where fish can experience a loss of behavioural control by ensuring sufficient water volume, proper oxygen levels, and appropriate distances from other fish and crowding equipment ¹.
- Avoiding situations that can trigger detrimental behaviours like panicking or crowd surging, which can lead to escape responses such as burrowing and can mean the fish injure themselves (e.g. the snout) by coming into contact with other fish or the tank and its infrastructure. Panic behaviours also increase oxygen demand.
- Preventing fish from being forced against static surfaces or becoming entangled in tank equipment, such as oxygen diffuser hoses.
- Initiating corrective actions to help fish adapt and avoid panic behaviours, like pausing the operation, increasing water inflow, reducing fish pumping rates, and allowing more space for swimming.
- Whilst there is a knowledge gap on the use of sedation during crowding, sedatives can be used to calm the salmon, and can lead to reduced cortisol and lactate levels and reduced scale loss ³⁸. If this is considered, gill health must be taken into account ²³, dosing recommendations from sedative producer/veterinarian should be followed, and the crowd operator should also be prepared for reduced oxygen consumption, and that the fish will likely resist pumping less, increasing the risk of higher fish densities in transfer pipes (and excess foaming in biofilters if crowding a RAS system).

4.3 A Welfare Indicator Toolbox to use during tank crowding

The CrowdMonitor welfare indicator toolbox for tanks can be applied in three stages, where first stage indicators are mostly non-invasive OWIs and relevant for fish farm personnel, whereas second and third stage indicators are increasingly more complex and can be more applicable to fish health specialists or technical experts (Figure 3). Second stage indicators are typically used if the first stage indicators suggest i) that something is not optimal, ii) the farm personnel are not able to explain and make an informed decision based on the deviation and/or iii) if the stakeholder wants even more detailed information on the health and welfare status of their fish. Third stage indicators are often analysed retrospectively and do not allow for any direct intervention during the crowding procedure (Figure 2). Monitoring the crowd is essential as many factors outside the control of the farm personnel can suddenly influence water quality, potential crowd management bottlenecks and fish behaviour inside the crowd. The farm personnel must therefore be ready to quickly adapt, implement corrective actions, pause or release the crowd if necessary.

	 Input-based WIs	 Outcome-based Group WIs	 Outcome-based Individual WIs																				
1 First stage OWIs	• Dissolved oxygen saturation (inlet, tank) • Water temperature and changes in temperature • Water velocity • Water flow rate (inlet, tank)	• Health status • Behaviour - Crowding intensity risk scale for above water • Red water / scales in water • Mortality and its cause • Skin colour change	• Laksvel injury-based OWIs (either monitoring using underwater cameras or minimally invasive assessment if the fish are individually sampled for other purposes anyway) • Mouth opening/Ventilation frequency																				
2 Second stage OWIs	• CO₂ • pH and change in pH • Gas supersaturation ▪ Severity ▪ Frequency ▪ Duration • If crowding in RAS also consider salinity, ammonia nitrogen (NH₃-N) and nitrite nitrogen (NO₂-N)	• A selection of the CrowdMonitor behavioural OWIs to consider if not using the intensity scale:<table><tr><td>▪ Burrowing</td><td>▪ Space between fish</td></tr><tr><td>▪ Gasping</td><td>▪ Distance to surface</td></tr><tr><td>▪ Lethargy</td><td>▪ Fins/back/heads out of water</td></tr><tr><td>▪ White side at surface</td><td>▪ Water surface state</td></tr><tr><td>▪ Contact with tank/equipment</td><td>▪ Swimming speed</td></tr></table>	▪ Burrowing	▪ Space between fish	▪ Gasping	▪ Distance to surface	▪ Lethargy	▪ Fins/back/heads out of water	▪ White side at surface	▪ Water surface state	▪ Contact with tank/equipment	▪ Swimming speed	• Detailed gill damages scoring • Laksvel injury-based OWIs:<table><tr><td>▪ First impression</td><td>▪ Eye opacity</td></tr><tr><td>▪ Scale loss</td><td>▪ Eye injuries</td></tr><tr><td>▪ Skin haemorrhaging</td><td>▪ Opercular injuries</td></tr><tr><td>▪ Body wounds</td><td>▪ Fin damage</td></tr><tr><td>▪ Snout injuries</td><td></td></tr></table>	▪ First impression	▪ Eye opacity	▪ Scale loss	▪ Eye injuries	▪ Skin haemorrhaging	▪ Opercular injuries	▪ Body wounds	▪ Fin damage	▪ Snout injuries	
▪ Burrowing	▪ Space between fish																						
▪ Gasping	▪ Distance to surface																						
▪ Lethargy	▪ Fins/back/heads out of water																						
▪ White side at surface	▪ Water surface state																						
▪ Contact with tank/equipment	▪ Swimming speed																						
▪ First impression	▪ Eye opacity																						
▪ Scale loss	▪ Eye injuries																						
▪ Skin haemorrhaging	▪ Opercular injuries																						
▪ Body wounds	▪ Fin damage																						
▪ Snout injuries																							
3 Third stage LABWIs	• Hydrodynamics of the rearing system • General tank design • Tank infrastructure and equipment		• Mucosal surfaces • Electrocardiographic predictors of heart disease • Skin, gill, heart histology • Keratocyte migration capacity – wound healing rate • Transcriptional analyses • Physiological markers e.g., plasma cortisol																				

Figure 3 An updated OWI and LABWI toolbox for crowding incorporating i) Input-based WIs (water quality parameters in this instance), ii) Outcome-based WIs at the group and individual level. Figure © Noble, C. Stien, L.H., Alvestad, R., Tschirren L. and Johansson, G.S. Adapted from figures outlined in ¹.

4.3.1 Input-based OWIs for detecting crowding risks in tanks

Dissolved Oxygen (DO) concentration and saturation levels. Increased fish density or poor water exchange in and around the crowd can lead to low DO saturation levels, which may be a welfare risk for the fish. This risk can be exacerbated by potential increases in the metabolic rate of the fish due to increased stress and/or activity levels, which in turn increase their oxygen demand. This is particularly relevant if the fish have not been fully fasted. Health issues, principally gill and heart health can also impact upon the oxygen requirements of the fish. Numerous authors have demonstrated that oxygen requirements are temperature dependent and ³⁹ has outlined an array of thresholds for salmon post-smolts at differing temperatures that can be considered either optimal (lower limits for DO saturations for maintaining maximal feed intake, termed DO_{maxFI}) or critical (the upper level of the Limiting Oxygen Saturation, LOS) at different temperatures for clinically healthy fish (see Table 3). However, caution should always be applied when considering specific DO saturation thresholds as numerous factors including the clinical health status of the fish and stress can have a marked impact upon how individual fish react to these limits ⁴⁰ and other authors have proposed that DO saturation *ranges* are utilised instead ⁴¹.

Building upon the work of Remen et al. ³⁹, Nilsson and colleagues ⁷ visualised and extended their DO thresholds from 4 – 20°C (see Figure 4 below, where the Remen et al., ³⁹ data are represented by the red/orange border). To account for potential variation in oxygen demand due to e.g. health status, stress levels etc., a margin of 10% has been added for the yellow level and 20% for the green level. Berntsson et al. ⁴² have recently suggested adding 40% margins to the DO_{maxFI} and LOS thresholds for practical farming operations to take fish health and activity status into consideration, based upon suggestions from Remen et al. ⁴⁰ who added this margin to the upper level of their LOS (see Table 3 for original and adjusted DO_{maxFI} and LOS data below).

DO saturations should at least be measured where the saturation can be expected to be the lowest, often near the tank outlet, but preferably at multiple points within the crowd, and if possible, over multiple depths, to help identify locations and times of highest risk. Dissolved oxygen supersaturation, leading to hyperoxia, is often a risk in tanks due to heavy use of emergency oxygenation systems. Hyperoxia has been shown to negatively affect fish health and welfare, yet little is known about acceptable target ranges for short term exposure (e.g. 6-12 hours).

Table 3 Showing the lower thresholds for DO saturation that maintain maximal feed intake (DO_{maxFI}) and the upper thresholds for Limiting Oxygen Saturation (LOS) levels for 300-500g post-smolt Atlantic salmon at differing temperatures, according to Remen et al. ³⁹, in addition to adjusted precautionary limits (as outlined by ⁴² based upon data from ⁴⁰).

Temperature (°C)	DO_{maxFI}	LOS	Adjusted DO_{maxFI}	Adjusted LOS
7	42%	24%	59%	34%
11	53%	33%	74%	46%
15	66%	34%	92%	48%
19	76%	40%	106%	56%

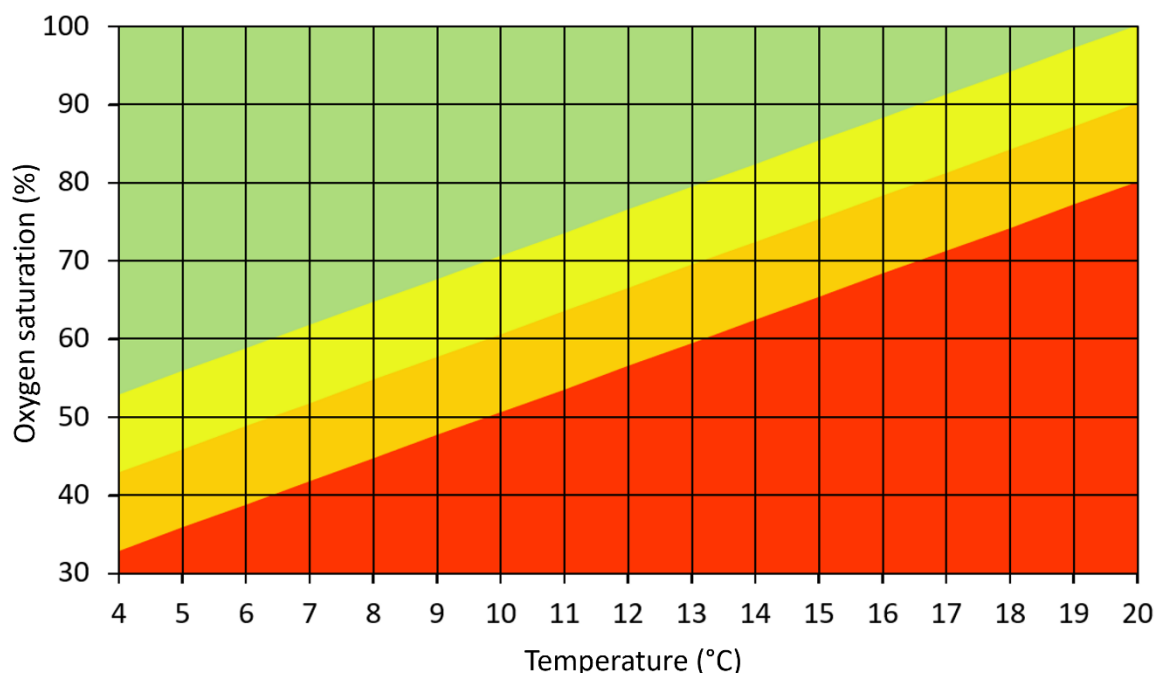


Figure 4 Outlining limiting values of oxygen saturation at water temperatures of 4 – 20 °C. This figure is reproduced with permission from Nilsson et al. ⁷. The values are based on known minimum levels that ensure normal activity in healthy, unstressed post-smolts, based upon work carried out by Remen et al. ³⁹, which here are represented by red/orange border. To account for potential variation in oxygen demand due to e.g. health status, stress levels etc., a margin of 10% has been added for the yellow level and 20% for the green level.

Water Temperature. Ambient water temperature drives the metabolism of many fish species ⁴³, wound healing rate ⁴⁴, affect oxygen solubility and pathogen and parasitic risks ⁷. Crowding risks increase with both high and low temperatures. Gill health and general fish health status should be considered when crowding at high temperatures (after Norwegian Food Safety Authority ²⁴) and low temperatures decrease wound healing rates ⁴⁴ and increase risks for skin ulcers e.g. ^{1,27} and references therein. Expected temperature changes after the operation e.g. whether the fish will be subjected to rising or falling temperatures should also be considered when evaluating welfare risk.

If crowding in RAS. See the FISHWELL handbook ¹ for water quality recommendations on salinity, gas supersaturation, ammonia nitrogen (NH₃-N) and nitrite nitrogen (NO₂-N), pH and CO₂.

Water velocity and exchange. Replenishes oxygen, dilutes or flushes out metabolites and aids the removal of e.g. any fish faecal material during a crowd if the fish haven't been fully fasted ¹. However, high velocities can lead to the fish losing behavioural control and their ability to hold their position within a crowd and can lead to stress, panic or exhaustion. The velocities that salmon can tolerate over time depend on e.g. fish size, fish status (especially gill status), water temperature, oxygen saturation and more. High velocity can also lead to fish being trapped up against crowding equipment which poses a welfare risk to the fish, particularly if they are tiring. If fish lose their ability to maintain their position during free swimming, or drift against the tank, water velocities should be reduced.

Poor water clarity. Poor visibility restricts visual observations of both the fish and the crowding operation, making it difficult to monitor and manage crowding risks. If the poor clarity is due to solids containing abrasive particles ⁴⁵ it can negatively affect gill health. Heightened levels of turbidity can also negatively affect water quality and reduce DO levels, e.g. ¹ and references therein.

4.3.2 Outcome-based OWIs for detecting crowding risks in tanks: group level

Health Status. Prior knowledge of the fish's health status will help an operator get a better overview of the potential health and welfare risks the fish will face during the crowding operation. The fish should be resilient enough to withstand the operation ¹. See Table 2 for guidelines on how risk can be documented in relation to this indicator. Engdal et al. ¹⁷ have refined and developed quantitative (addressing 5 traits) and qualitative (picture guide addressing 42 morphological traits) methods for auditing heart morphology, in addition to producing a pictorial guide for operational use as an appendix ¹⁷.

Mortality. A non-specific but appropriate indicator ⁷ to use around crowding operations. Can be followed during and after an operation to actively and retrospectively examine its welfare impacts ¹. Differing mortality levels are outlined in Table 2, after ⁷. Cause of mortality should also be documented.

Red water/Scales in water. Blood in the water (red water) can be a sign of gill injuries ¹. Scales in the water are an indicator of scale loss and skin damage (also to the mucosal surface). This damage can cause e.g. osmoregulatory issues and may provide access to opportunistic pathogens and/or ulcer development ¹.

Skin colour change. A non-invasive indicator for stress can be a change in skin colour from green to blue and this indicator can be monitored during crowding ^{1,25,46}.

Behaviour. Above water observational metrics have been developed in the CrowdMonitor project for assisting crowd management in tanks. CrowdMonitor observation metrics are predominantly behavioural and metrics consider how fish interact with the water surface, the tank, the crowding equipment and/or each other. Group structure is not included as a metric as fish can swim calmly but in an unstructured, irregular manner during crowding ^{4,5} and fish should be able to make a turn to avoid other fish or equipment/obstacles e.g. ²⁶. See Table 4 for overwater observations metrics.

Table 4 Observational metrics and surface observation descriptions for the CrowdMonitor crowding intensity risk scale for Atlantic salmon in tanks (adapted from Alvestad et al., in prep ¹⁴).

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 tank 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 3 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/back/heads out of water	Proportion of dorsal fins/back/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}

4.3.3 An updated crowding intensity risk scale for tanks

This section outlines an updated crowding intensity risk scale for tanks, refined from the crowding intensity scale proposed by Mejdell et al. ²⁵, the RSPCA welfare standards for farmed Atlantic salmon ²⁶ and adopted by the FISHWELL handbook ¹. The update predominantly i) increases the range of observational metrics that are included in the scale and attempt to standardise the terminology for each metric, and ii) proposes a pre-crowd or inter-crowd state (Level 1 - outlining the metrics that are e.g. desirable before the crowding begins or any inter-crowd period). We also aim to format it in a way that can be integrated into Standard Operating Procedures (SOPs). It is the same as the one we propose for net pens, but we reproduce it here to avoid the need for cross-referencing between different handbook versions.

We propose that:

- **level 1** addresses any potential acclimation and inter-crowd period and can be classified as **minimal risk - the crowding can proceed as planned**
- **level 2** can be classified as **low risk** and the crowding can **proceed as planned**
- **level 3** has **increasing risk** but the crowding can **proceed as planned (with increasing levels of diligence** from the crowding staff with **corrective actions being readied and applied if needed)**
- **level 4 poses even greater risks** and the crowding staff should **consider intervening with a corrective action/pausing/releasing the crowd**
- **level 5 is high risk, clearly unacceptable, and a corrective action should be immediately implemented/the crowd should be released** (this also equates to many of the metrics for level 5 crowding in the previous intensity scales ^{1,25,26}).

The suggestions we provide are no guarantee of crowding outcomes and are meant as guidance and support for personnel involved in monitoring and managing the crowd.

Observing one or more metrics is enough for defining the crowding intensity level in the risk scale.

The tank can also be divided into various risk zones e.g. area's around static oxygenation equipment and observational metrics can be especially monitored in these area's if needed or desired.

Table 5 The CrowdMonitor updated crowding intensity scale to use during tank crowding based on surface observations. NB: contrary to the heads out of the water, fins out of the water may not represent an overt risk but may still be used as early indicators of risk. Table © Noble, C. Stien, L.H. and Johansson, G.S.

Crowding intensity scale: tanks	LEVEL 1 – potential acclimation and inter-crowd	LEVEL 2 – proceed as planned	LEVEL 3 - proceed as planned/prepare corrective action	LEVEL 4 – implement corrective action/ pause/release crowd	LEVEL 5– implement corrective action/release crowd
BURROWING	None or	None or	None or	Potential burrowing observed from surface or	Extensive burrowing observed or
GASPING	None or	None or	None or	Sporadic instances of fish gasping or	Frequent instances of fish gasping or
LETHARGY	None or	None or	None or	Sporadic instances of lethargic fish or	Frequent instances of lethargic/exhausted fish or
WHITE SIDES AT WATER SURFACE	None or	None or	A few white sides occasionally in crowding risk zones (for 2-3 seconds per fish) or	High number of white sides in crowding risk zones (for 4-5 seconds per fish) or	High number of white sides across a large portion of the tank for > 5 seconds or
CONTACT WITH TANK AND EQUIPMENT	No fish in contact with the tank or tank equipment or	No fish in contact with the tank or tank equipment or	Fish occasionally in contact with the tank or tank equipment and fish have opportunity to move or	Fish frequently in contact with the tank or tank equipment but still have opportunity to move or	Fish pressed against the tank and tank equipment, no opportunity to move or
SPACE BETWEEN FISH	Fish at the surface have enough space to swim around each other or	Fish at the surface have enough space to swim around each other or	Fish are close together but fish are moving around each other or	Fish are very close together but they can still move around each other or	Many fish in contact with each other, fish cannot move around each other and are stuck or
DISTANCE TO WATER SURFACE	Clear gap between the surface and the fish group or	Clear gap between the surface and the fish group or	Small gap between the surface and the fish group or	No gap between the surface and the fish group, some fish backs exposed or	No gap between the surface and the fish group, high number of fish backs exposed or
DORSAL FINS/BACKS/HEADS OUT OF WATER	Low to none or	Some fins visible in crowding risk zones but not whole surface of crowd or	Moderate proportion of fins visible in crowding risk zones but not whole surface of crowd or	Occasional backs/heads out of the water in crowding risk zones but not whole surface of crowd or	Many fish backs/heads out of the water or
WATER SURFACE STATE	Occasional fish driven ripples, breaks and splashes from individual fish or	Modest frequency of fish driven ripples and breaks, occasional splashes or	High frequency of fish driven ripples, breaks and/or splashes or	The surface is covered with fish driven ripples, breaks and/or splashes or	Surface is "boiling" with fish splashing or
SWIMMING SPEED	Fish mainly swimming normally or	Fish swimming potentially slightly faster than normal or	Several fish swimming slower than normal with some observations of burst swimming or	Groups of fish stationary and/or increasing observations of burst swimming or	Nearly all fish stationary, burst swimming very difficult, but high number of burst attempts

4.3.4 Outcome-based OWIs for detecting crowding risks in tanks: individual level

For the first stage of the framework – deploying OWIs only – measuring and monitoring welfare indicators at the individual level may still be done either through observations with underwater cameras or when the fish are handled for other purposes. At such opportunities, the majority of the **LAKSVEL** ⁷ **external injury-based OWIs** are appropriate for monitoring and documenting crowding, especially the ones relating to i) the first impression of the fish, ii) scale loss, iii) skin haemorrhaging, iv) body wounds, v) snout injuries, vi) eye opacity, vii) eye injuries, viii) opercular injuries, ix) gill damage and x) fin damage.

Further OWIs to consider include:

Ventilation frequency/mouth opening frequency. Ventilation frequency has been used as a welfare indicator for crowding ⁴. Monitoring is most effective if fish are static or swimming slowly ¹. New technologies are available for calculating mouth opening frequency ^{13,50} using computer vision and machine learning or via the use of fish tags ⁵¹. When using ventilation frequency as a WI, dissolved oxygen saturations, water velocity or gill health status can affect absolute values. Noble et al. ¹ suggested using a percentage change in ventilation frequency when it is monitored before, during and after a crowding operation as a possible way around these challenges. This indicator may be difficult to apply in e.g. turbid RAS tanks.

For the second stage of the framework – deployed when the first stage results are unclear or reveal issues – a sub sample of the fish should be taken and the **LAKSVEL** ⁷ **external injury-based OWIs** outlined above can be monitored if they weren't evaluated during the first stage. Further OWIs to consider include:

Muscle pH can also be monitored as increased swimming activity or stress levels generate lactic acid in the muscle which lowers muscle pH ^{52,53}.

Lactate and glucose. Although these OWIs do take some time to peak following exposure to a stressor and their monitoring should be adapted accordingly, they have been used as OWIs for crowding ^{2,4,19,54}.

Muscle pH, lactate and glucose results are temperature dependent, and the information gained from sampling these indicators is more robust if they are sampled at intervals during and at the end of the crowding process.

For the third stage of the framework – used when the first and second stage results are unclear or revealed issues – more detailed indicators can be used to reveal potential causes of these issues. Other appropriate LABWIs at the third stage include:

Mucus. Crowding can affect the biological and chemical properties of the mucus layer on mucosal surfaces in close contact with the aquatic environment. Mucus can be collected from the skin and gills and may undergo targeted analysis of immune molecules or stress-related metabolites or it can be analysed using high-throughput profiling on advanced platforms such as metabolomics and proteomics ⁸.

Electrocardiogram (ECG). Several studies have shown that elevated heart rate can serve as an indicator of stress in various fish species, including farmed Atlantic salmon ¹⁰ and can be influenced by crowding intensity in tanks when the crowded using tank draining ²⁰. Electrocardiogram (ECG) analysis offers a valuable tool for real-time monitoring of cardiac electrical activity and provides insights into underlying conditions by tracking changes in ECG wave amplitude and duration. Recently, ECG recordings in anesthetized rainbow trout have demonstrated proof of concept for detecting heart disease

through rapid ECG screening ⁹, which suggests it could serve as a diagnostic tool for assessing cardiovascular disease risk and detecting stress-related crowding risks in farmed salmon.

Skin, gill and heart histology. Advanced imaging techniques and histological LABWIs that consider skin, heart and gill morphology may be used to check for micro-damage that is hard to determine with the naked eye ^{5,46}. Micro-damage may reduce oxygen uptake from the gills, heart functions and the barrier properties of skin. Skin damage may further reduce the skin's healing capacity, leading to the development of larger wounds and/or secondary infections ⁵⁵.

Keratocyte migration capacity. Increased crowding intensity induces, amongst others, micro-damage to the epidermis, which may decrease the migration capacity of keratocytes. If this persists over time, it may have a further impact on the skin's healing ability ⁵.

Transcriptional responses. Crowding can impact upon the gill transcriptome by triggering stress-related genes, influencing inflammation pathways, and potentially altering immune functions ⁴⁶. These changes can persist for several weeks, with the magnitude of the change dependent upon on the stressor.

Plasma cortisol. Crowding can stress the fish ^{2,5} and plasma cortisol levels and the time it takes for these levels to return to basal states can be a suitable indicator for crowding.

When considering outcome-based individual OWIs for crowding, Noble et al., ¹ state “*Although these parameters can be measured on the individual, a decision also has to be made at the group level, by comparing data from pre- and post- crowding*” and this approach is also suggested in the CrowdMonitor handbook.

5 After crowding and handling

Continued monitoring of fish health and welfare after the crowding and any subsequent operation allows for key insights about its effects, which then can inform future decisions on what corrective actions should be implemented. The following section therefore outlines a brief WI toolbox for monitoring fish welfare and managing welfare risks **after the crowding and handling operation in tanks**. Much of this toolbox can be monitored non-invasively e.g. behaviour using underwater cameras or the return of appetite.

5.1 Further risks to consider after crowding and handling

The risks before and after crowding may be similar, but drivers can be different, e.g. while impaired fish health before crowding can cause mortalities during the handling, the effect of the crowding and subsequent handling may cause impaired health and welfare afterwards. For example, it is well established that crowding can e.g. stress the fish, and lead to skin problems such as wounds, see ^{1,5} and references therein. Burrowing, gasping or panic driven behaviours can be an indicator that the fish have been stressed (and burrowing can also injure the fish, e.g. lead to snout wounds), and reduced water quality or poor weather during the crowd can lead to problems after the operation has been completed.

Box 5: Risks AFTER crowding and handling include, but are not limited to:

- Compromised health and welfare status:
 - Poor gill health
 - Poor heart health
 - Poor skin condition
 - Abnormal behaviour
 - Reduced appetite
 - Increased mortality
- Suboptimal water quality:
 - High temperature (and whether it is expected to rise or fall post handling)
 - Low temperature (and whether it is expected to rise or fall post handling)
 - Water velocities that are too high or too low
 - Presence of planktonic threats after sea transfer
 - High waves (and also wind speeds)

5.2 A Welfare Indicator Toolbox to use after crowding and handling

Evaluating health and welfare consequences for fish after a crowding operation is essential knowledge, to optimise the ongoing management of the fish population and to systematically improve crowding procedures for future operations. In this context, the outcome-based welfare indicators are especially relevant as they measure the result or outcome of what the fish is being or has been subjected to – in this case, a crowding operation followed by the rest of the handling operation (e.g. pumping + seawater transfer). Indicators include both the immediate health and welfare status of the fish after the operation, as well as how their health and welfare status develops during the days and weeks after the handling operation (Table 6).

The CrowdMonitor project has identified six key sets of welfare indicators and factors that a stakeholder should consider to gain a holistic insight into fish health and welfare status **after crowding and subsequent handling**. This post-handling toolbox can be used in both net pens and tanks. The grading of the welfare indicators follows the LAKSVEL protocol ⁷, where the indicators are divided into four levels from 0 to 3 (Table 1). The six welfare indicators are outlined in Table 6. NB water quality, water flow and velocity, wind speed, wave action and the presence of planktonic threats (following seawater transfer to marine net pens) should also be considered in the toolbox, but these are not assigned levels 0-3.

Table 6 A suggested toolbox for monitoring the health and welfare status of the fish **after crowding**. The toolbox focuses predominantly on outcome-based WIs. NB water quality and the presence of planktonic threats should also be considered in the toolbox.

Post-crowding/ handling	Level 0	Level 1	Level 2	Level 3	
Return of normal behaviour	Return of "normal group shoaling behaviour (outside feeding times) with no or very few individuals with deviating behaviour"	Return of "normal shoaling behaviour by the majority of fish, but small groups of individuals isolate themselves from the general fish group ("loser fish")"	There is still "clear deviating behaviour of large parts of the group or an increasing or significant part of the group with... [...] other abnormal behaviour"	There is still "very clear deviating behaviour, or large part of the group showing behaviour that indicates...[challenges with the operation]... often connected with increased mortality"	Adapted from Nilsson et al. ⁷
Return of appetite	Fish show normal feeding motivation and have eaten as much as expected over the past week based on fish size, water temperature and time of year	Fish show slightly lower feeding motivation and have eaten somewhat less than expected over the past week based on fish size, water temperature and time of year	Fish show markedly lower feeding motivation and have eaten noticeably less than expected over the past week based on fish size, water temperature and time of year	Fish are eating very little and have shown little feeding motivation over the past week based on fish size, water temperature and time of year	Adapted from Nilsson et al. ⁷
Mortality	Monthly: 0.0-0.3% Weekly: 0.0-0.7% Daily: 0.0-0.1%	Monthly: 0.3-0.7% Weekly: 0.7-1.7% Daily: 0.1-0.25%	Monthly: 0.7-2.0% Weekly: 1.7-4.9% Daily: 0.25-0.7%	Monthly: >2.0% Weekly: >4.9% Daily: >0.7%	Nilsson et al. ⁷
Gill health status	Free from injuries or deviations, no evidence of e.g. amoebic gill disease (AGD)	Minor injury or deviation	Clear injury or deviation	Severe injury or deviation	Adapted from Nilsson et al. ⁷
Heart health status	No evidence of HSMI/CMS. No deviation in autopsy findings/histology	HSMI/CMS suspected. Minor deviation in autopsy findings/histology	HSMI/CMS confirmed. Clear deviation in autopsy findings/histology	HSMI/CMS outbreak ongoing. Severe deviation in autopsy findings/histology	adapted from Fritzvold et al. ²⁹
Scale loss, body wound/ulcer, skin haemorrhaging, snout damage, fin damage	Free from injuries or deviations	Minor injury or deviation	Clear injury or deviation	Severe injury or deviation	Nilsson et al. ⁷

Other input based OWIs to consider not assigned a 0-3 score: Dissolved oxygen saturation levels, water temperature and changes in water temperature, water flow and velocity, wind speed, wave action and the presence of planktonic threats (following seawater transfer to marine net pens), see section on input-based indicators during crowding in the CrowdMonitor net pen handbook for more information on these parameters

5.2.1 Outcome-based OWIs to consider after the crowding and handling operation

Monitoring welfare indicators after crowding and handling can, to a large extent, follow those that are monitored during the operation, but with some further information to consider for certain outcome-based welfare indicators:

Return of normal behaviour. The time taken for normal behaviour to return can also be used as a retrospective indicator of how the operation affected the fish. Fish can return to normal activity levels 24 hours after crowding ^{19,49}. Behaviours to consider are outlined in Table 6, after ⁷.

Return of appetite. Appetite drops can be due to stress and health and welfare problems ^{7,56} and the time needed for appetite to return after the operation can be a relevant indicator for monitoring its severity ¹. A rapid return to normal appetite levels as outlined in Table 6, after ⁷ is desirable.

Mortality. Continue to follow up mortality after an operation to retrospectively examine its welfare impacts ¹. Differing mortality levels are outlined in Table 6, after ⁷. Cause of mortality should also be documented.

Health Status. An ongoing overview of any changes in health status after the crowding and handling operation will help an operator get a better overview of future potential health and welfare risks. See Table 6 for guidelines on how to follow health status after the crowding event.

The majority of the **LAKSVEL** ⁷ **external injury-based OWIs** are appropriate for monitoring and documenting the effects of crowding and handling on the fish after the operation, especially the ones relating to i) the first impression of the fish, ii) scale loss, iii) skin haemorrhaging, iv) body wounds, v) snout injuries, vi) eye opacity, vii) eye injuries, viii) opercular injuries, ix) gill damage and x) fin damage. **NB.** With regard to non-medicinal delousing treatments that involve handling (crowding, pumping, handling), the Norwegian Food Safety Authority ²⁴ have stated that if after 24 hours post-handling 0.1% of fish are observed with acute body wounds or eye damage that correspond with LAKSVEL ⁷ level 2 or 3, it is an undesired result of the handling.

Growth. Post crowding growth can be negatively affected by how stressful the operation was in both the short and long-term as the fish's motivation to eat might be negatively affected after the end of the operation ¹.

6 Discussion

This handbook builds upon and expands existing welfare indicator frameworks, toolboxes and recommendations for aiding the crowding of Atlantic salmon in tanks based upon the results of the CrowdMonitor project. It also proposes preliminary versions of an updated crowding risk scale for tanks. The handbook utilises the format of the existing welfare indicator toolbox outlined in the FISHWELL handbook ¹ and the LAKSVEL protocol ⁷ and further develops them for a crowding-specific purpose using the latest scientific insights and state-of-the-art knowledge. The goal of this handbook is to present stakeholders with a standardised framework from which tailored toolboxes can be created that fit their farm(s), personnel and operations.

The CrowdMonitor handbook authors would like to fully emphasise the preliminary nature of these revised intensity scales. The suggestions we provide are no guarantee of crowding outcomes and are meant as guidance and support for personnel involved in monitoring and managing the crowd. Whilst they are based upon state of the art generated in the CrowdMonitor project and other information sources, they are the first version of an updated scale that hasn't been extensively tested in a range of commercial crowding situations. We hope that we have been transparent with our revisions of previous crowding intensity scales for tanks. Feedback is very welcome, and we hope the scales can be tested, improved and built upon over time as momentum and experience builds with their use.

In light of this we would like to suggest the following:

6.1 Create your toolbox from the framework and use it

The presented health and welfare monitoring framework (Figure 1) arranges input- and outcome-based OWIs and LABWIs into three stages for the specific process of crowding farmed Atlantic salmon. From this standardised framework, a farm- and system-specific toolbox is created to monitor fish health and welfare during the whole process and offer decision guidance along the way.

In order to create a toolbox from the framework that is best suited to your systems and operations, please select the most appropriate WIs from the CrowdMonitor toolboxes when conducting a risk assessment of the crowding operation, paying attention to personnel responsibilities and competencies. For example, make lists of these indicators and add additional indicators that you consider relevant and/or are already part of your farm's routine if these are missing from the CrowdMonitor toolbox. Define how, how often and how frequently the WIs are measured and monitored. Write protocols, ensure all staff are trained in their use and both collect and interpret the data the same way. One should also consider what corrective actions to implement and when, especially e.g. relevant stop criteria. Clarify what the results of each indicator means and which indicators (alone or in combination) lead to which corrective action. Define when these actions should be implemented and also state who passes on, receives and has access to which information from the toolboxes to put potential actions in motion.

6.2 The value of long-term documentation

In addition to the immediate, actionable insights a fish health and welfare toolbox has, there is tremendous value in the long-term documentation of the data gathered. If the results generated by the toolbox are kept as digital data over time, additional insights can be gained by revealing patterns, correlations and causations. Knowing more about the causes and effects of factors before, during and after crowding can help improve future operations.

6.3 Key recommendations for crowding

Effective crowd management, diligent planning and smooth implementation of a crowding operation will help prevent and reduce the risk of poor fish welfare. This requires a clear understanding of potential risks and robust monitoring to give an operator a better overview of potential bottlenecks in the crowding operation and assist in decision support. Robust monitoring can help prevent problems with the crowd. When problems are encountered, effective and timely interventions should minimise their impacts.

Box 6: Some key recommendations when crowding fish in tanks

- Use welfare indicator toolboxes and crowding intensity risk scales if they are appropriate to your system/operation.
- Check all crowding equipment is in good working order before the crowd.
- Aim for a low-risk crowd, where fish have ample space and favourable conditions to control their movements and move amongst their conspecifics.
- Crowding at low water temperatures can be a risk for winter ulcers.
- Crowding at high water temperatures can increase oxygen demand and reduce oxygen availability to the fish.
- Monitor water quality parameters closely, especially oxygen levels, and use supplementary oxygen if necessary. Some authors suggest maintaining dissolved oxygen saturations at 80% ⁵⁷ or ca. 50 – 100% at temperatures 4 – 20 ° C ⁷, ca. 60 – 110% at temperatures 7 – 19 ° C ⁴² or 100% where possible ²⁴. 70% DO saturations are reported to be a widely used stop criteria in the industry ⁵⁸ and < 70% has also been proposed for fish in poor health ²³.
- Monitor fish behaviour from above the water surface (and also below if possible and feasible – see the ROV observation scale in the CrowdMonitor net pen handbook for pointers). Ensure that there is no contact between the fish and crowding equipment or the rearing system, and that the fish can move and reposition themselves in the crowd.
- A compact and dense crowd can still be low risk if water quality is sufficient, and the fish maintain behavioural control.
- Carry out the crowd in a methodical step-wise manner. Avoid rapid management actions that can trigger burrowing, surging and/or other panicking behaviours in the crowd. This must especially be avoided when the crowd is dense (e.g. if the fish begin to have limited space for movement or exert pressure upon each other due to close uncontrollable contact), or water quality is deteriorating or poor. Panic behaviours also increase oxygen demand. The crowd should be released in such situations.
- Areas where the fish can be stuck should be monitored and avoided if possible ^{e.g. 27}.
- Fish expelling bubbles via the mouth/gill openings can be a sign of acute stress ³⁷. Fish can also descend in the water column when this occurs, and it is important to give fish sufficient depth in the crowd to account for this.
- Crowding duration. Crowding is a stressful situation for the fish and some crowding recommendations and welfare standards suggest time limits for crowding ^{e.g. 23,26,30,32,58,59}. The crowd should be as short as possible but the wish for shortening crowding time should be balanced against the need for conducting the crowding operation in a calm and controlled manner.
- Crowding intensity and the maintenance of crowding intensity should also be balanced against the need for carrying out crowding in a calm and controlled manner.
- When procedures (e.g. pumping) or equipment failures cause delays and prolong the crowding period, the crowd should be loosened to allow the fish to rest.

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